



# Workshop on Plant Biology 2024

Centro Residenziale Universitario di Bertinoro  
21-23 February 2024



SOCIETA' ITALIANA di  
BIOLOGIA VEGETALE

# Workshop SIBV 2024

## Scientific and Organizing Committee

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<b>Maria Manuela Rigano</b>	University of Napoli Federico II
<b>Mirko Zaffagnini</b>	University of Bologna
<b>Michela Zottini</b>	University of Padova

### Conference venue

Centro Residenziale Universitario di Bertinoro (Forlì-Cesena)

### Conference Language

The official conference language is English

### Conference Dinner on February 21

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

GENERAL INFORMATION



# Wednesday, February 21, 2024

**01:30 pm**      **Registration and Welcome buffet**  
02:15 pm      Workshop Opening

## Session 1: Adaptation mechanisms to environmental stress

*Chairs: Daniele Coculo, Ginevra Marie Eloise Peppi*

### Invited:

02:30 pm      **Evolution of a unique O<sub>2</sub> sensing mechanism in land plants.**  
Laura Dalle Carbonare, *University Oxford*

### Oral Presentations:

03:10 pm      **Unveiling the role of second messengers in salt tolerance in rice (*Oryza sativa*)**  
Gabriele Pino, *Università degli Studi di Padova*

03:25 pm      **An Arabidopsis Regulator of G-protein like Receptor is required for the activation of defence responses induced by celotriose.**  
Valentina Scafati, *Università degli Studi dell'Aquila*

03:40 pm      **LISCL-dependent cellular detoxification: a pivotal pathway in plant-environment interactions**  
Maurizio Carnà, *Università degli Studi di Torino*

03:55 pm      **Role of ACA8 and ACA10 in wound-triggered systemic signaling leading to stomatal closure**  
Chiara Pedalino, *Università di Roma Tre*

**04:10 pm**      **COFFEE BREAK**

04:50 pm      **THE cAMP-dependent plant stress response**  
Eleonora Davide, *Università degli Studi dell'Insubria*

05:05 pm      **Plant-based biostimulants for soybean seed priming**  
Hisham Wazeer, *Università degli Studi di Pavia*

05:20 pm      **Role of CXE15 in strigolactone degradation under drought stress in tomato**  
Eva Campo, *Università degli Studi di Torino*

05:35 pm      **Novel "CYS-NDP" substrates identified in a yeast synthetic pathway**  
Noemi La Monaca *Sant'Anna School of Advanced Studies*

05:50 pm      **Scientific Quiz**

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

# Thursday, February 22, 2024

## Session 2: Plant Nutrition and Metabolism

Chairs: Adriana Furlani, Matteo Grenzi

### Invited:

09:00 am **Biofortification and Molecular Farming: two sides of the same coin.**  
Gianfranco Diretto, ENEA – Roma

### Oral Presentations:

09:40 am **Phytohormone involvement in *Medicago truncatula* seeds pre-germinative metabolism: from transcription to biochemical profiles**  
Federico Sincinelli, Università degli Studi di Pavia

09:55 am **Investigating Ca<sup>2+</sup> dynamics in nutrients-deficient plants**  
Chiara Dellapiana, Università degli Studi di Torino

10:10 am **In vitro degradation of plant polyphenols mediated by the versatile enzymatic activity of OSOXs**  
Anna Scortica, Università degli Studi dell'Aquila

10:25 am **Effects of sulfur limitation on photosynthesis and cell composition in *Dunaliella Salina* and *Phaeodactylum tricornutum***  
Minio Miles, Università Politecnica delle Marche

10:40 am **COFFEE BREAK**

11:00 am **Vita dopo il Dottorato di Ricerca, Possibilità e Prospettive per giovani ricercatori.**  
Stefano D'Alessandro (Università degli Studi di Torino), Fabrizio Doccula (JOVE), Maria Manuela Rigano (Università degli Studi di Napoli Federico II), Nico Betterle (Università degli Studi di Verona)

13:00 pm **LUNCH**

## Session 3: Development and signal transduction

Chairs: Francesca Resentini, Noemi Gatti

### Invited:

02:30 pm **Auxin dynamics during developmental programmed cell death events in the *Arabidopsis* root**  
Riccardo Di Mambro, Università degli Studi di Pisa

### Oral Presentations:

03:10 pm **The Dwarf 27 gene inactivation affects plant physiological traits in tomato**  
Alessia Cuccurullo, Università degli Studi di Napoli Federico II

03:25 pm **Characterization of chloroplast ribulose-5-phosphate-3-epimerase from the microalga *Chlamydomonas reinhardtii***  
Maria Meloni, Università degli Studi di Bologna

- 03:40 pm **Microbial volatile organic compounds (mVOCs) induce early and late responses in *Arabidopsis thaliana***  
*Francesco Caldo, Università degli Studi di Torino*
- 03:55 pm **BON1: a Ca<sup>2+</sup>-binding protein at the crosstalk between plant development and plant response to environmental stimuli**  
*Carlotta Nardi, Università degli Studi di Milano*
- 04:10 pm COFFEE BREAK**
- 04: 40 pm **Sketching Science**
- 08:00 pm DINNER**

## Friday, February 23, 2024

### Session 4: Emerging technologies

*Chairs: Martina Bussola, Nicola Bosco*

#### **Invited:**

- 09:00 am **Xylem structure-function relationships: insights from microCT-analysis**  
*Francesca Secchi, University of Torino*

#### **Oral Presentations:**

- 09:40 am **A biotechnology approach for the production of new protein bioplastics**  
*Pasquale Creanza, University of Urbino*
- 09:55 am **Zein-based nanoparticles as new formulation to deliver bioactive dsRNA for plant phytopathogens protection**  
*Sara Paola Nastasi, University of Milano*
- 10:10 am **Hydroponic cultivation of *Nicotiana* species for the production of biomass and bioproducts**  
*Kristina Ijumovic, University of Verona*
- 10:25 am **Liquid fraction of two-phase Olive pomace enriched in oligosaccharides activators of plant immunity**  
*Marco Greco, University of Roma Sapienza*
- 10:40 am COFFEE BREAK**
- 11:10 am **UV-B effects on the physiology of native microalgae from high land, low land and indoor locations**  
*Thais Huarancca, Reyes University of Pisa*
- 11:35 am **Investigating the mode of action of a new biofungicide against fungal and oomycete pathogens of tomato and grapevine**  
*Sofia Montanari, University of Trento*

11:50 am **New state-of-art imaging tools to study how crops adapt to environmental changes: *Lycopersicon esculentum* key study**  
Bianca Orlando, University of Milano

12:15 am **CONCLUDING REMARKS**

01:00 am **LUNCH**

## Sketching Science

February 22, 2024 - 04:45 pm - 06:30 pm

### Whiteboard 1: Adaptation mechanisms to environmental stress

*Chairs: Ambra Selene Parmagnani*

4:45 am – **“Dose-response” effects of prescribed burning on the functionality of *Pinus sylvestris* L. stem hydraulics**  
Rachele Gamba, Università di Torino

5:00 am – **Sucrose level is related to control of Flavescence dorée phytoplasma load in grapevine leaves**  
Cristina Morabito, Università degli Studi di Torino

5:15 am – **The interplay between mannitol and sucrose drives hydraulic recovery in *Fraxinus ornus* saplings exposed to drought and stem shading**  
Sara Gargiulo, Università degli Studi di Trieste e Udine

5:30 am – **Do SLs affect guard cell functioning via the miR319-LANCEOLATE module and gibberellin signaling?**  
Shuo Liang, Università di Torino

5:45 am – **GLR3.7 as negative regulator of GLR3.3 mediated stress responses**  
Stefano Buratti, Università di Milano

6:00 pm – **Polystyrene nanoplastics’ impact on crops: a new challenge for agriculture and food safety**  
Benedetta Pizziconi, Università Campus Bio-Medico di Roma

6:15 pm – **Under fungal attack on a microplastic-contaminated soil: a case study on *Arabidopsis thaliana***  
Marco Dainelli, Università di Firenze

### Whiteboard 2: Plant Nutrition and Metabolism

*Chairs: Silvana Francesca*

4:45 am – **Hypothetical interference of auxin-adjutant urea derivative**  
Giulia Giovanna Salerno, Università di Parma

5:00 am – **Morpho-Physiological and Molecular Effects of Exogenous Strigolactone-like Molecules Regulating Nutrient Use Efficiency in Tomato**  
Ruihan Shen, Università di Torino

5:15 am - **Durum wheat response under different phosphate fertilization regimes**  
Olga Vaccarella, Università di Bari "Aldo Moro"

5:30 am - **Deciphering Eriophyes tiliae's Biohacking Mechanisms: Transcriptomic Profiling of Tilia cordata Gall Formation**  
Filippo Costa, University of Oxford

5:45 am - **Investigating molecular mechanisms of drought memory in tomato (Solanum lycopersicum L.): role of mir156 and strigolactones**  
Diana Davydenko, Università di Torino

6:00 pm – **CaM-dependent NAD kinases: a hallmark of the green lineage**  
Elisa dell'Aglio, Università di Milano

### **Whiteboard 3: Development and signal transduction**

*Chairs: Teodora Chiara Tonto*

4:45 am – **FUTUREGRAIN: exploring the metabolic and resistance diversity among rice varieties in agricultural systems against global warming**  
Domenico Loperfido, Università di Milano

5:00 am - **CYB561-A and CYB561-B are likely involved in intracellular ascorbate redox homeostasis and ROS-mediated signalling**  
Edoardo Tosato, Università di Bologna

5:15 am - **Unravelling the physiological suitability of different tree species for restoration efforts in a Mediterranean protected area**  
Spennati Elisa, Università di Roma Sapienza

5:30 am - **In-silico identification of miRNAs targeting DNA Damage Response (DDR) genes in rice**  
Sria Amarnadh Gupta Tondepu, Università di Pavia

5:45 am- **Thallium tolerance in metallicolous and non-metallicolous accessions of Silene latifolia,**  
Gaia Regini, Università di Firenze

6:00 pm - **Characterization of different isoforms of glucose 6-Phosphate Dehydrogenase from plant and algal sources under abiotic stress**  
Concetta Di Napoli, Università di Napoli "Federico II"

6:15 pm - **Investigating the role of ATBBE oxidases and BBE-like oligosaccharide oxidases: radical cation scavenging activity, structural and physiological implications**  
Emilia Piccirilli, Università dell'Aquila e Università di Pavia

### **Whiteboard 4: Emerging technologies**

*Chairs: Davide Canini*

4:45 am – **The Impact of Coffee Silverskin on Metabolism and Phenotypes of Barley Seedling**

Rosanna Bossa, Università degli studi di Napoli, Federico II

5:00 am - **Innovative non-invasive methods to assess seed quality,**  
Adriano Griffo, Università di Pavia

5:15 am – **Investigating the role of endophytes in enhancing grapevine resilience to drought**  
Irene Doro, Università di Padova

5:30 am - **Plant cell cultures: a biotech platform for the production of ingredients for human healthcare**  
Francesca Bucci, IRB-Croda Italiana Spa

5:45 am - **Micropropagation and regeneration as new tools for *Cannabis sativa* L. breeding**  
Samuele Paganelli, Università di Modena e Reggio Emilia, CREA

6:00 pm - **Sustainable strategies to produce plant defensins with antiviral properties,**  
Lucia Franceschini, Università Campus Bio-medico di Roma

# **Session 1**

## **Adaptation mechanisms to environmental stress**

## Unraveling the role of second messengers in salt tolerance in rice (*Oryza sativa*, L.)

Gabriele Pino, Elide Formentin

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Soil salinization is one of the greatest threats to crop production and this problem is expected to increase in the coming years due to climate change. Rice, a staple food for more than half the world population, is the most salt-sensitive species among cereals. In a previous comparison between salt tolerant and sensitive rice varieties, Baldo (B) and Vialone Nano (VN), respectively, we found differences between the two genotypes both in the salt induced cytosolic  $\text{Ca}^{2+}$  and  $\text{H}_2\text{O}_2$  signals. Since plasma membrane is involved in mediating  $\text{Ca}^{2+}$  and ROS signals, it is possible that B and VN have a different membrane protein composition. In this work, transcriptomic data from VN and B roots were analysed to identify DE membrane proteins that could be related to B tolerance, identifying several ROS modulators and genes involved in  $\text{Ca}^{2+}$  (OSCA2.2, GLR3.1/4) and  $\text{H}_2\text{O}_2$  transport (PIP2;7). Our results show that differences in the plasma membrane components are present. Transgenic plants harbouring the ROS sensor roGFP-Orp1 were produced and for the first time, cytosolic ROS transients can be analysed in rice roots. Analyses are ongoing with plasmamembrane transporters inhibitors in order to verify the hypothesis of differences in signal generation or transduction between sensitive and tolerant plants. Preliminary results showed differences in the two backgrounds. Whether these differences are  $\text{Na}^+$  specific will be a focus of future experiments.

## **An Arabidopsis Regulator of G-protein like Receptor is required for the activation of defence responses induced by cellotriose**

Scafati V., Giovannoni M., Scortica A., Piccirilli E., Mattei B. and Benedetti M.

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The cell wall is a reservoir of different oligosaccharides that are perceived by plants as Damage-Associated Molecular Patterns (cwDAMPs). The ability of cwDAMPs to induce the defense responses in plants has been widely investigated, whereas the mechanisms that are at the basis of their perception remain still not fully clarified. Among the well-characterized cwDAMPs, oligogalacturonides (OGs) and cellodextrins (CDs), derived from the disruption of homogalacturonan and cellulose, respectively, have been the focus of extensive studies. Here, we report the identification of a putative Regulator of G-protein like receptor (RGLR) whose null mutation in Arabidopsis results in a decreased induction of defence responses triggered by the treatment with cellotriose (CD3). The *rglr* null mutant displays a reduced induction of defense marker genes RETOX, FRK1, and PAD3, and a compromised MAP kinase phosphorylation. Preliminary data also indicate impaired CD3-induced resistance to *Botrytis cinerea* infection in the *rglr* null mutant, whereas the same defense responses were comparable to those of wild-type plants upon treatment with the control elicitors OGs and bacterial flagellin. Our results strongly suggest that RGLR functions as a crucial component in the signaling system involved in the activation of the defense responses specifically induced by CD3.

## **LISCL-dependent cellular detoxification: a pivotal pathway in plant-environment interactions**

Carnà M., Cresta A., D'Alessandro S.

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Speaking Author: Carnà M. (maurizio.carna@unito.it) "Living organisms are exposed to many potentially toxic compounds, both from ectopic sources, like pollutants, and from endogenous sources. Under photooxidative stress, the oxidative burst in the chloroplasts generates many oxidative byproducts. Among these molecules, apocarotenoids and lipid peroxides exhibit contrasting effects: apocarotenoids can stimulate plant acclimation, while reactive carbonyl species derived from lipid peroxidation leads to cell death.

My work starts from the identification of the SCL14-dependent detoxification, triggered by apocarotenoids, in the response to excessive light. Based on these findings, we are studying the role of this mechanism in plant acclimation to environmental conditions and elucidating its molecular mechanism. To this aim, we selected Arabidopsis mutant lines for the members of the LISCL family of proteins and analyzed their performance under stress conditions.

Furthermore, molecules that induce LISCL expression may contribute to plant tolerance to stress conditions. In this direction, we are characterizing the effect of three known biostimulants in increasing Arabidopsis wild type and mutant tolerance to salt stress.

## **Role of ACA8 and ACA10 in wound-triggered systemic signaling leading to stomatal closure**

Pedalino C., Secchiero A., Angelini R., Cona A., Fraudentali I.

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After the perception of a mechanical damage, stomatal closure represents one of the first defence responses implemented by plants. Calcium ( $\text{Ca}^{2+}$ ) plays a pivotal role in this response, as stress-driven oscillations of its cytosolic concentration within stomata generate a specific  $\text{Ca}^{2+}$ -signature critical for stomatal movement regulation. Arabidopsis auto-inhibited  $\text{Ca}^{2+}$ -ATPases (ACAs) are plasma membrane pumps crucial in shaping the  $\text{Ca}^{2+}$ -signature. In this study, the role of ACA8 and ACA10 in regulating stomatal movement after the perception of leaf or root wounding was investigated, as they are both especially localized in guard cells among other tissues. Moreover, given its localization in the vascular bundle, the ACA8 involvement in leaf-to-leaf and root-to-leaf systemic signaling was evaluated exploiting *aca8* seedlings expressing the  $\text{Ca}^{2+}$  sensor R-GECO1. Interestingly, no stomatal closure after leaf/root wounding was observed in *aca8*, *aca10*, and *aca8aca10* mutants, as well as in a line overexpressing ACA8, in respect to wt. Moreover, RT-qPCR analyses showed an induction of ACA8 and ACA10 expression levels after both stimuli, stronger for ACA10 than for ACA8. Regarding systemic signaling, in wt×R-GECO1, an intense  $\text{Ca}^{2+}$ -wave propagated from the wounded to the unwounded cotyledon and from the crushed root to both cotyledons after leaf and root wounding, respectively, in a time interval of 1 minute. However, in *aca8*×R-GECO1 the  $\text{Ca}^{2+}$ -wave was completely impaired, as no signal was detected after both stimuli. Overall, data suggest not only the need of both pumps for the proper modulation of stomatal movement, but also the essential role of ACA8 in wound-triggered systemic signaling.

## The cAMP-dependent plant stress response

1Davide E., 1Domingo G., 1Marsoni M., 1Bracale M., 2de Pinto M.C., 3Blanco E., 1Vannini C.

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Numerous studies emphasize the progressively pivotal role of 3'-5' Cyclic Adenosine Monophosphate (cAMP) in the signaling pathways activated by plants in response to heat stress (HS), being cAMP implicated in multi-level regulatory networks that prevent cell damage and maintain cellular homeostasis.

In recent years, due to global warming, there has been a dramatic increase in the frequency, intensity, and duration of heat waves worldwide. Plants are highly sensitive to HS, especially during all stages of their growth and development. High temperatures alter fundamental physiological processes that contribute to crop yield and quality. However, deciphering these processes is extremely challenging, due to their complexity and multi-level nature. In this study, *A. thaliana* plants overexpressing the cAMP-sponge, a genetic tool that reduces intracellular cAMP levels, were used to achieve a more comprehensive characterization of the downstream actors of the cAMP signaling.

Two different HS conditions were established: mild heat and heat shock. Transmission electron microscopy revealed that the dampening of cAMP significantly increases alterations in leaf tissue ultrastructure due to the considered heat stress treatments. Moreover, to identify genes whose expression is altered by HS in a cAMP-dependent manner, a transcriptomic analysis is currently underway.

## **Plant-based biostimulants for soybean seed priming**

Wazeer H., Doria E., Macovei A.

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Plant-based biostimulants (PBs) are natural compounds with the potential to influence sustainable agriculture and bio-economy. PBs improve plant growth and stress resistance, while reducing the need for synthetic fertilizers. Such eco-friendly agricultural practices seek to mitigate environmental harm, ensuring long-term food production and economic stability. The economic worth of agricultural waste and plant by-products can be harnessed through recycling and employing precision farming technologies. Seed priming, a technology aimed at enhancing germination performance, is one such method that aids in establishing dynamic and sustainable agricultural practices. In this work, plant waste by-products have been used to produce biostimulants subsequently applied as seed priming agents to boost seed germination. Canola waste-derived extract was used to treat three soybean varieties (OL 996, NAV 270, EM PURA), along with hydropriming and unprimed controls. The germination efficiency was recorded both under physiological and osmotic stress conditions induced by polyethylene glycol (PEG). Germination performance was monitored for three days and multiple parameters (e.g., percentage, speed, synchrony, uniformity, stress tolerance, radicle growth) were measured. Additionally, reactive oxygen species (ROS) accumulation was measured at different time points during the priming process. The results evidence that the application of canola plant waste-derived biostimulant improved germinability, especially under PEG treatment, and reduced ROS accumulation, thus rescuing the germination under stress.

## Role of CXE15 in strigolactone degradation under drought stress in tomato

Campo Eva, Capezzali Caterina, Cardinale Francesca, Schubert Andrea

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Strigolactones (SLs) are plant hormones produced mainly in the roots and partially released into the soil. They play a crucial role in plant development, root architecture and interactions with other organisms, including mycorrhizae and parasitic plants. Additionally, they contribute to tolerance to abiotic stresses, making them valuable for various applications. However, extracting them is challenging, and synthesizing their analogues (e.g. GR24) is expensive and limited. Natural SLs produced by plants offer a wider range of bioactive molecules with diversified effects than synthetic analogues or mimics. Increasing their content in the plant could be an effective way to overcome these barriers. Recently, it has been demonstrated that specific carboxylesterases, such as AtCXE15, play a significant role in the catabolism of SLs in *A. thaliana*. Expression modulation of these proteins could be used to control SLs levels and release in the soil.

In this study, two putative orthologues of AtCXE15 in *Solanum lycopersicum* (SICXE15\_p1 and SICXE15\_p2) were selected by bioinformatic analysis. Preliminary qRT-PCR analysis revealed different responses to the artificial SL GR24, N/P starvation and osmotic stress. To further investigate the transcript profiles, a water stress experiment was performed on cv. M82 and on the SL-defective line 6936 (silenced in CCD7, a key SLs biosynthetic gene). Physiological data were collected, and plants were sampled at different water potentials. qRT-PCR in leaves and roots showed that both SICXE15\_p1 and SICXE15\_p2 expression is modulated upon stress, and that expression of the latter is significantly different between SL- and wild-type plants.

## **Novel “CYS-NDP” substrates identified in a yeast synthetic pathway**

La Monaca N.(1), Lavilla-Puerta M.(1,3), Perata P.(1), Giuntoli B.(1,2)

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In plants, the perception of molecular oxygen (O<sub>2</sub>) is controlled by the cysteine branch of the N-degron pathway (Cys-NDP), in which plant cysteine oxidase (PCO) enzymes, acting as O<sub>2</sub> sensors, promote the oxidation of the N-terminal Cys from substrate proteins. In *Arabidopsis thaliana*, only seven targets of this pathway have been identified so far. The discovery of novel Met-Cys-starting (MC-) proteins as PCO substrates is essential to understand the impact of this O<sub>2</sub>-sensitive regulation in both stress conditions (eg. flooding) and plant growth and development, since O<sub>2</sub> levels can be kept low within certain tissues. We devised a synthetic biology strategy to carry out a fast screening of Cys-NDP substrates *in vivo*, by expressing PCO enzymes in *Saccharomyces cerevisiae* along with MC- protein sequences in appropriate reporter constructs. With this approach, we surveyed a subset of the *Arabidopsis* MC- proteome and quickly attained the identification of four novel targets. The candidates were subsequently evaluated in *A. thaliana* by transient transformation of protoplasts, confirming their Cys-NDP dependence. This preliminary evidence suggests that our synthetic yeast platform can be a suitable heterologous tool for substrate mining. Among the novel substrates identified, further characterization of a transcription factor involved in leaf development is currently ongoing.

**Session 2**  
**Plant Nutrition and Metabolism**

## **Phytohormone involvement in *Medicago truncatula* seed pre-germinative metabolism: from transcriptome to biochemical profiles**

F. Sincinelli (1), A. Pagano (1), M. Benhamed (2), C. Raynaud (2), X. He (2), A. Macovei (1), J. Garcia (3), D. Vadivel (3), D. Dondi (3), And A. Balestrazzi (1)

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Seed quality improvement has become crucial to meet high-standard agricultural markets as well as responding to the challenges of climate change. Seed priming techniques are considered a valid approach to enhance overall germination performances of orthodox seeds as well as improving seedling stress resilience. Priming protocols involve a controlled imbibition phase, that allows to stimulate the pre-germinative metabolism. Imbibition must be stopped before radicle protrusion occurs, otherwise seeds lose desiccation tolerance, as well as the ability to survive the subsequent dehydration phase (dry-back). The main drawback of seed priming is the highly heterogeneous genotype- and seed-lot dependent responses that impair effective applications of the technique. Successful seed priming boosts the seed repair ability such as antioxidant response and DNA damage response, although such molecular events still lack a detailed description. Notably, the contribution of phytohormonal homeostasis in this context is often neglected although its implication during pre-germinative metabolism is crucial for a correct seedling establishment. In this study, we use RNA-Seq data from an experimental system including primed and overprimed *Medicago truncatula* seeds (Pagano et al. 2023, *Plant, Cell Environ.*; <https://doi.org/10.1111/pce.14295>) to highlight the expression patterns of genes involved in phytohormone metabolism/sensing, along the seed rehydration-dehydration cycle occurring during priming. Biochemical profiles of phytohormones will be also provided. This work will provide an additional perspective to investigate the pre-germinative metabolism and, possibly, to optimize priming protocols.

## Investigating Ca<sup>2+</sup> dynamics in nutrients-deficient plants

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In the context of sustainable crop production, understanding the nutrient sensing and signaling mechanisms in plants to better comprehend plant nutrient balance is a fundamental task. Multiple findings suggest that Ca<sup>2+</sup> serves as a signal for nutrient scarcity. However, few information is available about Ca<sup>2+</sup> signalling under iron (Fe) and sulfur (S) deficiency. Since their essential role in plants' metabolic processes, investigating the interaction occurring between Fe and S in plants is of great importance. Indeed, a close relationship between these nutrients in terms of functionality was demonstrated in plants specifically at mitochondrial level.

The aim of this work is to investigate the impact of single and combined Fe and S deficiency on Ca<sup>2+</sup> dynamics in Arabidopsis at cellular and subcellular level. Preliminary results showed that S-deficient and Fe/S deficient plants displayed altered cytosolic Ca<sup>2+</sup> dynamics in root tip cells in response to exogenous ATP. Furthermore, an increase of cytosolic Ca<sup>2+</sup> influx in leaf of Fe-deficient plants upon wounding stress was observed. Notably, a connecting point found between wounding stress, Fe and S deficiency and Ca<sup>2+</sup> signaling is jasmonic acid (JA) metabolism. It has been shown that JA-related signaling is involved in the modulation of Fe and S homeostasis in plants and interestingly JA metabolism has been affected in mutant's defective in mitochondrial calcium uniporter (MCU), which shows a strongly impaired mitochondrial Ca<sup>2+</sup> import. To explore the link between Fe/S deficiency-Ca<sup>2+</sup> signaling-JA, the impact of single and combined Fe and S deficiency on triple mutant lines (mcu123) is under investigation.

## **In-vitro degradation of plant polyphenols mediated by the versatile enzymatic activity of OSOXs**

Scortica A., Scafati V., Piccirilli E., Giovannoni M., Benedetti M. And Mattei B.

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Various plant oligosaccharides oxidases (OSOX) have been recently identified, i.e. enzymes able to oxidize fragments of cell wall polysaccharides at their reducing ends. The oxidation suppresses their ability of inducing plant defences and concomitantly releases H<sub>2</sub>O<sub>2</sub>. The concept that cell wall polysaccharides can be viewed as a reservoir of reducing energy available for OSOXs is bringing out new perspectives. In fact, it was recently shown that the cellodextrins (CDs) oxidase-1 (CELLOX1) is a conditional H<sub>2</sub>O<sub>2</sub>-producing enzyme [1]. In vitro, it stopped producing H<sub>2</sub>O<sub>2</sub> in the presence of a specific electron donor/acceptor combination (i.e. CDs with DP lower than 5 and the synthetic radical cation ABTS), switching its activity from oxidase to dehydrogenase. To further investigate the natural compound potentially acting as electron acceptors, we expressed and purified different OSOXs both from fungal and plant sources in *Pichia pastoris* and we tested their activity in the presence of a mixture of guaiacol (i.e. a coniferyl alcohol analogue) and tetra-guaiacol (i.e. a brownish tetra-phenolic compound derived from H<sub>2</sub>O<sub>2</sub>-peroxidase mediated guaiacol polymerization). Thus, by using the tetra-guaiacol depolymerization assay we demonstrated that an electron flux mediated by certain OSOXs exist between cell wall OSs and polyphenolic compounds speculating that the different activities of OSOXs against tetra-guaiacol may be related to their distinct roles in vivo.

## **Effects of sulfur limitation on photosynthesis and cell composition in *Dunaliella salina* and *Phaeodactylum tricorutum***

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Chlorophytes and diatoms are two groups of phytoplankton that evolved in different eras, the first during the Proterozoic Eon when sulphate levels were low in oceans, while the second radiated during the Mesozoic when ocean sulphate levels were similar to those of today's oceans. Sulphur is an essential element typically involved in redox and photosynthetic processes and its assimilation is one of the major metabolic energy sinks. Previous studies have suggested a diversified sulphur metabolism and allocation among different groups of algae based on their evolutionary history. In our study we investigate how the green algae *Dunaliella salina* and the model diatom *Phaeodactylum tricorutum* respond to growth limiting sulphur concentrations and how such changes impact photosynthesis. In order to assess differences in the physiological response of the two species, we characterized their growth in response to sulphur limitation and we performed a quantification of photosynthetic pigments and in vivo chlorophyll fluorescence analysis to investigate whether they differ in the acclimation of photosynthetic light reactions to sulphur limitation. We then evaluated how S limitation influenced the cells in their macroelemental and macromolecular composition by assessing changes in the carbon allocation pattern and shifts in the cell's elemental quota.

# **Session 3**

## **Development and signal transduction**

## **The Dwarf 27 gene inactivation affects plant physiological traits in tomato**

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The gene Dwarf 27 (D27) encodes for a  $\beta$ -carotene isomerase converting the all-trans  $\beta$ -carotene into the 9-cis form, initiating the strigolactone (SL) biosynthesis. Strigolactones are phytohormones finely tuning plant development and interaction with the environment, that are also involved in the response to abiotic stresses. In previous studies, abscisic acid (ABA) levels resulted altered in rice and Arabidopsis d27 lines, anticipating a contribution of this gene in mediating the crosstalk between SLs and ABA. Herein, tomato d27 CRISPR/Cas9 knock-out (KO) lines were evaluated in irrigated and drought conditions along with the canonical SL- mutant *ccd7* (Carotenoid Cleavage Dioxygenase 7). Main physiological traits, namely stomatal conductance (Gs) and photosynthesis (A) were significantly reduced in the d27 irrigated controls; additional analysis will be aimed at determine ABA and carotenoid content in leaf tissues. Overall, this study will contribute to clarify the role of D27 in the interaction between SLs and ABA, and on its potential involvement in further physiological processes in tomato.

## Characterization of chloroplast ribulose-5-phosphate-3-epimerase from the microalga *Chlamydomonas reinhardtii*

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Photosynthetic carbon fixation is realized by eleven enzymes in the so-called Calvin-Benson-Bassham cycle (CBBC), where essential epimerization of xylulose-5-phosphate (X5P) into ribulose-5-phosphate (Ru5P) is catalyzed by the ribulose-5-phosphate 3-epimerase (RPE) during the regeneration phase of the cycle.

In this study, we aimed at investigating the structural and functional characteristics of RPE from the model single-cell alga *Chlamydomonas reinhardtii*, which is demonstrated to participate both in the CBBC and in the oxidative pentose phosphate pathway (OPPP), thus acting as an amphibolic enzyme.

The crystal structure of CrRPE has been solved at a resolution of 1.96 Å. It revealed a homo-hexameric folding, where the monomer folds as a  $\alpha 8\beta 8$  TIM-barrel and contains an atom of zinc.

Reliable multi-step enzymatic assays were set up and optimized, allowing the determination the kinetic parameters relative to the activities of conversion of Ru5P to X5P and vice-versa and the subsequent evaluation of the effect on enzyme catalysis of different redox treatments. We demonstrated that protein catalysis is not responsive to oxidizing molecules, suggesting that the protein activity is not predominantly regulated by redox-based mechanisms despite the presence in the structure of four cysteines likely competent for redox exchange.

In conclusion, this work aims at expanding the basal knowledge on the functioning and regulation of CBC, which represents a crucial step to lay the basis for future improvements of carbon fixation and primary production.

## **Microbial volatile organic compounds (mVOCs) induce early and late responses in *Arabidopsis thaliana***

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Microbial volatile organic compounds (mVOCs) are low molecular weight molecules (<300 Da) produced by fungi and bacteria. These molecules are used by microorganisms to interact with other organisms both within the same kingdom and in interkingdom signaling. *Pseudomonas syringae* pv. tomato DC300 (Pst), a gram-negative bacterium, is an important phytopathogen, that causes significant crop economic losses. While the mechanisms of infection and the plant response are more studied, the effects of mVOCs produced by Pst on plant growth and development remain unclear. In the present study, the volatilome of Pst has been characterized by GC-MS headspace analyses. Pst mVOCs induce *Arabidopsis thaliana* Col0 plasma transmembrane potential (Vm) depolarization, cytosolic Ca<sup>2+</sup> fluctuations, K<sup>+</sup> channel activity, reactive oxygen species (ROS) and nitrogen reactive species (NRS) modulation. The *Arabidopsis* transcriptome revealed the presence of several genes modulated by Pst mVOCs. We used phytoalexin (*pad3*) and flagellin (*fls*) deficient mutants and analyzed early and late events upon mVOC exposure.

## **BON1: a Ca<sup>2+</sup>-binding protein at the crosstalk between plant development and plant response to environmental stimuli**

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Arabidopsis BON1 is a copine with two Ca<sup>2+</sup>-binding domains and a vWA domain responsible for interaction with targets. BON1 is plasma membrane (PM)-localised and is involved in plant immunity. Our analysis of two independent bon1 homozygous mutants show a drastically reduced adult plant size and clear defects in phyllotaxis that start to be visible 3 weeks after germination and are completely restored in the complemented line. Preliminary 3D reconstruction with MorphographX of the shoot apical meristem (SAM) structure in bon1, unveils that the KO mutant has a smaller and disorganized SAM, suggesting that BON1 has also a role in plant development during the progression from vegetative to reproductive growth.

The vWA domain of BON1 interacts with the autoinhibitory domain of two PM Ca<sup>2+</sup>-ATPases, ACA8 and ACA10 to possibly regulate their activity. By testing the effect of BON1 on ACA8, we demonstrate that it significantly activates ACA8 activity in a concentration-dependent manner being a positive regulator of this pump.

To investigate if the developmental defects of bon1 can be ascribed to its regulative effect on Ca<sup>2+</sup>-ATPases, hence, on cytosolic Ca<sup>2+</sup> homeostasis, we analysed in SAM resting cytosolic Ca<sup>2+</sup> concentrations as well as stimulus-induced Ca<sup>2+</sup> transients in bon1 and aca8aca10 expressing the Ca<sup>2+</sup> biosensor NES-YC3.6. Results reveal that both are altered suggesting that BON1 role in the floral transition process might be related to its impact on cytosolic Ca<sup>2+</sup> homeostasis in the cells of the SAM due to its regulative effect on PM-localised Ca<sup>2+</sup> pumps.

# **Session 4**

## **Emerging technologies**

## **A biotechnological approach for the production of new protein bioplastics**

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Plastic products derived from petroleum or chemical processes represent a problem for the planet: they are one of the major sources of pollution, essentially because they are not biodegradable. Therefore, the scientific community is constantly looking for strategies to produce environmentally friendly materials that can replace traditional plastic. The present work proposes the production that could partially replace synthetic polymers. To develop this idea, it was necessary start with genetic modification of the common bean seed storage protein (*Phaseolus vulgaris*) called phaseolin (PHSL). The protein was engineered by inserting a cysteine residue into the C-terminal tail of the polypeptide chain (PHSL\*) to stimulate the formation of interchain disulfide bridges. PHSL\* was used to transform the plastidial genome of tobacco plants (*Nicotiana benthamiana*) with biolistic transformation technology. Here, we indicate the validity of our strategy by demonstrating that modified phaseolin can form large molecular complexes ranging from 1.2 to 20 MDa in transplastomic tobacco leaves. Some plasticization tests were carried out, and the plastic material produced was analyzed to define some chemical-physical characteristics important in industrial or pharmaceutical processes. Our analysis suggests a possible application of this new biopolymer in different sectors, ranging from packaging for the food industry to producing material used in the biomedical sector as a carrier of protein-based drugs.

Keywords: biopolymer; disulfide bridges; phaseolin; transplastomic plants.

## **Zein-based nanoparticles as new formulation to deliver bioactive dsRNA for plant phytopathogens protection**

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To limit the use of pesticides and to develop new plant control strategies, environmentally and economically sustainable as well as socially acceptable, some bioactive molecules have been already isolated exploiting insect-natural antagonist interaction as source of bioinsecticide molecules. These include (1) a dsRNA able to silence an immune gene in chewing insects and (2) an ankyrin protein from a parasitoid of noctuid moth larvae that is toxic for caterpillars.

Due to the extreme sensitivity to environmental degradation, both dsRNA and proteins must be protected by biocompatible delivery strategies, to be efficiently accumulated into the plant and then reach undegraded the body of target insects. Different formulations are presently under development and analysis including (1) zein-based nanoparticles, (2) chitosan/humic substances nanoconjugates, and (3) alginate hydrogels.

Here, we present the fate of zein-based nanoparticles which are positively charged spheres of roughly 200 nm. These nanoparticles are synthesized in acidic pH 5.5 and are stable at the pH of the apoplast, while they become instable, degrading at the pH of the target insect gut (around pH 9).

By combining imaging (confocal and TEM microscopy) and molecular approaches, we show that zein-based nanoparticles marked with fluorescein, when administrated by foliar spray to 4-weeks old tomato plants:

- easily enter the leaf, accumulate in the first epidermal layer of leaves remaining stable for at least 96 hours,
- localize at the apoplast without degrading,
- don't induce macroscopic leaf damages.

Foliar photosynthetic efficiency measurement and transcript levels analysis for general stress marker genes are ongoing to address the plant stress condition.

## Hydroponic cultivation of nicotiana species for the production of biomass and bioproducts

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Indoor vertical farming involves growing crops in stacked layers, offering precise control over environmental factors. We focused on hydroponic soilless cultivation, mainly for *Nicotiana rustica*, *N. tabacum*, and *N. benthamiana* plants. We implemented a "cutting and regeneration" method in an industrial hydroponic pilot plant, resulting in a higher annual fresh weight production of *Nicotiana* plants (147kg/m<sup>2</sup>/year) compared to traditional complete growth cycles from seeds (120kg/m<sup>2</sup>/year). The initial setting of the procedure was achieved using *N. rustica* as an experimental system, focusing on synthesizing and extracting the primary alkaloid, nicotine.

This approach was then applied to transgenic *N. tabacum* expressing the gene for human  $\alpha$ -mannosidase, crucial for  $\alpha$ -mannosidosis therapy. In previous work, 10 200 units/kg of enzyme was achieved, with the "cutting and regeneration" method this amount can be obtained faster and possibly increased.

For *N. benthamiana*, a biotechnological platform, we used the spraying method of viral constructs to induce the synthesis of high added-value pharmaceutical proteins. Modified viruses prompted the plants to produce desired heterologous proteins, such as the small 12-aa domain of Liprin alpha 1 protein (LIP1), a candidate for rheumatoid arthritis treatment. Preliminary results showed significant viral particle accumulation (52% of the amount accumulated in positive control) using the spray method compared to traditional infiltration methods. These steps contribute to establishing an economically sustainable hydroponic plant cultivation process. Future perspectives include optimizing and scaling up the procedure for broader application."

## **Liquid fraction of two-phase olive pomace is enriched in oligosaccharides activators of plant immunity**

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Olive oil production generates significant amounts of biowastes. Despite they are characterized by a high polluting and phytotoxic degree, they are still mainly disposed directly on the soil, compromising the balance of ecological systems (Foti et al., 2021). The industrial process of olive oil extraction could promote the accumulation of molecules like the self-harm signals naturally generated upon pathogen infection, referred as damage-associated molecular patterns (DAMPs) (De Lorenzo & Cervone, 2022; Swaminathan et al., 2022). In this study, a process was developed for the isolation and characterization of a fraction enriched in cell wall oligosaccharides from the liquid fraction of two-phase olive pomace (Lionetti et al., 2017). Our results demonstrate that the liquid fraction of two-phase olive pomace contains a mixture of arabinose and mannose-enriched oligosaccharides and oligogalacturonides. Our results indicate that isolated elicitors trigger Arabidopsis immune responses and protect against *Botrytis cinerea* and *Pseudomonas syringae*, without affecting plant growth. These natural, non-toxic and environmentally friendly bioactive molecules can be used to reduce the large use of pesticides by integrating the chemicals into an integrated pest management (IPM) program, promoting a circular economy for more sustainable agriculture.

## UV-B effects on the physiology of native microalgae from highland, lowland, and indoor locations

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The differential effects of UV-B on the inhibition or activation of protective mechanisms to maintain cells photosynthetically active were investigated in native microalgae under an eco-physiological context. Four strains were used, including two *Chlorella sorokiniana* strains, F4 and LG1, isolated from a Mediterranean inland swamp and a recycled cigarette butt's substrate, respectively, and two isolates from an Ecuadorian highland lake related to *Pectinodesmus pectinatus* (PEC) and *Ettlia pseudoalveolaris* (ETI). Monocultures were exposed to acute UV-B (1.7 W m<sup>-2</sup>) over 18 h under controlled conditions. UV-B-untreated microalgae were used as the control. Comparative physiological responses, including photosynthetic pigments, non-enzymatic antioxidants, and chlorophyll a fluorescence, were evaluated at specific time points. Results showed that UV-B significantly compromised all the physiological parameters in F4, thereby resulting in the most UV-B-sensitive strain. Contrarily, UV-B exposure did not lead to changes in the PEC physiological traits, resulting in the best UV-B-resistant strain. This could be attributed to the acclimation to high light habitat, where maintaining a constitutive phenotype (at the photosynthetic level) is strategically advantageous. Differently, LG1 and ETI at 12 h of UV-B exposure showed different UV-B responses, which is probably related to acclimation, where in LG1, the pigments were recovered, and the antioxidants were still functioning, while in ETI, the accumulation of pigments and antioxidants was increased to avoid further photodamage. Consequently, the prolonged exposure in LG1 and ETI resulted in species-specific metabolic regulation (e.g., non-enzymatic antioxidants) in order to constrain full photoinhibition under acute UV-B.

## Investigating the mode of action of a new biofungicide against fungal and oomycete pathogens of tomato and grapevine

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Grapevine and tomato are two major crops worldwide and they are susceptible to many pathogens. Disease control involves the use of chemical fungicides hazardous to human health and the environment. The aim of this study is to evaluate the efficacy and mode of action of a new biofungicide based on choline pelargonate (CP, patent BE1026779B1) against fungal (*Botrytis cinerea* and *Erysiphe necator*) and oomycete (*Phytophthora infestans* and *Plasmopara viticola*) pathogens. CP was tested in vitro against *B. cinerea* and *P. infestans* and it was able to inhibit mycelium growth and spore germination. On tomato, CP reduced the disease severity of *B. cinerea* when tested at 5 mL/L and 10 mL/L. Moreover, CP reduced downy mildew severity in grapevine inoculated with *P. viticola*, reaching an efficacy of 57%, 80% and 83% when tested at 1 mL/L, 5 mL/L and 10 mL/L, respectively. Likewise, CP reduced the disease severity of powdery mildew caused by *E. necator* on grapevine with an efficacy of 55%, 91% and 94% when applied at 1 mL/L, 5 mL/L and 10 mL/L, respectively. The expression of defense-related genes will be studied in CP-treated grapevine plants inoculated with *P. viticola*, to investigate the ability of CP to directly induce defense-related genes and/or to activate a priming effect. In particular, a quantitative PCR analysis will be carried out to analyze the expression of two marker genes of the salicylic acid pathway (PR-1 and PR-2), two markers of jasmonic acid pathway (PR-4 and LOX-9), and a marker of the hypersensitive response (HSR).

## **New state-of-the-art imaging tools to study how crops adapt to environmental changes: *Lycopersicon esculentum* key study.**

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Plants, as sessile organisms, constantly face environmental challenges, including abiotic factors, like water scarcity, and biotic stresses, such as pathogen attacks, which significantly influences plant growth and crop yield. Consequently, understanding the mechanisms of plant responses to external stimuli becomes crucial.

So far, much research conducted on molecular pathways involved in the transmission of stimuli perception to plant responses comes from *Arabidopsis thaliana* studies. To reveal how crops perceive and cope with environmental stimuli we directed our focus toward tomato, *Lycopersicon esculentum*, due to its global economic importance and its susceptibility to adverse conditions.

To deepen our understanding of the roles played by different second messengers in signal transduction pathways within crops, we generated MicroTom tomato plants expressing the genetically encoded biosensors Cameleon YC3.6 and pH-GFP to analyse cytosolic calcium (Ca<sup>2+</sup>) and pH dynamics, respectively.

So far, we have successfully selected plants that exhibit favourable expression of the two sensors within guard cells and pollen tubes. Through fluorescence microscopy analysis of the Cameleon line, we observed spontaneous cytosolic Ca<sup>2+</sup> oscillations occurring in both guard cells and at the tip of growing pollen tubes. Concerning the pH-GFP plants, we were able to verify the proper sensor functionality in guard cells.

Whereas our preliminary analyses reveal the functionality of the sensors in MicroTom plants, we are facing difficulties on selecting plants with uniform expression of the sensors in different tissues, and not only limited to guard cells and pollen tubes. Attempts to improve the transformation protocol are undergoing.

# Sketching Science

February 22, 2024 - 04:45 pm - 06:30 pm

## Whiteboard 1: Adaptation mechanisms to environmental stress

### **Dose-response” effects of prescribed burning on the functionality of Pinus sylvestris L. stem hydraulics**

R. Gamba (1), A. Ascoli (1), S. Cavalletto (1), A. Bono (1), A. Crivellaro (2), A. Piermattei (1-2), A. Chicarella (1), S. Negri (1), E. Bonifacio (1), R. Motta (1), F. Secchi (1)

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Prescribed burning (PB) is a widespread fuel treatment technique based on the planned application of fire to manage understory fuels in forests and mitigate wildfire hazards. However, little is known about PB's physiological and anatomical impacts on one of the most widespread European tree species: Scots pine (*Pinus sylvestris* L.). This study investigated the ecological sustainability of the PB technique for this tree species by applying two different doses of fire (high and low intensity) and observing their effects on the functionality of tree hydraulics and wood structures. The PB was carried out in May 2022 in a mature Scots pine forest in Val Susa, Southwestern Italian Alps. Thermal sensors positioned at the tree stem base were used to quantify the two levels of fire intensity and assess the heat treatment (i.e. thermic sums above temperature thresholds). One, six and twelve months after the PB, branches and wood cores were collected in both PB-treated and untreated sites and analyzed. Our results show that while the two doses were reached (with half of the monitored plants exceeding the 400°C thermal sum threshold), the fire treatment did not considerably affect the xylem hydraulic properties of the vascular system and its anatomical features. This suggests the high resistance of *P. sylvestris* to the prescribed burning technique.

### **Sucrose level is related to control of Flavescence dorée phytoplasma load in grapevine leaves.**

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Flavescence dorée (FD) is an insect-borne phytoplasma disease severely affecting vineyards in Europe, as infected plants cannot be cured and must be uprooted to limit spread of the pathogen. Recovery of FD symptoms is occasionally observed in field-grown vines, more frequently in varieties highly susceptible to initial infection. We showed that grapevine genotypes exhibiting tolerance to the disease have a significantly higher leaf sucrose content. Thus, since sucrose concentration increases in FDp-infected compared to healthy plants, we hypothesized that high leaf sucrose levels may have a role in inducing a limitation of FDp load and in subsequent recovery in the plant. To provide causal evidence for this role of sucrose, we attempted to further increase sucrose content in leaves of infected plants, through trunk sucrose infusion and shoot girdling. Sucrose infusion induced a prompt sucrose hydrolysis, coupled with an increased expression of trehalose-6P, ABA and secondary metabolism biosynthetic genes. Girdling at mid shoot was effective in inducing decrease of infection rate and FDp load. The treatment promoted sucrose accumulation, followed by activation

of sucrose cleaving enzymes. Moreover, girdling promoted the expression of the same group of genes activated by trunk sucrose infusion. Taken together, these results suggest that a signal originated by elevated sucrose level, and possibly transduced through the signalling sugar trehalose-6P, may contribute to induce a limitation of FDP load during initial infection and in the recovering process.

### **The interplay between mannitol and sucrose drives hydraulic recovery in *Fraxinus ornus* saplings exposed to drought and stem shading.**

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Plants exposed to drought undergo a re-allocation of non-structural carbohydrates (NSC), and in some cases sugars accumulate in stem and roots. Recently, bark was proposed as the main site for sugar accumulation, suggesting the contribution of stem photosynthesis in this process. In addition, some species accumulate large amounts of poly alcohols (i.e. mannitol). We investigated how the interplay between NSC and mannitol drives the response of *Fraxinus ornus* saplings to water shortage and recovery coupled to a stem shading. Percentage loss of hydraulic conductivity (PLC), starch, soluble NSC pool, glucose, fructose, sucrose and mannitol were measured. Drought induced a decrease of total NSC in bark, wood and roots. Glucose was constant in bark but not in roots highlighting a different engagement during recovery. Sucrose and PLC were directly correlated, indicating the pivotal role of sucrose in plant hydraulic responses under drought. Fructose concentration was extremely low, suggesting a rapid conversion to mannitol, which was indirectly related to starch. Finally, mannitol increased in all organs at the onset of drought and remained high during recovery. Our findings confirm the role of stem photosynthesis during drought and recovery. Mannitol and sucrose emerged as key metabolites, revealing that the role of NSC during abiotic stresses should be reconsidered evaluating single saccharides and other osmotic active compounds derived from NSC.

### **Do SLs affect guard cell functioning via the miR319-LANCEOLATE module and gibberellin signalling?**

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Stomatal closure is an important strategy to prevent plants from water loss and death under drought. Strigolactones (SLs) are carotenoid-derived phytohormones engaged in plant development regulation and abiotic stress acclimation, which were also proven to modulate stomatal movement in ABA-dependent and ABA-independent ways. miR319 is an ancient and widely conserved miRNA family which targets and represses LANCEOLATE (LA) expression in tomato. A cause-effect link between SLs and miR319-LA module activation has been found in tomato leaves. Besides, LA activity correlates with bioactive GA (gibberellin) levels, and the GA signal negative regulator PROCERA (PRO), the only DELLA protein in tomato, physically binds to LA. Interestingly, PRO promotes stomatal closure in an ABA-dependent manner. Given the demonstrated connections between SLs, the miR319-LA module, and GAs (Visentin et al, submitted), combined with their consistent involvement in stomatal closure, our research aims to investigate whether miR319-LA and GAs mediate the SL signal in stomatal regulation in tomato. To test this hypothesis, genotypes expressing the gain- or loss-of-function PROCERA alleles, or the La-2 semidominant gain-of-function allele, are used along with their wild type (M82) to measure stomatal conductance after GR245DS (a synthetic SL-analogue), ABA or mock treatment. Further drought treatment, physiological, and molecular measurement will also be conducted. Our

research aims to investigate further the SL-dependent signalling modules acting in the guard cells, and provide an insight into the molecular mechanisms of stomatal modulation and drought acclimation in this important horticultural crop.

### **GLR3.7 as negative regulator of GLR3.3 mediated stress responses**

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During evolution, plants have developed sophisticated mechanisms to perceive and respond to the environment. Adaptability and responsivity to biotic and abiotic stresses is a key mechanism that involves multiple factors: second messengers, phytohormones, metabolites and a whole variety of molecules involved into the perception, distribution, and transduction of stress signals. Recent works showed that wounding-driven long-distance propagation of Ca<sup>2+</sup> waves depend on the activity of the cation permeable channel Glutamate Like Receptor 3.3 (GLR3.3), but further information on how this key factor is regulated is still scarce.

GLRs are homologous to the animals' ionotropic glutamate receptor channels (iGluRs), which can form both homo and hetero tetramer. This allows us to hypothesize that different assemblies of plant GLRs as well can bring to the formation of channels with different properties.

Here we present multiple lines of evidence supporting the molecular and functional interdependency between GLR3.3 and another relatively uncharacterized GLR isoform, GLR3.7.

Our analyses, including response to exogenous AAs administration and burning stress, revealed that GLR3.7 plays a negative regulatory role on GLR3.3-mediated responses.

Colocalization and close proximity of GLR3.3 and GLR3.7, both at tissue and cellular level, has been demonstrated by GUS reporter assay, complemented by a FLIM analysis. Moreover, Co-Immunoprecipitation experiments demonstrated direct protein-protein interaction between the two GLRs.

In summary, our results suggest that GLR3.7 can modulate the activity of GLR3.3 by possibly forming, in planta, a heteromeric channel.

### **Polystyrene nanoplastics' impact on crops: a new challenge for agriculture and food safety.**

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Polystyrene nanoplastics (PSNPs) originating from humans spread and linger into the environment affecting soils used to grow crops widely used for human and animal nutrition, raising concerns about their toxicity.

In this light, the rationale of this work is to assess the impact of PSNPs on crop growth and metabolism, focusing on durum wheat, employed as a model plant.

Two genotypes were selected, Kronos and a mutant (MRP3) characterized by the silencing of the genes encoding for the phytic acid (PA) transporters. MRP3 has a higher ability to accumulate micronutrients and a different root architecture than Kronos. Precisely, MRP3 displays reduced root length and tips number while showing increased root volume and average diameter.

In this work, newly germinated plants were subjected to short-term exposure to 10 mg/L PSNPS during growth for three weeks. Both genotypes exhibited increased growth and protein synthesis. However, diverse effects were observed in the sugar metabolism with increased sucrose in Kronos and higher free D-glucose content in MRP3. Long-term exposure of adult plants to PSNPs revealed increased flavonoid content and reduced nitrogen balance index (NBI) in both genotypes suggesting signs of stress induced by prolonged exposure; Kronos showed higher photosynthetic capacity when exposed to PSNPs, while this process was reduced in

MRP3, possibly underscoring a more pronounced stress-response for this genotype. Our preliminary data suggest that PSNPs exerted diverse effects on crops in correlation to the genotype and length of exposure.

### **Under fungal attack on a microplastic-contaminated soil: a case study on *Arabidopsis thaliana***

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Nowadays the accumulation of microplastics (MPs, 100 nm - 5 mm) and nanoplastics (NPs, < 100 nm) in the environment represents one of the most threatening conditions for plant organisms. The aim of this work was to test if two widespread plastic polymers, polyethylene terephthalate (PET) and polyvinyl chloride (PVC), are able to modify plant-pathogen interaction. To do this, seeds of *Arabidopsis thaliana* were germinated in soils with different concentrations of PET/PVC MPs (0.2% w/w and 0.5% w/w; particles size 40-50 µm) and grown for three weeks. At the end of the treatment, *A. thaliana* leaves were independently exposed either to ceratoplatenin (CP), a harmful protein secreted by *Ceratocystis platani*, or to conidia of the pathogenic fungus *Botrytis cinerea*.

Even if plant growth and physiological parameters were not significantly affected by MPs, plants treated with 0.2% PET/PVC showed a higher production of phytoalexins after CP exposure. Moreover, plants treated with 0.2% PVC exhibited a better resistance to *B. cinerea*, as demonstrated by a lower development of leaf lesions. A deeper untargeted metabolomics analysis was performed to identify the activation of metabolic pathways involved in disease resistance or susceptibility. Preliminary results emphasized a substantial metabolic reprogramming in leaves of *A. thaliana* grown in PET/PVC-contaminated soil and subsequently subjected to the simulated biotic stress, especially at 0.5% concentration. An interaction between MP pollution and pathogen attack was thus verified, but the direction (positive or negative) of such interference is not easily predictable without further investigations on different species, polymers and concentrations."

## **Whiteboard 2: Plant Nutrition and Metabolism**

### **Hypothetical interference of auxin-adjutant urea derivative on vascular pattern formation: preliminary results.**

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The vascular system consists of interconnected veins that form a hierarchical network. It is essential for plants because it provides long-distance transport of several water-soluble compounds, as signal molecules, nutrients, proteins etc. Its formation seems to be a self-organizing process, driven by the main plant hormone, auxin, through its binding to the receptor Auxin Binding Protein 1 (ABP1). This binding, together with directional

transport of the hormone facilitated by the PINFORMED (PIN) proteins, auxin-efflux carriers, induce the formation of the vascular pattern. As a consequence, vascular tissues differentiate where auxin concentration is high. Hierarchical network formation may be affected by the supplementation either of exogenous auxin or of auxin transporter inhibitors. In this scenario, we would like to verify if an auxin-adjutant urea derivative, the N,N'- bis-(2,3-methylenedioxyphenyl)urea (2,3-MDPU), could interfere with vascular pattern formation, as it has been recently shown that it interacts with apoplastic portion of ABP1. Moreover, without showing any auxin- and/or cytokinin-like activity, it enhances adventitious root formation in Gymnosperms and Angiosperms with poor callus formation, it doesn't directly interfere with polar auxin transport, it inhibits cytokinin oxidase/dehydrogenase activity. Here, the first results on the effect of the urea derivative in the development of the vascular system are shown as the formation of vascular lobes analyzed in cotyledons of the model plant *Arabidopsis thaliana*, cultured in vitro in different conditions.

### **Morpho-Physiological and Molecular Effects of Exogenous Strigolactone-like Molecules Regulating Nutrient Use Efficiency in Tomato**

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In the context of frequent extreme weather events, crops are frequently stressed, which greatly affects their productivity. Adequate nutrients are necessary for proper plant growth, especially N; and yet, applying too much fertilizer is expensive and can pollute the environment. Plant hormones can regulate the acclimation of plants to nutrient deficiencies. It is well known that strigolactones are important endogenous growth regulators in plants and play a role in plant acclimation to abiotic stress. Karrikins (KARs) are highly stable strigolactone-like compounds that can be extracted from smoke produced by burning vegetation; they share part of the molecular structure and signalling pathway with strigolactones and are bioactive on plants at low concentrations. They hold great potential to maximize the utilization efficiency of resources, including nutrients, in crops. If they were to be proven active in improving nutrient use efficiency (NUE), they could be added to the toolbox of crop management strategies, because compared with expensive fertilizers that pollute the environment, KARs are low cost and eco-friendly.

The overarching scientific purpose of my project is to pinpoint the physiological and molecular mechanisms controlling tomato NUE under regular and nutrient-deprivation conditions, as influenced by KARs compounds. To this purpose, NUE, morpho-physiological and molecular responses of tomato plants to N deprivation and/or KARs is being assessed under hydroponic conditions.

This project has received funding from the European Union Horizon Europe Innovation programme under the Grant Agreement No. [101081858] (ECONUTRI)

### **Durum wheat response under different phosphate fertilization regimes""**

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Durum wheat (*Triticum durum* Desf.) is a cereal crop of fundamental importance in Mediterranean regions such as Italy, constituting a cornerstone of agricultural economies. Given its significance, durum wheat has long been the subject of numerous studies, focused on reducing fertilizer input and improving yields. Despite numerous projects, the mechanisms of absorption, assimilation and utilization of phosphorus are still partially unclear. Based on these premises, we used a multidisciplinary approach to analyze the molecular response of two

durum wheat cultivars (Ciccio and Svevo) to different phosphorus fertilization regimes (Control, no fertilization; P120, phosphorus fertilization; NPK, nitrogen, phosphorus, and potassium fertilization). Biometric data were combined with data on phosphorus accumulation, differential expression of genes involved in phosphorus metabolism, along with biochemical parameters to comprehensively understand the response of the different genotypes to the treatments. Additionally, the proteins involved in response to different phosphorus fertilization in leaves (collected during the stem elongation phase) were investigated by a proteomic approach, utilizing two-dimensional electrophoresis coupled with mass spectrometry. Proteomic data were validated through qPCR analysis linked to the identified phosphorus-specific proteins and transporters. The results indicate that statistically significant differences occur among the employed fertilization regimes and in the response of each genotype to the treatments.

### **Deciphering Eriophyes tiliae's Biohacking Mechanisms: Transcriptomic Profiling of Tilia cordata Gall Formation**

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Plant galls are specialized structures induced by a range of organisms, including mutualistic, parasitic, or pathogenic entities, which manipulate plant development to create a nutrient-rich and protective environment. The formation of galls is believed to be driven by effector molecules released by the gall-inducers that hijack the plant's developmental pathways. However, the specific functions of these effectors in gall development are not fully understood. While modern omics approaches have shed light on the genetic underpinnings of gall formation at a molecular level, definitive insights into the identity of gall-inducing agents and the early cellular events in gall initiation are still forthcoming.

Eriophyid mites, especially *Eriophyes tiliae*, which induce distinctive galls on *Tilia cordata*, offer a distinctive model to investigate the complex interplay between plants and gall-inducing arthropods.

Our research focuses on analyzing the transcriptomic shifts in *Tilia cordata* induced by *Eriophyes tiliae* to identify the effector molecules and understand their roles. We employ RNA-Seq to examine the transcriptome from various gall development stages, utilizing next-generation sequencing and bioinformatics for gene expression profiling. Initial findings indicate the involvement of genes related to cell cycle, hormone signaling, and stress response, suggesting a mite-influenced regulatory network.

Our aim is to clarify the molecular processes behind gall formation, offering potential targets for synthetic biology applications and contributing to the development of innovative plant defense strategies. Our findings lay the groundwork for future studies on the co-evolution of plants with their gall-inducing parasites and may lead to novel approaches in agricultural pest management."

### **Investigating molecular mechanisms of drought memory in tomato (*Solanum lycopersicum* L.): role of mir156 and strigolactones**

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The escalating global climate crisis poses a significant threat to crop productivity, with drought stress emerging as a key factor impacting plant survival. In response to these environmental pressures, plants exhibit a unique phenomenon known as drought memory, where prior stress experiences shape their subsequent responses, suggesting the existence of a molecular memory mechanism enabling plants to adapt to recurring stress events.

Many stress memory instances involve intricate changes to the chromatin structure of crucial regulatory genes, including modifications to the epigenetic landscape like DNA methylation and histone modifications. These

enduring epigenetic alterations allow plants to "remember" past stress encounters, enabling fine-tuning of responses to subsequent challenges. Phytohormones, such as abscisic acid (ABA) and strigolactones (SLs), play pivotal roles in drought-memory mechanisms. SLs serve as a molecular link between drought and miR156, a conserved microRNA induced by drought, influencing stomatal activity through ABA-dependent pathways. Moreover, miR156 modulates plant development, with its expression in transition from vegetative to reproductive phases mediated by specific epigenetic marks at MIR156A and C gene loci in Arabidopsis. Understanding these processes is vital as they may provide insights into how plants inherit and transmit their responses to subsequent generations, potentially enhancing their ability to cope with environmental stressors.

### **CaM-dependent NAD kinases: a hallmark of the green lineage"**

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NAD kinases are enzymes found in all living organisms, since they produce the essential cofactor NADP. In 1978 it was reported that plants possess two types of NAD kinases: those whose activity was dependent on Calcium-loaded Calmodulin (CaM) and those active in its absence.

While the genes coding for CaM-independent NAD kinases were identified twenty years ago following the publication of the Arabidopsis genome, the CaM-dependent ones remained elusive until we identified the gene coding for the Arabidopsis one in 2019. This revealed that CaM-dependent NAD kinases constitute an independent class of genes that we called "NADKc". Their sequences feature: i) a target sequence that addresses the proteins to the mitochondrial outer membrane, ii) a N-terminal domain of unknown function, iii) a catalytic C-terminal domain and iv) an experimentally- validated CaM binding peptide linking the N- and C-terminal domains.

Phylogenetical analysis showed that the catalytic domain is present in all divisions of Archaeplastida, including white, red and green algae, as well as higher plants. In contrast, the CaM binding site is only found in the green lineage. Enzymatic validation of selected candidate homologues confirmed that the CaM-dependent NAD kinase activity is a hallmark of the green lineage only.

A combination of plant reporter lines for Calcium, ROS and the NAD(P)/H pool have been made and will help elucidate the physiological role of these enzymes in various model organisms. Crystallography and biochemical techniques are being employed to unravel the CaM activation mechanism on Arabidopsis NADKc.

## **Whiteboard 3: Development and signal transduction**

### **FUTUREGRAIN: exploring the metabolic and resistance diversity among rice varieties in agricultural systems against global warming**

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Global warming has a profound impact on agriculture and Italian crops. In general, the combination of low water availability and supra-optimal temperatures detrimentally affects plant reproductive processes, impeding normal fruit or seed development and consequently restricting both crop yields and the quality of fruit/grain production. Specifically, in Italy, climate change affects the nation's agriculture, with commercial rice crops being vulnerable to alterations in soil salinity or drought, thereby influencing seed germination. Certain ancient varieties of European rice, when compared to Italian commercial lines, demonstrate either heightened resistance or increased sensitivity to water and salt stress during germination. These varieties

exhibit a corresponding upregulation or downregulation in the expression of specific genes, both during germination and seed development.

Recently, we have identified factors and genes associated with oxidative stresses and metabolic regulation, potentially implicated in drought and heat stress in rice grains. These factors may contribute to shielding plants from damage caused by abiotic stress, ensuring growth performance under stress by activating signaling cascades for response mechanisms, or inducing protection for protein structure at the cellular level during osmotic and oxidative stresses.

Through morphological and physiological characterization of specific ancient and modern rice lines, we aim to generate practical knowledge in seed biology that can be useful in the early stages of the plant's life in contrast to an increasingly adverse climate. Our objective is to comprehend, at the molecular, cellular, and developmental levels, the role of genes in seed quality and plant establishment, as well as their potential involvement in abiotic stress tolerance, particularly in response to heat and drought."

### **CYB561-A and CYB561-B are likely involved in intracellular ascorbate redox homeostasis and ROS-mediated signalling**

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Besides being one of the most important antioxidants, ascorbate (ASC) acts as a substrate for several enzymes, including cytochromes b561 (CYB561s). ASC is biologically active in its reduced state but certain cellular compartments (e.g., vacuoles, ER, apoplast) lack the enzymes that catalyze its reduction from the oxidized forms. CYB561s catalyze the transmembrane transfer of single electrons, thus they could perform the regeneration of ASC in those organelles, reducing monodehydroascorbate on one side of the membrane with electrons from ASC on the other side. The genome of *Arabidopsis thaliana* contains 4 CYB561s-encoding genes (CYB561-A, -B, -C and -D).

We recently demonstrated that CYB561-A catalyzes the bidirectional electron transport across the tonoplast in vivo. Our research is now focused on the investigation of the physiological role of CYB561-A and -B by phenotyping T-DNA mutant plants at two distinct developmental stages. Compared to wild-type plants, the mutants show an alteration in the intracellular ASC redox homeostasis: despite having small differences in ASC content, the mutants display a slight decrease in dehydroascorbate reductase activity but a significant increase in ASC peroxidases activity, correlated with a higher content of cytosolic ASC peroxidases. Additional preliminary phenotyping results suggest the involvement of CYB561s in ROS-mediated signalling: all the mutants show a decreased ROS content, a one-week delay in the flowering onset, a slower dehydration rate and a three-fold higher increase in anthocyanin accumulation under high-light.

Finally, we are producing antisense mutants for CYB561-C and -D to get further insights into the physiological role of this protein family.

### ***In-silico* identification of miRNAs targeting DNA Damage Response (DDR) genes in rice**

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Rice is a staple food crop grown all around the globe in tropical and temperate climatic conditions, and it requires high amounts of water and high temperatures. Its wide distribution of cultivation puts it under various

environmental stresses that may cause DNA damage. This DNA damage must be repaired to safeguard plant genome integrity and survival. This is achieved by the activity of the DNA Damage Response (DDR) mechanisms.

DDR is a complex and highly coordinated network of cellular pathways that cooperate to sense and repair DNA lesions. DDR is regulated at several levels, including transcriptionally and post-transcriptionally. The latter can involve the activity of microRNAs as single-stranded RNA molecules complementary binding to mRNA targets and leading to cleavage or translational inhibition. Since the knowledge on the miRNA regulation of DDR-associated genes in plants is still scanty, the current study proposes an in-silico approach to identify putative miRNAs that are able to target genes involved in DDR. To do so, the sequence of *Oryza sativa* DDR genes was retrieved from the Rice Genome Annotation Project (<http://rice.uga.edu/>) while the psRNATarget tool (<https://www.zhaolab.org/psRNATarget/>) was used to predict putative miRNAs targeting the selected genes. Additionally, a data mining approach was used to investigate the expression of DDR genes during rice developmental stages, using the RiceXPro (<https://ricexpro.dna.affrc.go.jp/>) database. The retrieved data will be subsequently tested under appropriate experimental conditions.

### **Thallium tolerance in metallicolous and non-metallicolous accessions of *Silene latifolia***

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Thallium (Tl) is both an emerging pollutant and a valuable element for the ecological transition. The species *Silene latifolia* has been indicated as a potential Tl hyperaccumulator for phytoextraction from contaminated soils with a possible economic return but its features of Tl tolerance and accumulation have never been characterised. To this aim, the metallicolous accession from the Tl-rich mine dump of Saint-Laurent-le-Minier (France) was compared to an accession from a non-contaminated area (Barraux, France). Seeds were sown in peat soil and then plantlets were transferred to hydroponics. Samples were exposed to a growing series of TlNO<sub>3</sub> concentrations (0, 2.5, 5, 30, 60 µM) for seven days. Leaf area and root length increment were calculated, chlorophyll a fluorescence and indices of leaf pigments were measured. While the growth of the non-metallicolous accession was negatively impacted by the presence of Tl, the metallicolous accession was scarcely affected. Biometric data were successfully fitted to a logistic model and significant differences were found between the EC<sub>50</sub>s of the non-metallicolous (2.5 ± 0.3 µM) and the metallicolous accession (22.5 ± 0.8 µM). The reduced growth of the non-metallicolous accession was partly explained by the negative effect of Tl on the photosynthetic parameters and the pigment content, significantly decreased only in these samples. Therefore, the metallicolous accession showed Tl tolerance, probably as an adaptation to the high Tl concentration in its substrate of origin. Ongoing analysis of Tl accumulation in roots and shoots will reveal if *S. latifolia* tolerance is linked to the metal hyperaccumulation.

### **Characterization of different isoforms of glucose 6-Phosphate Dehydrogenase from plant and algal sources under abiotic stress.**

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Glucose 6-Phosphate Dehydrogenase (G6PDH) is the key enzyme of the pentose phosphate oxidative pathway (OPPP). G6PDH catalyses the first step of the cycle, converting the glucose 6-phosphate (G6P) into 6-phosphogluconolactone and producing reducing power as NADPH. In plants, different isoforms of G6PDH have been

described, playing important roles in plant development and stress responses. High or low temperatures, salt stress, drought, nutrient limitation, and pathogens are the main factors to which plants can be exposed, also simultaneously. In response to these factors, plants have developed mechanisms of tolerance and/or resistance that limit permanent and irreversible damage.

This study aims to identify isoforms and clarify the role(s) of plant G6PDH in the response to the abiotic stress in WT and mutant *Arabidopsis thaliana*, deficient of chloroplastic isoform (P1-G6PDH). Next, we will focus on the regulation and post translational modification of in recombinant G6PDH proteins from plant and algae, such as *Populus trichocarpa* and *Chlamydomonas reinhardtii*, obtained by heterologous expression and site-directed mutagenesis techniques. Furthermore, we will try to define the post translational modifications present on different G6PDHs when acting to counteract stress, in particularly phosphorylation, nitrosilation and, their effects on G6PDH activity and regulation.

These results will hopefully help to describe the basal mechanisms regulating the primary metabolism in plant cells during stress response and resilience.

### **Investigating the role of ATBBE oxidases and BBE-like oligosaccharide oxidases: radical cation scavenging activity, structural and physiological implications**

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During plant cell wall degradation, oligosaccharides produced can be perceived by plants as DAMPs (Damage-associated molecular patterns), e.g. Oligogalacturonides (OGs) and cellodextrins (CDs), inducing the activation of defense response. Various FAD-linked oxidases, such as OGOX and CELLOX in *Arabidopsis thaliana*, can oxidize OGs and CDs, abolishing their activity as DAMPs. The oxidation produces H<sub>2</sub>O<sub>2</sub> that may be used by extracellular peroxidases to reinforce cell wall in a direction that is proportional and opposite to the ongoing degradation.

Recently, it has been demonstrated that OGOX and CELLOX scavenged the radical cation ABTS<sup>•+</sup> with an efficiency inversely related to the length of oxidized oligosaccharides, suggesting their implication in plant defense against oxidative stress[1]. Unfortunately, OGOX and CELLOX X-ray structures have not been solved yet. Nevertheless, FAD-linked oxidases are also produced by various fungal pathogens and, like plant counterparts, they oxidize cell wall fragments producing H<sub>2</sub>O<sub>2</sub>. Our preliminary results indicate that the activity of fungal enzymes, i.e. GOOX and XOOX, on short pectin/cellulose fragments can also result in the scavenging of the radical cation ABTS<sup>•+</sup>.

However, their role in plant-pathogen interactions and physiological events is unclear. A Molecular Dynamics (MD) approach is being carried out to identify amino acid residues involved in the interaction with the respective substrates, looking for structural homologies between fungal and plant enzymes active site. Structure-function relationships may give insight into the role of plant and fungal oxidases in plant-pathogen interaction or during plant cell wall remodeling.

## **Whiteboard 4: Emerging technologies**

### **The Impact of Coffee Silverskin on Metabolism and Phenotypes of Barley Seedlings**

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This study aimed to assess the potential of coffee silverskin (CS), the primary byproduct of coffee roasting, as a soil fertilizer for barley (*Hordeum vulgare* L.). Barley plants were cultivated for 10 days after germination in soils containing 0, 2, 6 or 10% (w/w) CS. We specifically investigated the effects of CS addition on phenotypic,

physiological and biochemical responses of barley leaves and roots. Barley root and shoot length decreased in plants grown with increasing percentages of CS. Total biomass was less affected by CS and even slightly increased at a 2% CS. Photosynthesis (calculated on an area basis) increased in all CS treatments with respect to control, whereas Chlorophyll b was reduced. The total leaf protein content was similar to controls in plants grown at 2 and 6% CS, but a significant reduction in total protein and Rubisco was observed under 10% CS, when a substantial increase of antioxidant capacity was also observed. In conclusion, this study suggests that the addition of low concentrations (2%) of CS has the potential to positively influence barley physiology and growth. These preliminary findings may contribute to understanding whether coffee waste can be used as an environmentally friendly biostimulant for sustainable agriculture.

### **Innovative non-invasive methods to assess seed quality**

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Seed quality encompasses the set of physical, genetic, and physiological characteristics, reflecting the overall germination potential. Considering the influence of seed quality on agriculture, the development of novel methods to assess it in a non-invasive manner is of great interest of the Seed Industry.

In this context, the measurement of two biological phenomena related to the physiological state of seeds, namely ultra-weak photon emission (UPE) and delayed fluorescence (DL), represents a potential approach for non-invasive seed quality detection. In the present work, seeds of five legume species were stored at different conditions for several years, then analysed using a light analyser, and finally used for germination tests. For each species, datasets including the germination percentage, the UPE and DL measurements, were obtained and used for machine-learning model development. The validation of the following models reveals positive accuracy values that underline a link between seed quality and specific DL and UPE patterns, thus supporting the possibility of using photons emission phenomenon to detect seed quality in a non-invasive manner.

Moreover, other techniques, involving the production of reactive oxygen species (ROS) and volatile organic compounds (VOCs), can aid in developing more and diverse non-invasive and sustainable approaches for seed quality assessment. These techniques will be further validated in a system using aged and non-aged seeds, in a work supported by the SIBV Seed Grant 2023 that will be performed in collaboration with the INFRA-VOL platform.

### **Investigating the role of endophytes in enhancing grapevine resilience to drought**

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Grapevine is a crop of great economic importance for several countries. The intensification of grapevine production has mostly been sustained by the increasing use of water resources at the expense of the environmental water balance. Moreover, in the last decades, climate change and the consequent expansion of drought have further compromised water availability, making current agricultural systems even more fragile both ecologically and economically. Recently, many research groups have highlighted the important role of endophytes in facilitating plant growth under optimal or stressful conditions. Within the framework of the PRIMA project, we aim to investigate the possible exploitation of the natural endophyte biodiversity as a sustainable tool to make grapevine plants more resilient to water deficit environmental conditions. Cultivable bacterial communities of field grapevine plants growing in the arid regions of Italy and Algeria have been isolated from leaf tissues. Endophytes were characterised and screened for their plant growth-promoting traits and used to

generate endophyte consortia to inoculate endophyte-free grapevine plants. In a parallel approach we tested the possibility of using the grafting procedure to transfer endophytes between plants. Preliminary data are presented showing the efficiency of this procedure and the dynamics of the endophyte community in the destination plant.

## **Plant cell cultures: a biotech platform for the production of ingredients for human healthcare**

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Plants extracts have been used since ancient times for cosmetic, pharmaceutical and nutraceutical applications. While plants used to come from agriculture, more recently the exploitation of plant cell cultures (PCC) have been proposed for their unique properties and sustainability. With respect to agriculture, they allow to save freshwater and do not compete for the use of fertile lands, limiting therefore the overexploitation of soils. Moreover, the productive process does not require the application of pesticides and fertilizers, avoiding the pollution of groundwater and microbial communities. Linked to these aspects, they preserve both flora and fauna biodiversity. Lastly, the extraction of active compounds from PCC uses the whole cells, limiting waste of undrawn biomass and the massive use of solvents to digest recalcitrant polymers (i.e. lignin).

The green biotechnological approach developed by IRB, takes advantage of PCC to produce high quality and reproducible products in a controlled environment. Additionally, the amount of actives that are currently produced with our production processes is higher than the corresponding obtained from plants.

Due to its robustness combined with the decades-long expertise, the method potentially applies to the overall plant biodiversity, allowing to work with plants out from their seasonality, stage and latitude of growth, but also with endangered species. A good representative is Majestem, one of the first IRB products that has been developed starting from *Leontopodium alpinum* (Edelweiss), which produces Leontopodic Acids A and B, Chlorogenic Acid and 3,5-dicaffeoylquinic acid, characterized by a strong antioxidant activity, three times higher than Vitamin C.

## **Micropropagation and regeneration as new tools for *Cannabis sativa* L. breeding**

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The possibility of improving plant varieties for traits of interest through biotechnology is one of the objectives of the new European regulatory framework on agrifood development and research. Through tools such as micropropagation and in vitro tissue culture it is possible to maintain, multiply and transform, in sterility and absence of pathogens, species with high nutraceutical or medicinal value. Among these, *Cannabis sativa* L., thanks to its therapeutic properties and its high market value, is a crop of interest. Given its incredible genetic heterogeneity, many *Cannabis* varieties are recalcitrant to in vitro culture and regeneration and gene editing protocols are still insufficient. As part of the PhD entitled 'Breeding and Innovation in Medicinal Cannabis' within the Research in Food and Agricultural Science, Technology and Biotechnology - STEBA - XXXVII cycle, a new cultivation medium (PP) was created which differs from the commercial synthetic media (like MS and DKW) due to the different ratio between the nutritional components. By balancing the ratios between macro and micronutrients in new ways, excellent results have been obtained in the management of plants recalcitrant to in vitro culture. Furthermore, through the evaluation of eight hundred explants from certified hemp seeds, we tested the regeneration capacity of three different tissues in the presence of different cytokinins. The creation of efficient regeneration protocols will allow the application of assisted evolution technologies which could quickly improve the knowledge and quality of this crop.

## **Sustainable strategies to produce plant defensins with antiviral properties**

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Plant defensins represent a class of antimicrobial peptides with antiviral action that can be useful for the production of phytotherapies for the treatment of human viral diseases. Since they are naturally involved in plant defense responses against a wide range of environmental injuries, the expression of defensin genes from *Nicotiana tabacum* (FST, S13, THIO, J1) has been stimulated by subjecting cultured Tobacco Bright Yellow 2 (TBY-2) cells to different elicitation treatments (JA, SA, ABA, NaCl, H<sub>2</sub>O<sub>2</sub>, heat stress). Unfortunately, undifferentiated cells failed to express genes involved in the biosynthetic pathways of these metabolites, probably because most of the secondary metabolites are produced in differentiated tissues. In the context of molecular farming, large-scale agroinfiltration of *N. benthamiana* is used as a fast and efficient system of delivering transgenes into plant cells for recombinant protein production in plants. In this research, the AtD90 plant defensin gene from *Arabidopsis thaliana*, which sequence was engineered to optimize the isolation and purification steps, was cloned in pK7WG2 vector under CaMV35S promoter and NOS terminator. *Agrobacterium tumefaciens* LBA4404 and *Rhizobium rhizogenes* A4 strains were used for agroinfiltration of defensin gene into *Nicotiana benthamiana*. PCR analysis indicated the presence of AtD90 transcript in the *N. benthamiana* leaves.

SI RINGRAZIANO PER IL LORO CONTRIBUTO



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