



# Workshop on Plant Biology 2023

Centro Residenziale Universitario di Bertinoro  
(Forlì-Cesena)  
22-24 February 2023



SOCIETA' ITALIANA di  
BIOLOGIA VEGETALE

# Workshop SIBV 2023

## Organizing and Scientific Committee

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**Francesca Secchi**

University of Turin

**Mirko Zaffagnini**

University of Bologna

### Conference venue

Centro Residenziale Universitario di Bertinoro (Forlì-Cesena)

### Conference Language

The official conference language is English

### Conference Dinner on February 22

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

GENERAL INFORMATION



# PROGRAMME

**Wednesday, February 22, 2023**

01:30 pm Registration and welcome buffet  
02:15 pm Workshop Opening

## **Session 1: Emerging technologies and sustainability**

*Chairs: Benedetta Bottiglione and Azzurra Di Bonaventura*

### **Invited:**

02:30 pm **Regulation of photosynthesis under fluctuating light conditions in the moss *Physcomitrium patens***  
Alessandro Alboresi, University of Padua

### **Oral Presentations:**

03:20 pm **Metabolic engineering of *Nannochloropsis oceanica* to produce astaxanthin**  
Davide Canini, University of Verona

03:35 pm **Dissecting the physiological role of the enzyme phosphoribulokinase in the green microalga *Chlamydomonas reinhardtii***  
Maria Meloni, University of Bologna

03:50 pm **Optimization of synthetical photorespiratory bypasses in *Physcomitrium patens***  
Eleonora Traverso, University of Padua

04:05 pm **Crispr/cas9-mediated genome editing: a tool to sustainably develop biofortified rice to address hidden hunger**  
Conrado Jr. Duenas, University of Pavia

04:20 pm **COFFEE BREAK**

05:00 pm **New state-of-the-art imaging tools to study how crops adapt to environmental changes: *Lycopersicon esculentum* key study**  
Bianca Maria Orlando Marchesano, University of Milan

05:15 pm **Analysis of the role and functionality of plant-derived exosome-like nanovesicles: a new research strategy and therapeutic application**  
Clarissa Zanotti, University of Rome Tor Vergata

- 05:30 pm **The use of plant cell cultures as an alternative method for the production of novel food**  
Carmen Laezza, University of Naples
- 05:45 pm **Impact of environmental conditions on lettuce seed quality**  
Francesca Messina, University of Pavia
- 06:00 pm **ELEVATOR PITCHES**
- 08:00 pm **GET TOGETHER PARTY**  
**DINNER AT CA' DE BÈ RESTAURANT**

## Thursday, February 23, 2023

### Session 2: Plant Stress Resilience and memory

*Chairs: Silvana Francesca and Francesca Resentini*

#### **Invited:**

- 09:00 am **Synthetic biology strategies to investigate plant signal transduction**  
Beatrice Giuntoli, Sant'Anna School of Advanced Studies of Pisa

#### **Oral Presentations:**

- 09:50 am **Arabidopsis NAD kinase C (NADKc): a missing link between Ca<sup>2+</sup> signaling, metabolism, and plant stress response**  
Elisa Dell'Aglio, University of Milan
- 10:05 am **Can microplastics threat plant productivity and fruit quality? Insights from MICRO-TOM and MICRO-PET/PVC**  
Marco Dainelli, University of Florence
- 10:20 am **The cAMP-dependent plant stress response**  
Eleonora Davide, University of Insubria
- 10:35 am **Can a tunisian-related olive tree be a promising candidate as *Xylella fastidiosa*-resistant plant?**  
Giambattista Carluccio, University of Salento
- 10:50 pm **COFFEE BREAK**
- 11:30 am **Involvement of GUN1 in the acquisition of basal thermotolerance in *Arabidopsis thaliana***  
Cecilia Lasorella, University of Bari

- 11:45 am **ARGONAUTE3 is involved in hypoxia tolerance in *Arabidopsis thaliana***  
Elisa De Meo, Sant'Anna School of Advanced Studies of Pisa
- 12:00 pm **Karrikin priming affects responses to drought stress in tomato**  
Cristina Morabito, University of Turin
- 12:15 pm **Phenotypical, biochemical and molecular characterization of durum wheat genotypes under salt stress**  
Irene Sbrocca, Campus Bio-Medico University of Rome
- 12:30 pm **LUNCH**

### Session 3: Plant Environment Interactions

*Chairs: Sara Natale and Matteo Grenzi*

#### **Invited:**

- 02:00 pm **Redox signaling in plant environment interaction**  
Vittoria Locato, Campus Bio-Medico University of Rome

#### **Oral Presentations:**

- 02:50 pm **Mild drought treatments prime the recovery from severe drought modifying the tradeoff between soluble non-structural carbohydrates and growth in black poplars**  
Sara Gargiulo, University of Trieste and Udine
- 03:05 pm **ISCA-LIKE 3: a putative plant magnetosensor**  
Ambra Selene Parmagnani, University of Turin
- 03:20 pm **Healthy mitochondria are needed for optimal photosynthesis: the role of respiration in plant bioenergetics and primary metabolism**  
Antoni Mateu Vera Vives, University of Padua
- 03:35 pm **AtCuAO $\beta$  and RBOHD distinct roles in mechanical wounding-induced stomatal closure**  
Chiara Pedalino, University of Roma Tre
- 03:50 pm ***Tulbaghia violacea* var. alba routinely emits high quantity of signaling GLV (Z)-3-hexenyl acetate**  
Alessandro Frontini, University of Salento
- 04:05 pm **A simple methodology to study the effects of plant nutrient homeostasis on Ca<sup>2+</sup> signaling**  
Stefano Buratti, University of Milan

- 04:20 pm **COFFEE BREAK**
- 05:00 pm **“PLANT TALKS”  
YOUNG SCIENTISTS BRAINSTORMING**
- 08:00 pm **DINNER**

**Friday, February 24, 2023**

## **Session 4: From Seed to Seed: Plant Physiology and Development**

*Chairs: Libero Gurrieri and Edoardo Tosato*

### **Invited:**

- 09:00 am **Role of biostimulants in enhancing seed germination, plant growth and abiotic stress tolerance in crops**  
Cinzia Margherita Berteà, University of Turin

### **Oral Presentations:**

- 09:50 am **Salt stress response in rice: the involvement of sulfur metabolism**  
Michela Molinari, Campus Bio-Medico University of Rome
- 10:05 am **Unveiling the functional and regulatory properties of a new class of enzyme controlling the GSNO homeostasis**  
Ginevra Peppi, University of Bologna
- 10:20 am **Water and nutritional savings shape non-structural carbohydrates in grapevine (*Vitis vinifera* L.) cuttings**  
Alessandro Pichierri, University of Trieste
- 10:35 am **COFFEE BREAK**
- 11:15 am **Impairing of lipid reservoirs mobilization in *Eruca sativa* germination under salt stress conditions**  
Emilio Corti, University of Florence
- 11:30 am **Evaluation of D27 and D27-likes genes in tomato and their potential role in fruit quality**  
Alessia Cuccurullo, University of Naples

- 11:45 am **Neighbors detection and transcriptional response, does chromatin accessibility count?**  
Alessandra Boccaccini, IRCCS-Fondazione Bietti
- 12:00 pm **ELEVATOR PITCHES**
- 01:00 pm **TALK PRIZE**  
**CONCLUDING REMARKS**
- 01:30 pm **LUNCH**

## ELEVATOR PITCHES

**February 22, 2023**

**06:00 pm - 07:00 pm**

### Session 1: Emerging Technologies and Sustainability

Benedetta Bottiglione, University of Bari

**Monochromatic led lighting affecting morphological and physiological parameters in lentil (*Lens culinaris medik.*) seedlings**

Azzurra Di Bonaventura, University of Udine

**Production of secondary metabolites from coffee spp. cell cultures**

Irene Doro, University of Padua

**Grafting as a new strategy for endophyte transferring in grapevine**

Lucia Franceschini, Campus Bio-Medico University of Rome

**Sustainable strategies to produce bioactive molecules with antiviral properties in plant cultures**

Noemi Gatti, University of Turin

**The application of a biostimulant based on tannins enhances fruit nutraceutical properties of a tomato plants cultivated under salt stress**

Shraddha Shridhar Gaonkar, University of Pavia

**Screening approaches to enhance storability of primed legume seeds**

### Session 2: Stress Resilience and Memory

Caterina Capezzali, University of Turin

**Application of abiotic stress to tomato (*Solanum lycopersicum* L.) to increase the extraction yield of secondary bio-based compounds**

Filippo Costa, University of Bologna

**Characterization of hypoxic stress effects on the symbiotic relationship between *Medicago truncatula* and *Sinorhizobium meliloti* through root transcriptome analysis**

Silvana Francesca, University of Naples "Federico II"

**Flowering time and leaf morphology traits are key for heat tolerance in one tomato genotype**

Davide Greco, University of Salento

**Crop renewal for the restoration of the agroecosystems compromised by *Xylella fastidiosa***

Michela Manai, University of Rome Tor Vergata

**Role of salt tolerance-related protein (STRP) in salt stress responses in *Arabidopsis thaliana***

Miriam Negussu, University of Florence

**Gaining insight into the drought tolerance mechanisms in chickpea**

Yuri Telara, Sant'Anna School of Advanced Studies

**Plant responses to complex environmental stresses: investigation of iron deficiency response in plants exposed to flooding**

Niccolò Tricerri, University of Turin and University School for Advanced Studies IUSS Pavia

**Impact of drought progression rate on embolism recovery in poplar**

**February 24, 2023**

**12:00 pm - 01:00 pm**

### **Session 3: Plant Environment Interactions**

Claudia Beraldo, University of Padua

**Multi-strategy study of non-photochemical quenching in the moss *Physcomitrium patens***

Silvia De Rose, University of Turin

**Plant and fungal gene expression coupled with stable isotope labelling provide novel information on sulfur uptake and metabolism in orchid mycorrhizal protocorms**

Matteo Grenzi, University of Milan

**Long-distance turgor pressure changes induce local activation of plant glutamate receptor-like channels**

Sara Natale, University of Trieste and University of Padua

**Novel approaches to study photosynthetic characteristic of chloroplasts found along the radial profile of *Fraxinus ornus* stems**

Francesca Resentini, University of Milan

**Functional characterization of the calcium-binding protein BONZA11 in *Arabidopsis thaliana***

Teodora Chiara Tonto, Campus Bio-Medico University of Rome

**Nutritional quality of tomato fruits grown under different agronomic conditions**

### **Session 4: From Seed to Seed: Plant Physiology and Development**

Giuseppe Gabellini, University of Bologna

**What's new in *Arabidopsis thaliana* nitrosylation catabolism: AKR (aldo-keto reductase) are capable to catalize the reduction of nitrosilated species**

Libero Gurrieri, University of Bologna

**Glutathionylation as determinant of stomata opening and starch degradation in guard cells**

Sara Paola Nastasi, IPSIM and University of Milan

**Ca<sup>2+</sup> levels in cytosol and tonoplast during stomatal opening**



Paola Pagano, University of Pavia

**The involvement of DNA damage response in plant stress: a snapshot into the Arabidopsis TDP1 genes function**

Edoardo Tosato, University of Bologna

**The molecular phenotyping of *Arabidopsis thaliana* mutants suggests a role for cytochromes b561 in the regulation of intracellular ascorbate redox homeostasis and ROS-mediated signaling**

## **Session 1**

# **EMERGING TECHNOLOGIES AND SUSTAINABILITY**

# METABOLIC ENGINEERING OF NANNOCHLOROPSIS OCEANICA TO PRODUCE ASTAXANTHIN

<sup>1</sup>Canini D., <sup>1</sup>Martini F., <sup>1</sup>Pacenza B., <sup>1</sup>Malesci D., <sup>2</sup>D'Adamo S., <sup>1</sup>Ballottari M.

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<sup>2</sup>Bioprocess Engineering, Wageningen University (NL)

Presenting Author: Davide Canini, [davide.canini@univr.it](mailto:davide.canini@univr.it)

Astaxanthin is a ketocarotenoid with high antioxidant activity [1] primarily produced by microalgae. Despite its bioavailability, 95% of the market relies on synthetic astaxanthin produced from petrochemicals while microalgae-derived astaxanthin (mainly from *Haematococcus lacustris*) represents less than 1% of the market [2]. Synthetic astaxanthin has an antioxidant activity much lower than the natural one [3] but its production cost is lower [2]: *H. lacustris* has a slow growth, is prone to contamination and its cultivation and extraction costs are high. To develop sustainable alternatives for natural astaxanthin production, we focused on *Nannochloropsis oceanica*, a marine alga widely used for aquaculture, to obtain an engineered strain with higher content of ketocarotenoids, in particular astaxanthin.

We used *Nannochloropsis oceanica* LP-tdTomato [4], an engineered strain optimized for nuclear transformation, to express the two key enzymes for astaxanthin production, a carotene  $\beta$ -ketolase (CrBKT) from *Chlamydomonas reinhardtii* and a carotene  $\beta$ -hydroxylase (CrtZ) from *Brevundimonas* sp. SD212 [5]. Transgene insertion by homologous recombination in a highly transcribed genomic locus can be screened in LP-tdTomato background by negative fluorescence [4].

Transformants with CrBKT have a red-brown phenotype due to reduced content of violaxanthin, and higher content of adonirubin, canthaxanthin and astaxanthin, compared to wild type. While the expression of both CrBKT and CrtZ further increased astaxanthin accumulation. Moreover, we performed through Cas12a endonuclease-mediated genome editing, the knock-out of two putative genes encoding for zeaxanthin epoxidases (NoZEP1 and NoZEP2) to redirect the metabolism toward ketocarotenoid production. The results obtained demonstrate that *Nannochloropsis oceanica* is a suitable platform for metabolic engineering to produce high-value metabolites as ketocarotenoids.

<sup>1</sup>Naguib, Y. M. A. Antioxidant activities of astaxanthin and related carotenoids. *J Agric Food Chem* 48, 1150-1154 (2000)

<sup>2</sup>Panis, G. & Carreon, J. R. Commercial astaxanthin production derived by green alga *Haematococcus pluvialis*: A microalgae process model and a techno-economic assessment all through production line. *Algal Res* 18, 175-190 (2016)

<sup>3</sup>Capelli, B., Bagchi, D. & Cysewski, G. R. Synthetic astaxanthin is significantly inferior to algal-based astaxanthin as an antioxidant and may not be suitable as a human nutraceutical supplement. *Nutrafoods* 12, 145-152 (2013)

<sup>4</sup>Sudfeld, C. et al. The nucleolus as a genomic safe harbor for strong gene expression in *Nannochloropsis oceanica*. *Mol Plant* 15, 340-353 (2022)

<sup>5</sup>Wu, Y. et al. Combinatorial expression of different  $\beta$ -carotene hydroxylases and ketolases in *Escherichia coli* for increased astaxanthin production. *J Ind Microbiol Biotechnol* 46, 1505-1516 (2019)

## DISSECTING THE PHYSIOLOGICAL ROLE OF THE ENZYME PHOSPHORIBULOKINASE IN THE GREEN MICROALGA *CHLAMYDOMONAS REINHARDTII*

<sup>1</sup>Meloni M., <sup>2</sup>Favoino G., <sup>2</sup>Boisset N., <sup>2</sup>Crozet P., <sup>1</sup>Zaffagnini M.

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Presenting Author: Maria Meloni, mariameloni9@gmail.com

Photosynthesis is an essential process that provides most of our planet primary production through the fixation of atmospheric carbon into organic molecules. The Calvin-Benson cycle (CBC) relies on 11 enzymes and constitutes the metabolic phase of photosynthesis where carbon fixation takes place through the action of the Rubisco enzyme. Regeneration of the Rubisco substrate (ribulose-1,5-bisphosphate, RuBP) is ensured by the other ten enzymes of the CBC, among which phosphoribulokinase (PRK) catalyzes the ATP-dependent phosphorylation of ribulose-5-phosphate into RuBP. Over the last decades, many efforts have been made to deepen the study of the CBC, with the ultimate goal of improving its efficiency as it is considered a partially inefficient process. In fact, while the cycle functioning is understood, the regulatory mechanisms along with limitations and improvement possibilities are still largely unexplored.

In this context, our aim is to study the enzyme PRK from the green microalga *Chlamydomonas reinhardtii* by means of two main strategies. First, we aim to understand whether the PRK-catalyzed reaction could represent a kinetic bottleneck by generating mutant lines with differential accumulation of the enzyme through a MoClo-based synthetic biology approach. Variable levels of PRK have been then correlated with growth analysis as a read-out of the CBC efficiency. Finally, we intend to broaden the research on the CBC through a paleo-biochemical approach which involves the resurrection of PRK ancestral forms, whose biochemical and structural characterization would allow the identification of the critical amino acids that defined the characteristics of extant enzymes during evolution.

## OPTIMIZATION OF SYNTHETICAL PHOTORESPIRATORY BYPASSES IN *PHYSCOMITRIUM PATENS*

Traverso E., Smaldone A., Ciampanelli A., Morosinotto T.

*Dipartimento di Biologia, Università degli studi di Padova.*

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Crops' productivity has strongly increased in the last decades thanks to more sophisticated agricultural practices and genetic selection. However, it is expected that these improvements will plateau without being able to keep the pace with the constant increase in world population and the subsequent food demand. Therefore, it is necessary to find innovative strategies to further increase plants productivity. The major targets are RuBisCO oxygenase activity and Photorespiration. The latter is deputed in 2-phosphoglycolate (2-PG) detoxification and, even if indispensable, it reduces the conversion efficiency of light into biomass by 20 to 50%. The recently developed idea is to exploit non-native pathways able to recycle 2-PG without any C and N loss. Whether the aim is to increase crops yield, these alternative mechanisms have to be enough efficient to bypass photorespiration, becoming the preferential 2-PG detoxification pathway.

An interesting pathway is the Beta-Hydroxy-Aspartate-Cycle (BHAC) one. Before introducing it into crops' chloroplasts, it is necessary to test its functionality and possible advantages in simpler organisms. For this purpose, two alternative recipient lines of the model organism *Physcomitrium patens* has been identified: a photorespiratory mutant (plgg1/2 KO) and a WT one. The phenotype rescue of the first will give us information about bypass proper activity while the introduction into WT will show BHAC higher efficiency in 2-PG detoxification. A first Fv/Fm screening allows to identify putative plgg1/2-BHAC-positive clones while a WT-BHAC-positive one already shows a higher photosynthetic efficiency than WT recipient line.

**CRISPR/CAS9-MEDIATED GENOME EDITING:  
A TOOL TO SUSTAINABLY DEVELOP BIOFORTIFIED RICE TO ADDRESS HIDDEN HUNGER**

<sup>1,2</sup>Duenas C.Jr., <sup>2</sup>Ludwig Y., <sup>2</sup>Arcillas E., <sup>2</sup>Macove A., <sup>1</sup>Slamet-Loedin I.H.

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Genome editing via CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9) has become a versatile tool for both basic and applied science ranging from the biomedical field to agriculture. In this study, we aimed to develop biofortified rice by editing the promoter region of Nicotianamine Synthase 2 (OsNAS2) gene, known to be involved in the production of phytosiderophores in roots. The induced INDELS in the promoter sequence disrupted cis-regulatory elements associated with inhibitors of iron- and zinc-related (IZR) transport genes leading to increased gene expression. Consistent 4 base pair deletions in the OsNAS2 gene promoter were associated with elevated grain zinc concentration and increased spikelet number, thus indicative of higher yield as well. The enhanced expression of these IZR transport genes can be related to achieving the primary target of iron and zinc biofortification, thus mitigating the micronutrient deficiency associated to the hidden hunger concept. In the context of product development, this genome editing approach can circumvent the arduous deregulation process necessary when transgenic methods are used, since it represents a transgene-free promoter editing approach. The development of a product with improved nutritional quality and enhanced yield potential, is highly-desired in the context of the Agenda 2030 Sustainable Developmental Goals. Hence, biofortification approaches implemented through the use of genome editing tools can be envision as emerging and sustainable solutions to address hidden hunger, taking into consideration its impacts on the agri-food system.

## **NEW STATE-OF-THE-ART IMAGING TOOLS TO STUDY HOW CROPS ADAPT TO ENVIRONMENTAL CHANGES: LYCOPERSICUM ESCULENTUM KEY STUDY**

Orlando Marchesano B. M., Luoni L., Bonza M. C., Costa A.

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Being sessile organisms, plants are continuously subjected to environmental challenges and consequently adjust their development. Moreover, adverse conditions could reduce crop yield.

Therefore, understanding plant response to biotic and abiotic stresses is of vital importance.

In the last decades, molecular signalling from the stimuli perception to plant response was largely investigated. However, most of the available knowledges on plant signalling derive from studies performed on *Arabidopsis thaliana* model plant.

Thus, we decided to focus our attention on *Lycopersicon esculentum*. Tomato was chosen as model plant since it represents a worldwide economically important vegetable crop and its growth and productivity are sensible to inappropriate external conditions.

To better understand how crops deal with environmental stimuli, such as drought, salt, or osmotic stresses, we aimed at combining the development of tomato lines expressing genetically encoded biosensors with tailored in-vivo imaging approaches. Indeed, the generation of a battery of tomato biosensor lines will give us the possibility to study the role of important plant regulators, such as Ca<sup>2+</sup>, pH, ROS and phytohormones, and to evaluate how crops cope with environmental stresses. The ongoing research allowed us to obtain the first tomato lines through transformation and regeneration of tomato explants. In this way, it will be possible to perform preliminary analysis to understand crop response to external stimuli.

## **ANALYSIS OF THE ROLE AND FUNCTIONALITY OF PLANT-DERIVED EXOSOME-LIKE NANOVESICLES: A NEW RESEARCH STRATEGY AND THERAPEUTIC APPLICATION**

<sup>1</sup>Zanotti C., <sup>2</sup>Samperna S., <sup>3</sup>Sennato S., <sup>2</sup>Marra M.

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Plant-derived exosomes-like nanovesicles (PELNVs) are similar for characteristics to mammalian exosomes: they consist of a lipid membrane with a diameter of 50-150 nm and are composed by proteins, lipids and nucleic acids. They are involved in many physiological processes, indeed participating in plant cell crosstalk and in cross-kingdom communication. Plant exosomes perform multiple activities into the biological environment and carry biochemical cargo to the receiving cells, giving them new properties or functions. Moreover, for their natural source PELNVs are minimally cytotoxic and immunogenic and show good tissue-specific targeting. Growing evidence indicates that PELNVs exert many therapeutic effects in the treatment of diseases, with anti-inflammatory, anti-carcinogenic and anti-oxidant activities. For these reasons, a better understanding of molecular activities of plant-derived exosomes could provide a new and alternative nanotherapeutic platform to deliver bioactive components in clinical applications, especially in the nutraceutical, cosmeceutical, and therapeutic fields.

For these purposes, plant exosomes preparations have been carried out from different edible plants or sprouts as well as from hairy roots cultures, cultivated in laboratory under controlled environmental conditions. Typically, the starting material for exosomes isolation is obtained from homogenized plant tissues and, although different methods are available for PELNV purification, differential ultracentrifugation (dUC) currently represents the gold standard method. Depending on the natural origin, each PELNV has different characteristics, components and biochemical properties. Hence their morphological, compositional and biochemical characterization is fundamental for appropriate nutraceutical and therapeutical applications.



## THE USE OF PLANT CELL CULTURES AS AN ALTERNATIVE METHOD FOR THE PRODUCTION OF NOVEL FOOD

<sup>1</sup>Laezza C., <sup>2</sup>Tenore G., <sup>3,6</sup>Monti S. M., <sup>4,6</sup>Di Loria A., <sup>5,6</sup>D'Amelia V., <sup>1,6</sup>Rigano M. M.

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Current evidence state that alterations in climate have strongly challenged food security and safety. In particular, traditional farming may not be able to cope with the increasing food demand. Therefore, alternative strategies should be taken into account, as plant cell cultures (PCCs). This method has recently attracted attention for the production of novel food, as it offers several advantages such as: (i) rapidity in obtaining results; (ii) no seasonal dependence; (iii) no waste of water and land; (vi) no use of fertilizers and pesticides. Given this, the aim of our research was to develop novel protocols for the production of PCCs accumulating high-value secondary metabolites (SMs) from *Malus pumila* peel and pulp. As results, we were able to develop effective apple peel- and pulp-derived callus cultures. Thereafter, the content of these cell cultures was analyzed. Interestingly, outcomes revealed that the amount of certain bioactive compounds provided by calli was comparable or higher than apple. Future work will concentrate on in-depth study of SMs biosynthetic pathway within the developed *M. pumila* cell cultures to optimize the production of natural molecules. The final goal of this research is the production of novel food rich in valuable SMs that may have positive effects on human diet.

## IMPACT OF ENVIRONMENTAL CONDITIONS ON LETTUCE SEED QUALITY

<sup>1</sup>Messina F., <sup>2</sup>Leonarduzzi C., <sup>1</sup>Pagano A., <sup>1</sup>Macovei A., <sup>1</sup>Balestrazzi A.

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<sup>2</sup>*ISI Sementi S.p.A. a sole shareholder subject to direction and coordination of Mitsui & CO., Ltd (IT)*

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Seed germination is a complex process, influenced by several environmental factors, such as light, temperature, and moisture. Among these, temperature is an important environmental component able to condition seed germination. Values below or above the optimal temperature of germination will delay or inhibit germination process. Seeds that fail to germinate at supra-optimal temperatures are called thermoinhibited. Lettuce (*Lactuca sativa* L.) seeds exhibit thermoinhibition at temperatures above 20°C, depending on the variety and production site. During seed development and maturation, the environment affects the ability of lettuce seeds to germinate at suboptimal temperatures. Lettuce is cultivated in open field, any type of plastic-house/plastic tunnel and in greenhouse. Regardless of the cultivation system, the upper limit of germination temperature can be improved by pre-sown seed treatments like seed priming. The production of lettuce seeds with increased thermotolerance will be extremely useful, also in view of the current global emergency linked to climate change. The present work provides a first picture of the germination behaviour of different seed lots collected from different production sites where the same commercial lettuce variety is grown. Results show that differences among the tested seed lots are related to the distinctive components of the production process: geographic origin site, year, and method.

## **Session 2**

# **PLANT STRESS RESILIENCE AND MEMORY**

## ARABIDOPSIS NAD KINASE C (NADKc): A MISSING LINK BETWEEN $Ca^{2+}$ SIGNALING, METABOLISM, AND PLANT STRESS RESPONSE

<sup>1</sup><sup>2</sup><sup>3</sup>Dell'Aglio E., <sup>1</sup>Giustini C., <sup>1</sup><sup>3</sup>Tocci C., <sup>4</sup>Kraut A., <sup>4</sup>Couté Y., <sup>3</sup>Costa A., <sup>5</sup>Decros G., <sup>5</sup><sup>6</sup>Gibon Y., <sup>1</sup>Finazzi G., <sup>1</sup>Curien G.

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Plant perception and propagation of stress signals depend on calcium ions ( $Ca^{2+}$ ) and reactive oxygen species (ROS). The current model establishes that, in stressed cells,  $Ca^{2+}$  binds to NADPH oxidases localized on the plasma membrane, generating apoplastic ROS that can move to the neighboring cells. In turn, ROS, and in particular  $H_2O_2$ , trigger the activation of  $Ca^{2+}$  permeable channels located at the plasma membrane, allowing the influx of  $Ca^{2+}$  that again stimulates ROS production via NADPH oxidases. Thus, a long distance signal is established thanks to a ROS/  $Ca^{2+}$  positive feedback loop, leading to signal propagation within minutes.

By a combination of proteomics, enzymology and physiological observations, we have shown that the pathogen-triggered ROS burst in Arabidopsis seedlings is dependent on a newly-discovered NAD kinase (NADKc) whose enzymatic activity is dependent on  $Ca^{2+}$ -loaded calmodulin (CaM). In vivo studies showed that the ROS burst induced by the pathogen elicitor flagellin22 is completely absent in *nadkc* seedlings. In parallel, in concomitance with the ROS burst, wild-type but not *nadkc* seedlings, show an increase in NADP(H)/NAD(H) ratio. The NADKc-dependent NADP/H increase is therefore required for the apoplastic ROS production in response to bacterial elicitors, and constitutes a new, unexpected bridge between primary metabolism and plant stress signaling.

Conservation of NADKc sequences in the plant lineage and enzymatic activity tests on representative species suggested that NADKc evolved among algae but its CaM-dependent activation is a feature that has emerged only in land plants.

In summary, we propose that the sudden surplus of NADP(H) produced in the presence of NADKc is essential to sustain the activity of NADPH oxidases and that this tight  $Ca^{2+}$ -CaM control is a recent evolutionary acquisition. Further studies will elucidate NADKc mechanism of action in the presence of  $Ca^{2+}$ -CaM, and its role in orchestrating an efficient response to pathogens.

## **CAN MICROPLASTICS THREAT PLANT PRODUCTIVITY AND FRUIT QUALITY? INSIGHTS FROM MICRO-TOM AND MICRO-PET/PVC**

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About 80% of plastic waste is land-based and the abundance of plastic particles (i.e. microplastics and nanoplastics) in agroecosystems has made it necessary to study the effects of these contaminants on commercially important crops. In this study, *Solanum lycopersicum* L. cv. Microtom, one of the most worldwide diffused crops with a high value for human diet, was used as model plant to test the impact on plant growth, productivity and fruit quality of two among the most represented microplastics in soils, i.e. polyethyleneterephthalate (PET) and polyvinylchloride (PVC). Tomato plants were grown in pots with an environmentally realistic concentration of microplastics and, during the whole life cycle, photosynthetic parameters, number of flowers and fruits was monitored. At the end of the cultivation, plant biometry and ionome were evaluated, along with fruit production and quality. The two pollutants had negligible effects on the plant traits analysed, with only PVC causing a significant reduction in plant fresh weight. Despite the apparent absent or low toxicity, both microplastics decreased the number of fruits and, always in the case of PVC, their fresh weight as well. The plastic polymer-induced decline in the net fruit production was coupled to wide variations in the fruit quality in terms of ionome. Specifically, the fruit concentration of Ni and Cd increased in fruits from microplastic-treated plants, thus posing serious concern about possible risk for human health, in addition to the threat of yield reduction and economic loss.

## THE cAMP-DEPENDENT PLANT STRESS RESPONSE

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To counteract the effects of high temperatures and maximize the chance of survival, plants activate the Heat Stress Response (HSR) aimed at restoring cellular homeostasis. HSR comprises complex signalling networks that affect gene expression, post-translational modifications, and protein synthesis. In particular, a cAMP involvement has been established in biotic and abiotic stress responses.

The research project aims to provide new insights into the role of cAMP in plant responses to heat stress and set the foundation for novel approaches in plant protection. To achieve these objectives, *Arabidopsis thaliana* plants overexpressing the chimeric protein, cAMP-sponge, have been produced. This "sponge" is a non-invasive genetic tool that allows in vivo cAMP depletion, and it was also used to identify specific cAMP-dependent components of HSR, like the expression profile of several Heat Shock Proteins (HSP18, HSP26, HSP70 and HSP101), key components of thermotolerance response. Other key points of this research project are the involvement of cAMP in autophagy and the involvement of cyclic AMP in ABA production, both conditioned by heat stress.

Then, in order to study the effects of cAMP transients in plant cells and tissues during challenges, new *Arabidopsis thaliana* lines harbouring the cAMP sponge regulated by an inducible promoter will be generated. These plants will be subject to biomolecular analysis too, in order to highlight the effect of HS.

## **CAN A TUNISIAN-RELATED OLIVE TREE BE A PROMISING CANDIDATE AS XYLELLA FASTIDIOSA-RESISTANT PLANT?**

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*Xylella fastidiosa* is a systemic bacterium that colonizes exclusively xylem tissue, causing scorching and stunting diseases worldwide. It causes the Olive Quick Decline Syndrome (OQDS) which consists of an initial desiccation of the branches and which over time leads to the desiccation of the entire canopy and the death of the plant, representing one of the main threats to not only in Apulia (Italy) where the pathogen was firstly detected on this host, but to the whole Mediterranean area.

The great extent of the damage in Apulia is given by the fact that most of the trees grown in infected areas belong to the susceptible cultivars Cellina di Nardò and Ogliarola salentina. Currently there no cure is available, thus one of the management strategies rely in using resistant plants. Currently only Leccino and FS17 cultivars are considered resistant and their planting in infected areas is authorized. Exploratory missions in the Apulia, resulting in the identification of several paucisymptomatic or asymptomatic plants in olive orchards severely affected by OQDS. In particular, research led to the identification in Apulia of the genotype SX\_32, closely related to the Tunisian cultivar Chemlali, which shows a marked tolerance to the bacterium. Comparative studies with respect to resistant and susceptible cultivars (Leccino and Cellina di Nardò) were carried out considering parameters such as bacterial count, symptoms development, endophytic population, metabolomic and transcriptomic analysis, and histological and anatomical parameters, confirming the peculiar characteristics of this genotype, such as unique metabolomic profile and a high concentration of quercetin-3-O-rhamnoside.

## INVOLVEMENT OF GUN1 IN THE ACQUISITION OF BASAL THERMOTOLERANCE IN ARABIDOPSIS THALIANA

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Heat stress (HS) severely affects different cellular compartments operating in metabolic processes and represents a critical threat to plant growth and yield. Chloroplasts are crucial in heat stress response (HSR), signalling to the nucleus the environmental stress and adjusting metabolic and biosynthetic functions, accordingly. GENOMES UNCOUPLED 1 (GUN1), a chloroplast-localized protein, has been recognized as one of the main players in chloroplast retrograde signalling. The aim of this study was to investigate the HSR in Arabidopsis wild-type and *gun1* plantlets subjected to 2 hours of HS at 45°C. The data showed that in wild-type plants, Reactive Oxygen Species (ROS), accumulating promptly, acted as signalling molecules, inducing the expression of heat stress-responsive genes. On the other hand, *gun1* mutants failed to induce the oxidative burst immediately after HS and accumulated ROS and oxidative damage after 3 hours of recovery at 22°C, resulting in increased sensitivity to HS. The results supported the idea that GUN1 is required to oxidize the cellular environment, participating in the acquisition of basal thermotolerance through the redox-dependent plastid-to-nucleus communication.



## **ARGONAUTE3 IS INVOLVED IN HYPOXIA TOLERANCE IN ARABIDOPSIS THALIANA**

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In plants, submergence can induce hypoxia, and limited oxygen availability affects mitochondrial respiration, thus unbalancing the plant's energy status. Hypoxia severely alters gene expression, triggering key responses that help the plant coping with the stress. At the same time, RNA interference is involved in gene expression regulation at several levels. Nowadays, still little is known about the role of RNA signaling under hypoxia. In this work, we show the link between ARGONAUTE 3 (AGO3) and the hypoxic response in *Arabidopsis thaliana*. We found that ago3-3 mutants are more sensitive to submergence and gene expression analyses show that AGO3 expression is induced under hypoxia. On the other hand, AGO3 protein stability is negatively regulated by the lack of oxygen. Additionally, the investigation of mutant and transgenic plants affected in different steps of the hypoxic response proved that AGO3 expression is regulated by ERFVII transcription factors. We also identified a protein arginine dimethylase, PRMT5, as an interactor of AGO3 involved in its destabilization. Moreover, using cPTIO, a NO scavenger, we proved that the destabilization of AGO3 by hypoxia might be PRMT5 dependent. As very little is known about the biological function of AGO3 and its specific role in the RNA interference machinery, we performed AGO3 immunoprecipitation and high-throughput sequencing of co-immunoprecipitated sRNAs. The output of this analysis will lead to the identification of AGO3 biological targets, shedding light on the function of the protein. Altogether, our results identify a role of AGO3 as one possible link between RNA interference and the hypoxic response.

## KARRIKIN PRIMING AFFECTS RESPONSES TO DROUGHT STRESS IN TOMATO

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Climate change-related issues, such as the increase of drought events, are expected to intensify throughout many areas of the world in the next few years (Swann, 2018). Many recent studies have experimented priming as a useful strategy to help plants coping with abiotic stresses such as drought. In the last decade, a group of molecules known as karrikins (KARs) has been identified in charred or burnt plant material and its smoke (Flematti et al., 2011). These compounds, previously studied as promoters of seed germination (Waters et al., 2017), have also been discovered to play a role against abiotic stresses (Li et al., 2017). *Solanum lycopersicum* is one of the most relevant crops in the Mediterranean basin for its economic importance and value (FAO, 2018), whose final yields are severely affected by drought. In this study, we primed tomato plants with a 5  $\mu$ M KAR1 solution and we exposed treated and not-treated plants to moderate water stress. KARs were effective in inducing an early stomata closure under stress, but also an anticipated stomata opening at the onset of recovery. Several parameters of recovery from drought follow a delayed dynamics ("after effect") and our results confirmed that KARs affect these patterns, in particular regarding osmoregulation. Indeed, KARs-sprayed tomato plants maintained higher contents of osmolytes and proline even after accomplishing full physiological recovery. Under drought, plants primed with KARs adopted a stress-avoiding strategy, which allowed them to accumulate useful compounds to promptly recover their physiological functions.

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## **PHENOTYPICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF DURUM WHEAT GENOTYPES UNDER SALT STRESS**

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Climate changes, such as the rising of temperatures, are contributing to soil salinization of arable lands. Salinity affects seed germination by limiting water imbibition, hence disturbing the progression of germination and exerts osmotic stress as well as ion imbalance. Salt stress, affecting water uptake, transpiration, photosynthesis, enzyme activities, and metabolism of protein and lipids, significantly reduces crops yield. The identification of new genetic resources and the study of genetic variability leading to a better salt tolerance, could contribute to increase the stability of crops productivity in future adverse climatic conditions. In this study, we performed a screening of 18 different durum wheat genotypes resulting from a breeding program involving "Primadur" and "T1303" as parental lines. The salt-stress tolerance was evaluated at two different NaCl concentrations (50 and 100 mM) during seed germination and seedling developmental stages. According to germination and shoot and root length, we were able to identify three salt-resistant genotypes and three salt-sensitive ones. In order to better understand the physiological mechanisms involved in the salinity tolerance of durum wheat, we decided to focus on the antioxidant and redox metabolism. Total polyphenol content and antioxidant capacity were analyzed. The presence of SSRs previously associated with salt-stress tolerance was also investigated to complete the characterization of the selected genotypes and to identify at least one marker that can be used for further breeding programs. Our results could contribute to the identification of durum wheat genotypes with improved salinity tolerance that can be used in future breeding programs.

## **Session 3**

# **PLANT ENVIRONMENT INTERACTIONS**

# **MILD DROUGHT TREATMENTS PRIME THE RECOVERY FROM SEVERE DROUGHT MODIFYING THE TRADEOFF BETWEEN SOLUBLE NON-STRUCTURAL CARBOHYDRATES AND GROWTH IN BLACK POPLARS**

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Recovery from drought relies on the plant's ability to restore water transport. Non-structural carbohydrates (NSC) are drivers in plant energy supply and osmotic adjustment processes. We hypothesized that mild drought treatments can prime a faster recovery of plants experiencing a further severe drought, as consequence of a trade-off between NSC accumulation and plant growth.

One group of *Populus nigra* plants were exposed to two cycles of mild drought treatments followed by a severe water stress and drought relief, while a second group underwent only to severe drought and recovery. Poplars exposed to priming treatments showed a reduced growth and a higher NSC content in bark, wood and xylem sap compared to not primed plants. During drought, both groups of plants displayed similar loss of hydraulic conductivity (PLC). Although, after 5 hours of stress relief, both groups showed a recovery of xylem pressure, only a partial drop in the level of PLC was measured. However, the primed poplars exhibited a higher level of hydraulic recovery compared to not primed plants. During drought and recovery, the NSC content in bark and xylem sap increased in both groups, being significantly higher in primed than untreated poplars.

Furthermore, the primed plants exhibited, during stress, a higher NSC/plant DW and, during recovery, a starch decrease.

We suggest that the reduction of plant growth, as a consequence to priming, leads to the formation of a pool of "ready-to-use" sugars that can be used for allowing a faster and more efficient recovery of xylem functionality.

## ISCA-LIKE 3: A PUTATIVE PLANT MAGNETOSENSOR

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The geomagnetic field (GMF) is an intrinsic environmental factor for all living organisms on our planet and protects Earth from the solar wind by deflecting most of its charged particles (Maffei, 2014). While many animal species are able to sense the magnetic field and exploit it to orient themselves over long distances, plants react to varying magnetic field intensity and direction by altering their growth (Occhipinti et al., 2014). Yet, the biological nature of plant magnetoreception remains unknown. A putative magnetoreceptor protein (MagR) has been identified in *Drosophila melanogaster*, and homologues of MagR exist in animals, plants, and microorganisms (Quin et al., 2016). We suggest that plant Iron-Sulfur Complex Assembly (IscA) proteins, homologues of animal MagR, are good magnetoreceptor candidates and their physiological characterization could contribute to a better understanding of plant magnetic induction (Parmagnani et al., 2022). In *Arabidopsis thaliana*, among the four IscA proteins, IscA-like 3 has been found to be the closest MagR homologue and our data show that IscA-like 3 mutant lines are insensitive to MF variation. Moreover, the expression levels of ISCA genes in wt plants are correlated with MF intensity, and IscA-like 3 might be involved in their regulation.

## HEALTHY MITOCHONDRIA ARE NEEDED FOR OPTIMAL PHOTOSYNTHESIS: THE ROLE OF RESPIRATION IN PLANT BIOENERGETICS AND PRIMARY METABOLISM

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Photosynthetic organisms use light as the main source of energy to support their metabolism, but respiration is needed to support metabolism during the night and in non-photosynthetic tissues. Respiration is also active under illumination, although the molecular pathways connecting photosynthetic and respiratory metabolisms are far from clear. We used the moss *Physcomitrium patens* as a model. We monitored cytosolic ATP levels during dark-to-light transitions using the ATP sensor ATeam and observed a light-driven increase of ATP that involves both chloroplast and mitochondria. We also isolated lines lacking a functional respiratory complex I or IV. The mutants show impaired growth, unbalanced carbon metabolism and a rearrangement of the respiratory chain. Despite not showing drastic differences in the composition of the photosynthetic apparatus, the respiratory mutants are photosynthetically less efficient, with reduced rates of both net CO<sub>2</sub> fixation and net O<sub>2</sub> evolution. Our data confirm the importance of mitochondria and respiration in photosynthetic performance. Further studies on these mutants could lead us to identify key players in the interaction between chloroplasts and mitochondria, and potential targets to optimize photosynthesis by genetic engineering.

## **AtCuAO $\beta$ AND RBOHD DISTINCT ROLES IN MECHANICAL WOUNDING-INDUCED STOMATAL CLOSURE**

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In plants, stress-driven modulation of stomata opening/closure is a primary response to achieve rapid whole plant acclimation to changing environmental conditions, in order to prevent excessive water loss, regulate leaf cooling via transpiration and restrict microbial entry into leaves. The propagation of abiotic stress-induced signaling involves the production of Reactive Oxygen Species (ROS), among which hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), that are considered key players in controlling stomatal movement. In *Arabidopsis thaliana*, respiratory burst oxidase homologs NADPH oxidases (RBOHs) and amino oxidases (AOs) greatly contribute to H<sub>2</sub>O<sub>2</sub> accumulation. Particularly, both the D isoform of RBOH proteins (RBOHD) and the copper containing amine oxidase  $\beta$  (AtCuAO $\beta$ ) are especially localized in stomata guard cells and have been reported to be involved in stress-mediated stomatal modulation. Here, the distinct role of AtCuAO $\beta$  and RBOHD in stomatal closure triggered by leaf and root mechanical wounding has been investigated at both injury and distal sites. Analysis of wound-triggered stomatal closure in *AtCuao $\beta$*  and *rbohd* insertional mutants showed that AtCuAO $\beta$ -driven H<sub>2</sub>O<sub>2</sub> production mediates both local and systemic stomatal closure, while RBOHD-driven H<sub>2</sub>O<sub>2</sub> production is involved only in systemic leaf-to-leaf and root-to-leaf stomatal closure. The specific involvement of RBOHD and AtCuAO $\beta$  in local and systemic stomatal responses as ROS generators has been further demonstrated using a chloromethyl derivative of 2,7-dichlorodihydrofluorescein diacetate (CM-H<sub>2</sub>DCFDA). Taken together, results obtained suggest that RBOHD may act downstream of and cooperate with AtCuAO $\beta$  in the oxidative burst elicitation that leads to systemic wound-triggered stomatal closure.



## **TULBAGHIA VIOLACEA VAR. ALBA ROUTINELY EMITS HIGH QUANTITY OF SIGNALLING GLV (Z)-3-HEXENYL ACETATE**

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*Tulbaghia violacea* Harv. (1837) is a widespread ornamental plant belonging to Amaryllidaceae family. While analyzing by SPME (Solid Phase Micro Extraction) and GC/MS the volatile compounds produced by flowers of two varieties of this species, a white-flowered variety (var. Alba) and a purple-flowered variety (var. Violacea), we observed a higher production of (Z)-3-Hexenyl acetate (Z3HAC, a green-leaf volatile, GLV) by var. Alba. Therefore, we determined the production of volatile compounds by whole plants (both in unaltered state and after mechanical wounding) by keeping them confined under a glass bell for 5 h together with a SPME fiber. Whole plants (both varieties) emit main five volatile compounds: Z3HAC, benzyl alcohol, nonanal, decanal, (Z)-3-Hexenyl- $\alpha$ -methylbutyrate; as for flowers, var. Alba emits ten times more Z3HAC than var. Violacea. The wounding (achieved by cutting the 50% of leaves at half-height) results in a high increase in Z3HAC emission and a small production of (Z)-3-Hexenyl- $\alpha$ -methylbutyrate for both varieties. Z3HAC is a GLV capable of stimulating plant defenses, attracting herbivores and their natural enemies, and it is also involved in plant defense priming. Thus, *T. violacea* could represent a useful model for the study of GLVs production and a 'signal' plant capable of stimulating natural defenses in the neighboring plants. For this reason, we aim to deepen this mechanism, by searching any differences in plant defense related genes expression between the two varieties and between whole and wounded plants.

## **A SIMPLE METHODOLOGY TO STUDY THE EFFECTS OF PLANT NUTRIENT HOMEOSTASIS ON Ca<sup>2+</sup> SIGNALING**

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Increasing crop yields by using eco-friendly practices is of high priority in tackling problems regarding food security and malnutrition worldwide. The scientific community aims to understand plant nutrients homeostasis by deciphering the nutrient sensing and signaling mechanisms of plants. Several lines of evidence about the involvement of Ca<sup>2+</sup> as the signal of impaired nutrient availability have been reported. Moreover, an interesting aspect is to test how the nutrient status can affect Ca<sup>2+</sup> signaling in response to environmental challenges. To do so, we aimed at developing a reliable approach to combining plant hydroponic cultures with in vivo Ca<sup>2+</sup> imaging. Whereas hydroponics offers precise control of the media composition and easy access to every plant tissue for phenotypical and molecular analysis, modern genetically encoded Ca<sup>2+</sup> indicators (GECIs) allow to carry out whole plant imaging at low magnification. Here we report the development of a pipeline to perform Ca<sup>2+</sup> imaging analysis in adult Arabidopsis plants grown in hydroponic conditions. To standardize and automatize the image analyses we report the development of a customized data analysis pipeline, a python based napari plugin, that will be shared with the community and the design of a 3D-printed custom-made chamber for a quick exchange of the nutrient solution as well as osmotic or salt stress treatments. To test the effects of nutrient availability on the generation and propagation of long-distance Ca<sup>2+</sup> waves we are performing leaf wounding experiments in wild type and mutants Arabidopsis plants, grown in media with different compositions.

## **Session 4**

# **FROM SEED TO SEED: PLANT PHYSIOLOGY AND DEVELOPMENT**

## **SALT STRESS RESPONSE IN RICE: THE INVOLVEMENT OF SULFUR METABOLISM**

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Salinity is one of the major abiotic stresses which negatively affects crops production. Rice (*Oryza sativa*) represents one of the most important staple food crops and a model monocot plant. However, rice sensitivity to soil salinity still requires finding strategies to increase its capability to cope with this stress. The comparison of molecular mechanisms activated by tolerant and sensitive plants in response to salt stress could allow the varietal improvement of rice against salt stress.

We analyzed the phenotypical, biochemical and molecular response to salt stress of four Italian rice varieties: Onice and Baldo selected as tolerant varieties and Selenio and Vialone Nano as sensitive ones. It is known that antioxidant systems's involvement is crucial in abiotic stress tolerance. Previously we identified glutathione as the redox metabolite mainly involved in rice response to salt stress. As glutathione is the major organic sulfur compound in plants, in this study we focused on sulfur metabolism regulation at transcriptional and post transcriptional levels, involving miRNA395. Tolerant varieties up-regulate glutathione level in response to salt stress and show lower levels of inorganic sulfur compared with the sensitive ones. These data are consistent with the higher expression levels of sulfur metabolism genes observed in Baldo and Onice compared to Selenio e Vialone Nano. These results suggest that sulfur assimilation efficiency is related to salt tolerance in rice.

The different activation of sulfur assimilation pathway between sensitive and tolerant varieties supports the correlation between sulfur metabolism, antioxidant response and salt stress resistance in rice.

## UNVEILING THE FUNCTIONAL AND REGULATORY PROPERTIES OF A NEW CLASS OF ENZYME CONTROLLING THE GSNO HOMEOSTASIS

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In the cell, S-nitrosoglutathione (GSNO) homeostasis is primarily controlled by the ubiquitous NADH-dependent enzyme S-nitrosoglutathione reductase (GSNOR). Recently, human aldo-keto reductases (AKRs) have been identified as a new class of enzymes also implicated in GSNO catabolism by a mechanism resembling that of GSNOR, but using NADPH as a source of reducing equivalent. Here, we aim to determine the biochemical features of plant AKRs belonging to the 4C subclass, which exhibit the greatest sequence similarity to the well-characterized human enzyme, AKR1A1. To this end, we expressed and purified four isoforms of AKR4C from *A. thaliana* (AtAKR4C8-11). Subsequently, we kinetically characterized each protein to determine the Michaelis-Menten constants ( $K_m$ ) and turnover numbers (kcat) for both GSNO and NADPH. In addition, we have also evaluated the ability of AtAKR4Cs to use alternative substrates such as a nitrosylated form of truncated glutathione (i.e., nitrosylated  $\gamma$ -Glu-Cys and Cys-Gly). Overall, these kinetic analyses revealed that plant AKR4Cs exhibit variable catalytic parameters. Notably, AtAKR4C8 appears to be the most catalytically efficient isoform with an affinity toward GSNO similar to that measured for GSNOR ( $\sim 30 \mu\text{M}$ ). Finally, we examined the redox sensitivities of each enzyme to evaluate the activity response to oxidative molecules such as hydrogen peroxide, oxidized glutathione (GSSG), and GSNO. Intriguingly, only the C8 isoform was found to be inactivated by oxidant treatments, suggesting that it may contain one or more redox sensitive cysteines. Globally, these data allow for a deeper understanding on AKR4Cs, highlighting their potential role in controlling GSNO homeostasis in plants.

## **WATER AND NUTRITIONAL SAVINGS SHAPE NON-STRUCTURAL CARBOHYDRATES IN GRAPEVINE (VITIS VINIFERA L.) CUTTINGS**

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Global changes and sustainability challenge researches in saving water and nutrients. The response of woody crops, which can be forced at facing more drought events during their life, is particularly important. *Vitis vinifera* can be an important model for its relevance in countries subjected to climate changes and its breeding, requiring cuttings plantation and strong pruning.

Drought leads to an impairment between growth and reserves which can be a key point in the survival of plantings. This work aims at understanding the role of non-structural carbohydrates (NSC) in: i) the maintenance of hydraulic function in cuttings subjected to water deficit and limited nitrogen: ii) identify the best regime of water and nitrogen to achieve a correct compromise between plant growth and NSC. Cuttings of two different cultivars and three rootstocks of *V. vinifera*, were grown in pots under different water and N regimes. During the vegetative season, morphological and physiological traits were measured. In particular, NSC content analysis were performed in roots, rootstocks, shoots and canes on blooming and on cane ripening period.

During early vegetative phases, plants under water deficit shown a reduced growth and lower water potential respect well-watered plants. At the end of the vegetative season, NSC was affected also by the type of cultivar or rootstock. Nitrogen does not seem to have any effect on carbohydrates content.

Our results suggest that imposing a controlled water deficit to grapevine from budburst, can support plants to accumulate NSC, useful to help cutting survival and face incoming drought events.

## IMPAIRING OF LIPID RESERVOIRS MOBILIZATION IN ERUCA SATIVA GERMINATION UNDER SALT STRESS CONDITIONS

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Over the past few years Mediterranean areas are getting more affected by soil salinization resulting in reduced crop yields. *Eruca sativa*, commonly known as arugola or rocket, is an erbaceous species widely cultivated in this area with an increasing commercial value. The purpose of this study was to investigate the salinity effect on seedling development by growth and germination parameters as well as by morphological and ultrastructural observations of cotyledons. Seeds were subjected to salinity starting from 137 mM up to 548 mM NaCl. Delaying in seed germination was observed in all the treatments although seedlings growth was strongly affected only over 137 mM. Light microscopy results highlighted the wide occurrence of lipid bodies in mesophyll cells of seedlings treated with salinity levels above 137 mM, confirmed by ultrastructural investigations. It can be hypothesized a detrimental effect of salt in lipid reservoirs mobilization during seedlings development. Moreover, a reduction in intercellular spaces and structural changes chloroplast and peroxisomes were observed in treated plants. Finally, the highest salinity levels promoted autophagic processes especially involving chloroplasts. Intriguingly, the seedlings showing the highest values of fresh weight and mesophyll thickness were grown at 137 mM, suggesting a possible salt adaptation in germination process.

## EVALUATION OF D27 AND D27-LIKES GENES IN TOMATO AND THEIR POTENTIAL ROLE IN FRUIT QUALITY

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Comparative analysis on a panel of independent CRISPR/Cas9 knock-out lines for each of the genes involved in the strigolactone (SLs) biosynthesis in tomato - D27, CCD7, CCD8 and MAX1 - revealed an altered phenotype for all the edited lines except d27, which resulted comparable to the WT for all the examined traits. DWARF27 (D27) encodes for a  $\beta$ -carotene isomerase, and mediates the reversible conversion of alltrans- $\beta$ -carotene into 9-cis- $\beta$ -carotene. Even though this isomerization step could occur spontaneously, phylogenetic and similarity-based analysis suggests the presence of two additional D27-LIKE genes (D27-LIKE 1 and D27-LIKE 2) in tomato, whose function is currently unknown. They could likely catalyse the isomerization of all-trans- $\beta$ -carotene in redundancy with the "canonical" D27, but the activity on other substrates and/or the consequent production of different carotenoid-derived molecules is possible as well, thus influencing fruit quality. Furthermore, an increased susceptibility to drought was recently observed for a d27 line in rice. At the present, the production of tomato CRISPR/Cas9 knock-out for all the D27 genes is underway. These lines will be subsequently analysed in order to produce tomato genotypes with an increased content of fruit carotenoids. Moreover, the response to abiotic stresses will be considered too.



## NEIGHBORS DETECTION AND TRANSCRIPTIONAL RESPONSE, DOES CHROMATIN ACCESSIBILITY COUNT?

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Plants can sense the proximity of other plants because leaves reflect the Far-Red component of the sunlight reducing the ratio between Red light and Far-Red (LowR/FR). This shaded environment, typical of dense vegetation, is not tolerated by plants loving full sunlight, such as *Arabidopsis thaliana*, which developed a series of elongation responses to escape from this situation known as shade avoidance syndrome (SAS). SAS is achieved by gene expression reprogramming, which is controlled by different classes of transcription factors (TFs). Given the lack of information about the chromatin organization of the TFs binding sites and their regulatory role, this work aimed to characterize it through an INTACT/ATAC-seq experiment. The analysis showed that genes with a fast, but transient upregulation in LowR/FR, have nucleosome-free regions of 150-200pb on their promoters that do not change depending on light conditions. The accessible chromatin conformation of these regions facilitates the TFs binding, ensuring a quick, but also reversible response depending on TFs availability. We also found that the chromatin of the TSS of one of the most important SAS factor, called HFR1, get more open by long LowR/FR treatment. This could explain its peculiar expression in LowR/FR, which does not correlate with TFs levels. In fact, among the early LowR/FR-induced genes, HFR1 is the one showing a persistent upregulation in prolonged LowR/FR conditions. Overall, this work helped a better understanding of the transcriptional reprogramming happening during SAS, which is a beautiful example of how plants translate an external stimulus into a physiological response.

# **ABSTRACTS**

## **Session 1**

### **EMERGING TECHNOLOGIES AND SUSTAINABILITY**

## MONOCHROMATIC LED LIGHTING AFFECTING MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS IN LENTIL (*LENS CULINARIS* MEDIK.) SEEDLINGS

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In the last decades, light-emitting diode (LED) proved to be a sustainable tool for plant growth and development. Knowledge about plant response to light increased during the last years, also thanks to the diffusion of LEDs, which offer several advantages compared to conventional light sources, such as the possibility to tailor the light spectrum and regulate the light intensity allowing to investigate the responses of plants to light in a different way.

This study aims to evaluate the effects of light intensity and spectra composition on physiological parameters in lentil (*Lens culinaris* Medik.) seedlings grown in hydroponics. Two monochromatic LED lights (red and blue) and three different light intensities (100, 300, and 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were used, under 24 h of continuous light and with darkness as reference. The effects of LED lighting on 3- and 5-day-old lentil seedlings were investigated by assessing biometric (fresh and dry matter, root and aerial part length) and biochemical (ascorbate, phenols, photosynthetic pigments, hydrogen peroxide, lipid peroxidation and antioxidant enzymes activities) parameters. The degrees of interaction between light quality and intensity were highlighted, turning into statically significant. Applied Red light promoted elongation growth, whereas blue light enhanced the bioactive compounds of lentil seedlings, although the promotion effects varied according to light intensities. Further research may be required to deeper understand the interaction between LED lighting and plants and establish the optimal guideline for improving seedlings's quality and productivity, in a sustainable agricultural approach.

## PRODUCTION OF SECONDARY METABOLITES FROM COFFEA SPP. CELL CULTURES

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Plants synthesize an array of phytochemicals, also described as secondary metabolites (SMs), to fulfil important physiological tasks as well as to face abiotic and biotic stresses. Furthermore, bioactive phytochemicals are widely used in pharmaceutical, nutraceutical and cosmetic sectors. Coffee (*Coffea* spp.) has potential applications in the therapeutic field as a source of SMs including phenolic compounds, alkaloids, as well as terpenoids.

Plant tissue cultures provide an eco-friendly option for large scale production of high-value SMs. Callus induction from plant explants is the first step required. Plant growth regulators (PGRs) are added to the basal growth medium to stimulate and control cell development. Among these, auxins and cytokinins are the most extensively used and studied in the context of callus formation. The texture and type of callus depend on explant type as well as on the combinations of PGRs.

In order to investigate both the best concentration and type of PGRs required for a friable callus induction from leaf explants in different varieties of *Coffea arabica*, the experiments were arranged in three steps: i. Selection of the best concentration of PGRs, ii. Selection of the best cytokinin, iii. Selection of the best auxin. At the end of the experiments, the percentage of callusing and the mean fresh weight of explant + callus were measured for each treatment. On the basis of these results, the optimal medium was selected to establish the suspension cell culture for the induction of SMs production.

## **GRAFTING AS A NEW STRATEGY FOR ENDOPHYTE TRANSFERRING IN GRAPEVINE**

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Grapevine is a perennial woody plant of global economic importance. In the field, grapevine is colonized by endophytes, which are bacteria or fungi that reside in plant tissues and promote plant growth. Grafting is a common practice for grapevine propagation and trait improvement, so we want to verify whether it can be used to mediate the transfer of endophytes in plants of interest to improve their growth. In this context, grapevine plants populated by specific endophytes are used as rootstocks in the grafting procedure of grapevine plants lacking that endophytic population. To demonstrate the efficiency of endophyte transfer, GFP-labelled endophytic bacteria are inoculated in endophyte-free plants that are used as rootstock for endophyte-free grapevine plants. At different times after grafting, stems and leaves of the plant to which the bacteria should have been transferred are analysed by confocal microscopy.

## SUSTAINABLE STRATEGIES TO PRODUCE BIOACTIVE MOLECULES WITH ANTIVIRAL PROPERTIES IN PLANT CULTURES

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The identification of plant metabolites with antiviral action can be useful for the production of new drugs or supplements and phytotherapies for the treatment and prevention of human viral diseases. In this context, plant defensins are promising antimicrobial peptides involved in plant defense responses against a wide range of environmental injuries. The expression of four 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, gene expression analysis showed that tobacco endogenous defensins' genes were not expressed in TBY-2 cells, neither in the elicited cultures nor in those grown under basal conditions. These results can be due to the difficulties of cultured cells to express genes involved in biosynthetic pathways of defense metabolites, because most of the secondary metabolites are produced or accumulated in differentiated tissues, rather than undifferentiated cells. To get around the problem, the use of hairy roots' cultures obtained from *Petunia hybrida* cv. Mitchell W115 subjected to *Rhizobium rhizogenes* infection has been chosen as alternative strategy to produce putative antiviral defensins. Actually, this kind of organ culture is widely used as plant biotechnological approach to obtain bioactive compounds. Based on the reported antimicrobial activity, the heterologous genes selected for transformation in *P. hybrida* were a defensin from *Arabidopsis thaliana* (AT3G05727), which sequence was engineered to optimize the isolation and purification steps, and the KALATAB1 cyclotide precursor from *Oldenlandia affinis*.

## **THE APPLICATION OF A BIOSTIMULANT BASED ON TANNINS ENHANCES FRUIT NUTRACEUTICAL PROPERTIES OF A TOMATO PLANTS CULTIVATED UNDER SALT STRESS**

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The productivity of tomato (*Solanum lycopersicum* L.), one of the most important crops worldwide, can be strongly affected by abiotic stresses. Among these, salinity represents one of the main critical factors in tomato cultivation losses, since high salt concentrations can decrease plant physiological performance and lowering fruit production and quality. Potential solutions aimed to protect plants from abiotic stresses are currently employed, however, the excessive use of agrochemicals causes a significant environmental impact, which in long term will result in the amplification of environmental pollution. A promising strategy to transform agriculture into a more sustainable activity is represented by biostimulants, products not only beneficial for plant growth and productivity, but also able to enhance abiotic stress tolerance, with a consequent increase of crop quality traits. The aim of this work was to evaluate the effects of the application of a tannin-based biostimulant (VIVEMA TWIN, Greenhas Group, Canale, Italy) on nutraceutical profile of fruits harvested from five different tomato genotypes (two tolerant and three sensitive to salinity) cultivated under standard or salt stress conditions. Several plant bioactive compounds, such as carotenoids, lycopene, and polyphenols were quantified. Moreover, the biostimulant effect was also investigated through the evaluation of the reducing and radical scavenging activity of the ethanolic fruit extracts. In conclusion, the overall results indicate that the biostimulant application is not only able to mitigate the negative effects caused by salinity, but also enhance the nutraceutical properties of fruits harvested from plants cultivated in standard and salt stress conditions.

## SCREENING APPROACHES TO ENHANCE STORABILITY OF PRIMED LEGUME SEEDS

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High germination performance is essential for seed quality as it impacts crop yield. Seed priming is a well-established technology for seed quality enhancement, based on the transient activation of the pre-germinative metabolism, including the antioxidant mechanisms and DNA damage repair pathways. There is an increased reactive oxygen species (ROS) production at the onset of germination, however ROS levels should be stringently regulated to avoid deleterious oxidative damage and genotoxic stress. Seed priming improves germination, seedling establishment and enhances stress response. Despite these advantages, the storability of primed seeds is negatively affected. In this study, we have developed an experimental system using accelerated aging (AA), named "Petite assay" as a tool to elucidate the cellular and molecular mechanisms underlying the deterioration of primed seeds in the model legume *Medicago truncatula* and the fodder crop *Medicago sativa*. Germination data showed an expected decrease in germinability and presence of aberrant phenotype in the primed seeds after AA. ROS levels were explored in this experimental system, during the pre-germinative metabolism and post-aging re-imbibition. Further molecular analysis will be conducted to provide insights on the dynamics of pre-germinative metabolism in order to better characterize the system and then test its potential as screening tool for the identification of treatments able to mitigate the deterioration of primed seeds in storage.



# **ABSTRACTS**

## **Session 2**

### **PLANT STRESS RESILIENCE AND MEMORY**

# **APPLICATION OF ABIOTIC STRESS TO TOMATO (*SOLANUM LYCOPERSICUM* L.) TO INCREASE THE EXTRACTION YIELD OF SECONDARY BIO-BASED COMPOUNDS**

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Tomato (*Solanum lycopersicum* L.) production of secondary metabolites is essential for the plant to face both biotic and abiotic stresses. Among them, steroidal glycoalkaloids (SGAs), are of great scientific and applicative interest, both for their role in plant defence against pathogens and for their useful properties for human health.

Unharvested green tomato parts, typically left in the field and discarded without any valorisation, are a potentially sustainable source of these alkaloids which could feed back in industry, enabling a circular economy approach. A few preliminary reports indicate that environmental factors may affect SGA accumulation, and this opens the possibility that sub-optimal cropping conditions (e.g. deficit irrigation, partially salty water), which are increasingly required to reduce the resource use impact of tomato cultivation, could also increase the content of these valuable substances.

The main objectives of this PhD project are thus i) the refinement of a process to extract SGAs from tomato plant wastes, and ii) the enhancement of SGA accumulation in tomato green tissues. The first objective will be followed by testing extraction techniques in collaboration with a specialized company. The second objective will be pursued by addressing three sub-objectives: a) CRISPR/Cas editing of genes involved in SGA catabolism and transport; b) study of the influence of abiotic stresses and of hormones, in particular strigolactones (SL), on SGA biosynthesis, and c) identification and study of miRNA-encoded micro peptides (miPEPs) as potential regulators of SGA accumulation due to their ability to affect miRNA-dependent regulation of genes involved in SGA metabolism.

# CHARACTERIZATION OF HYPOXIC STRESS EFFECTS ON THE SYMBIOTIC RELATIONSHIP BETWEEN MEDICAGO TRUNCATULA AND SINORHIZOBIUM MELILOTI THROUGH ROOT TRANSCRIPTOME ANALYSIS

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In plants, hypoxia sensing occurs through group VII Ethylene Response Factors (ERF-VII) oxygen-dependent destabilization: under normoxic conditions these factors are continuously degraded by the proteasome through the N-degron pathway, which is blocked in case of oxygen deficiency leading to the nuclear accumulation of ERF-VII. The primary effect of ERF-VII stabilization is the transcriptional regulation of genes involved in hypoxia adaptive response, through energy metabolism modifications counterbalancing energy deficiency caused by oxidative phosphorylation downregulation.

The increase of heavy rainfalls and flooding events, causing hypoxia in plants and jeopardizing the agricultural sector, demands for scientific focus on the characterization of the molecular nature of hypoxia on cultivated species.

Fabaceae (Legumes), contains a high number of agronomically important species. Plants in this family develop symbiotic relationships with nitrogen-fixing bacteria (Rhizobia). In this symbiotic system, bacteria are located within nodules, specialized root organs characterized by a microoxic environment. However, hypoxia appears to have a negative impact on nodule development.

*Medicago truncatula*, a well-established model legume for the study of the rhizobia-legume symbiosis, is nodulated by *Synorhizobium meliloti*. With a compact (465Mb) and fully sequenced genome, paired with the availability of abundant gene expression data, *M. truncatula* is exceptionally suitable for studying RNA-mediated gene regulatory networks. In this study we aim at revealing the molecular mechanism underlying hypoxia response in plants and their involvement in the establishment of symbiosis through transcriptome profiling. This approach will allow for an unprecedented resolution in the understanding of hypoxia-induced regulatory networks within plant root nodules, leading to improved strategies to enhance submergence stress tolerance in cultivated plants.

## **FLOWERING TIME AND LEAF MORPHOLOGY TRAITS ARE KEY FOR HEAT TOLERANCE IN ONE TOMATO GENOTYPE**

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The increase in heat waves is putting global food security at great risk. Crop improvement for tolerance to increased temperatures is therefore of vital importance. High temperatures can cause several physiological changes in plants including flower abscission and photosystems damages, which are related to a decrease of crop production. Previously, we selected a tomato genotype (E42) that demonstrated to have a stable yield under elevated temperatures. We analyzed plant functional traits, growth parameters and physiological responses of E42 to high temperatures in open field conditions with late transplant. E42 was able to maintain a long flowering period, producing more flowers than E42 in the early season, exhibiting a good pollen viability as well. Moreover, E42 grown during hot periods changed leaf morphology in order to optimize its carbon gain, decreasing leaf area and increasing specific leaf area (SLA). Since SLA is related to strategies of resource acquisition and use, we hypothesize that E42 leaf may be a key organ correlated to acclimation mechanism. Further investigation will allow us to confirm these initial outcomes and to better characterize the response of E42.

## **CROP RENEWAL FOR THE RESTORATION OF THE AGROECOSYSTEMS COMPROMISED BY XYLELLA FASTIDIOSA**

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*Xylella fastidiosa* is a quarantine pathogen that causes numerous diseases, and one of the most threatened plants is the olive tree as demonstrated by the mass deaths in thousands of hectares caused by the strain De Donno. To restore the agricultural sector affected by the epidemic, besides varietal renewal of the olive tree using resistant cultivars, the crop renewal using plant species considered immune or resistant to the pathogen represents a key strategy for the restoration of the agroecosystem. For this purpose, we evaluated the almond and pistachio, two trees which adapt well to the soil and climatic conditions of the infected areas, and for which there is a fair amount of interest by growers. Besides disease assessment (eg. the incidence of the bacteria and the severity of the disease), analysis were carried out to evaluate susceptibility to cavitation, physiological responses to xylem occlusion and production parameters. Different cultivars of both species were also considered, with the aim of identifying the most tolerant varieties towards the pathogen. Our finding suggest that the almond tree is a resistant species against the strain presents in Apulia, showing very mild symptoms and a low bacterial concentration regardless cultivar. A similar trend was observed for the pistachio, which was found to be very resistant and asymptomatic. Furthermore, preliminary observations suggest that these plants, as well as being anatomically less susceptible to cavitation, could be able to activate more efficient refilling. Long-term observations are requested to confirm the good aptitude of cultivars for crop renewal.

# ROLE OF SALT TOLERANCE-RELATED PROTEIN (STRP) IN SALT STRESS RESPONSES IN ARABIDOPSIS THALIANA

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Despite its self-explicative name, the role played by the Salt-Tolerance Related Protein (STRP) in salt stress remains largely unknown. STRP has been previously characterized as a Late Embryogenesis Abundant (LEA)-like, intrinsically disordered protein, involved in plant responses to cold stress. STRP localizes in the cytosol, nucleus, and plasma membrane. Protein levels strongly increase upon different stress conditions. In the nucleus, STRP interacts with DEK3, a chromatin-related protein that modulates DNA structures and gene expression. STRP levels are strongly increased by exogenous ABA application, and it is also involved in ABA signaling and perception. In this background, our goal was to deeply investigate the role of STRP in *A. thaliana* salt stress responses. Our experiments show a strong increase of STRP levels in response to salt stress, due to a reduction of proteasome-mediated degradation of the protein. Physiological and biochemical responses to salt stress were studied by comparing wild type (wt) plants with the *strp* mutant and *STRP*-overexpressing plants. We observed that several salt stress-related alterations are more marked in the *strp* mutant than in the wt plants, whereas they are reduced in the *STRP*-overexpressing plants. Moreover, *strp* mutant accumulates more H<sub>2</sub>O<sub>2</sub> and is more susceptible to the oxidative damage induced by salt stress, compared to wt plants. Results obtained suggest a role for STRP in plant protection against salt stress, proposing this protein as a multitasking regulator acting at different levels in the response mechanisms to abiotic stress in *A. thaliana*.

## GAINING INSIGHT INTO THE DROUGHT TOLERANCE MECHANISMS IN CHICKPEA

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Latest genomics-based research has laid the foundation for further understanding of the activation of different genes associated with drought tolerance and sensitivity. Chickpea (*Cicer arietinum* L.) is an important grain-legume crop that is mainly grown in rainfed areas, where terminal drought and heat stress at flowering and seed filling are major constraints to its productivity. Recent advancements in coping with these challenges comprise the application of RNA-Seq approaches to the understanding of the differential gene regulation in desi and kabuli chickpea varieties. This work aims to decipher molecular mechanisms underlying drought stress mechanisms in chickpea through an integrative multi-omics approach (RNA-seq and whole genome bisulfite sequencing) combined with phenotypic analysis (chlorophyll content, photosynthetic measurements), biochemical analysis and agronomic traits (qualitative parameters). Two varieties of chickpeas: desi (brown-coloured small seed) and kabuli (white or beige-coloured large seed), represented by 4 selected genotypes each, and one cultivar (Blanco lechoso) were used for the experimental setup. A total of 10 biological replicates were grown for each genotype, under two different hydric conditions combined with heat stress during the last growth phase and the flowering time. Results indicate a tendency of better photosynthetic performance for the kabuli type than the desi chickpea type with differences among used genotypes. Moreover, 4 out of 9 genotypes show a decrease of chlorophyll content under drought stress, 5 genotypes display no statistical difference in chlorophyll content and two genotypes have a significant increase of chlorophyll production. According to the results of this study it was possible to select two genotypes demonstrating contrasting performance under different irrigation regimes and heat stress: KABULI FLIP07 318C and DESI PI598080. The analysis of proline, soluble sugar and starch content confirmed the better performance of KABULI FLIP07 318C than DESI PI598080 under this abiotic stress.

Keywords: Phenotypic parameters, Biochemical parameters

## PLANT RESPONSES TO COMPLEX ENVIRONMENTAL STRESSES: INVESTIGATION OF IRON DEFICIENCY RESPONSE IN PLANTS EXPOSED TO FLOODING

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Waterlogging occurs when the soil gets saturated with water; this complex stress condition impacts on soil properties in several ways, in particular leading to a decrease in the redox potential, reduced nutrient uptake and a decline of oxygen (O<sub>2</sub>) availability. O<sub>2</sub> is an essential substrate for many biochemical reactions in plants including photosynthesis and metabolism, moreover it is an indispensable co-substrate for many enzymatic activities. The main oxygen sensors in *Arabidopsis thaliana* are represented by plant cysteine oxidase (PCO) enzymes, which oxidize ethylene-response factor VII (ERF-VII) members and target them for degradation, depending on oxygen availability. During waterlogging, PCO reaction is inhibited due to the lack of oxygen; therefore, ERF-VII members are stabilized and able to induce the expression of anaerobic genes. PCOs belong to dioxygenases superfamily, and as such they require a form of nonheme iron (Fe<sup>2+</sup>) as cofactor. Thus, being O<sub>2</sub> and Fe<sup>2+</sup> required for their activity, they might represent a convergence point between low oxygen and nutritional stimuli. In this study we investigated the possible involvement of known enzymes and transcriptional factors implicated in the low-oxygen perception in the iron-deficiency response; our approach has been based on correlating the physiologic and cellular effects of their inactivation to iron availability in plant tissues, in order to shed light on the interplay between these two forms of stress. The final goal of this study is to deepen our understanding about stress-perception pathways in plants.



## IMPACT OF DROUGHT PROGRESSION RATE ON EMBOLISM RECOVERY IN POPLAR

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During water deprivation woody plants are challenged with the complex dilemma of reducing energy requirements while preparing for the recovery of xylem functions after the inevitable vessels embolism formation. The hypothesis that we are testing focuses on how the rate of stress progression may have an impact on the plant responses to drought and the recovery process. The same stress level was obtained either over a long or short time period to reach a similar embolism formation to then evaluate how the ability to reinstate xylem hydraulic conductivity was affected. In-vivo image captures performed using x-ray micro-CT technology were used to measure the percentage area of non-functioning vessels in respect to the total xylem area. Mean values of 7,5% were observed on plants recovered from fast developed drought while the other condition reached a value of 10,9%. As expected, prolonged stress was also causing a reduced growth implying an altered nonstructural carbohydrate production and consumption. Our preliminary analysis on the rate of recovery from embolization in plants indicate that slow drought events lower the ability to recover from damage compared to plants that reached the same water stress level but in a shorter period. The data suggest an important role of carbohydrate status on the plant ability to recover from embolism formation and reinstate the water column function. Further biochemical and molecular analysis will deepen our knowledge on the actual mechanisms guiding this phenomenon.

# **ABSTRACTS**

## **Session 3**

### **PLANT ENVIRONMENT INTERACTIONS**

## MULTI-STRATEGY STUDY OF NON-PHOTOCHEMICAL QUENCHING IN THE MOSS *PHYSCOMITRIUM PATENS*

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Sunlight provides energy to photosynthetic organisms through the activity of pigment-protein supercomplexes Photosystem (PS) I and II. Light absorbed by light harvesting complexes (LHCs) associated to PS fuels an electron transport chain to produce reducing power and ATP which are necessary for the carbon fixation. When the absorbed light exceeds photochemistry needs, plants can experience photo-oxidative stress with harmful effects for fitness and productivity. The reversible mechanism of non-photochemical quenching (NPQ) of chlorophyll a fluorescence around photosystem II (PSII) enables dissipation of excess absorbed energy as heat and plays a prominent role in photoprotection. In plants, NPQ requires the presence of the membrane protein PsbS that interacts with LCH, inducing their dissipative conformation. In this project, NPQ is studied with a multi-strategy approach in the moss *Physcomitrium patens*. First, we exploited base editing to introduce in vivo specific amino acid changes and assess their impact on PsbS activity. This work demonstrates the potential of base editing approaches for in planta gene function analysis and identifies a cluster of hydrophobic aminoacids impacting NPQ activation. Now, to mimic natural variable environments, we are studying the acclimation to high (HL) and fluctuating (FL) light conditions on *P. patens* wild-type plants and npq-less strains. Preliminary results show that NPQ mutants are unable to modulate their photosynthetic activity when exposed to HL and FL and have lower values of PSII maximum efficiency with respect to WT plants, suggesting the importance of this process in acclimation.

## **ANAC102 CHLOROPLASTIC LOCALIZATION, ARE WE ANTICIPATING EVOLUTION?**

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ANAC102 is a transcription factor involved in stress response and brassinosteroids signaling, with circadian regulation controlled by phytochromes. ANAC102 has been proposed to have a role in downregulating chloroplast transcription, which may be very useful in reducing photosynthesis and chloroplast energy demand under stress conditions. However, its localization in the chloroplast has mainly been demonstrated by using constitutive promoters. In this work, we recapitulate the literature, clarify which are ANAC102 isoforms in Arabidopsis and analyze their expressions under control conditions and in response to stress. Based on our results, the most highly expressed ANAC102 isoform is nucleocytoplasmic and the N-terminal chloroplast targeting peptide appears to be a recent evolutionary acquisition, present only in Brassicaceae, and not involved in stress response.

# PLANT AND FUNGAL GENE EXPRESSION COUPLED WITH STABLE ISOTOPE LABELLING PROVIDE NOVEL INFORMATION ON SULFUR UPTAKE AND METABOLISM IN ORCHID MYCORRHIZAL PROTOCORMS

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Orchid endomycorrhiza (OM) represents an unusual symbiosis between plants and fungi since carbon is provided to the host plant by the mycorrhizal fungus during early orchid development, the protocorm stage, and in adults of achlorophyllous or partially photosynthetic orchid species. The cells intracellularly colonized by fungal coils, known as pelotons, are assumed to be the site of nutrition transfer, where the fungal partner provides to mycorrhizal orchid protocorms other crucial nutrients such as phosphorus and nitrogen. While the exchanges of these vital nutrients have been thoroughly studied, little is known about the transfer of sulfur (S) in the OM symbiosis. Targeted gene expression analysis in combination with a stable isotope tracer approach coupled with ultra-high spatial resolution secondary ion mass spectrometry (SIMS), and the application of a laser microdissection protocol, allowed us to decipher S metabolism and exchange in orchid mycorrhiza, focusing on the model system represented by the Mediterranean orchid *Serapias vomeracea* and the mycorrhizal partner *Tulasnella calospora*. By correlating data on the translocation of <sup>34</sup>S with those of differential expression of plant and fungal genes involved in S uptake and metabolism in symbiotic and asymbiotic protocorms, we have demonstrated for the first time that the fungal partner is actively involved in S supply to the host plant, and that the transfer most likely occurs through S reduced organic forms. Our study will improve the knowledge on the regulation of S primary and secondary metabolism, as well as on the whole nutritional framework in orchid mycorrhizal symbiosis.

## LONG-DISTANCE TURGOR PRESSURE CHANGES INDUCE LOCAL ACTIVATION OF PLANT GLUTAMATE RECEPTOR-LIKE CHANNELS

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In *Arabidopsis thaliana*, local wounding and herbivore feeding provoke leaf-to-leaf propagating  $\text{Ca}^{2+}$  waves that are dependent on the activity of members of the glutamate receptor-like channels (GLRs). In systemic tissues, GLRs are needed to sustain the synthesis of jasmonic acid (JA) with the subsequent activation of JA-dependent signaling response required for the plant acclimation to the perceived stress. Even if the role of GLRs is well established, the mechanism through which they are activated remains unclear. Here we report that in vivo the amino acid-dependent activation of the AtGLR3.3 channel and systemic responses require a functional Ligand Binding Domain (LBD). By combining imaging and genetics we show that leaf mechanical injury, such as wound, burn as well as hypo-osmotic stress in root cells induce the systemic apoplastic increase of L-Glutamate which is largely independent of AtGLR3.3 that is instead required for systemic cytosolic  $\text{Ca}^{2+}$  elevation. Moreover, by using a bioelectronic approach we show that the local release of minute concentrations of L-Glutamate in the leaf lamina fails to induce any long-distance  $\text{Ca}^{2+}$  waves.

## DEFENCE SIGNALLING PATHWAYS IN PLANTS WITH ALTERED HOMOGALACTURONAN CONTENT

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The cell wall (CW) is a complex extracellular matrix, mostly composed of polysaccharides, with crucial roles during plant life cycle and in defence against pathogenic microorganisms and herbivores. We have shown that pectin, a major CW component, plays a central role in plant development and response to biotic stress. Plants with reduced content of homogalacturonan (HG), a major pectin component, due to the expression of a fungal polygalacturonase or to the *qua2-1* (*qua2-1*) mutation impairing HG biosynthesis, show enhanced resistance to the fungal pathogen *Botrytis cinerea*, over-accumulation of reactive oxygen species (ROS), loss of cell adhesion and loss of cuticle integrity. Throughout genetic and molecular analysis we aim to elucidate which defence signaling pathways lead to the increased resistance toward *Botrytis*. Preliminary results show that *qua2-1* plants have constitutive activation of the jasmonate (JA) pathway, which does not affect cuticle integrity and cell-cell adhesion but contributes to restrained growth and to increased fungal resistance. Yet other mechanisms appear to contribute to this resistance, as *f* gene involved in the biosynthesis of camalexin, as *PAD3*, a gene required for camalexin biosynthesis and basal resistance to *B. cinerea* is strongly up-regulated in *qua2-1* plants independently of JA. Current work aims at elucidating the cross-talk between CW alterations and plant defence to necrotrophic pathogens.

## NOVEL APPROACHES TO STUDY PHOTOSYNTHETIC CHARACTERISTIC OF CHLOROPLASTS FOUND ALONG THE RADIAL PROFILE OF FRAXINUS ORNUS STEMS

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Leaves are generally the most important photosynthetic organs in woody plants, but chloroplasts can also be found in stem tissues. Anyway, their role is still unclear and we therefore decided i) to characterize photosynthetic properties of these chloroplasts, showing that they are capable of photosynthetic activity ii) to apply microsensor technology to assess the oxygen production and iii) to apply fluorescence-lifetime imaging microscopy (FLIM) to better understand the proportion of photosystem I and II (PSI/PSII ratio) of stem samples of different ages of *Fraxinus ornus*. Blue light was mainly absorbed by the outer bark, and far-red enriched light reached the xylem and pith. The stem photosynthetic apparatus was apparently acclimated to the red-enhanced and low-light environment, as shown e.g. by higher amounts of LHCII, PSII, more abundant grana compared to leaves. Both intact stems and isolated bark/wood were able to perform a light-induced changes in O<sub>2</sub> production, and bark resulted as the greatest contributor to oxygen production. We also show that FLIM technology can be applied to stem samples, and we detected a lower ratio of PSI/PSII in bark than in wood samples. We finally present the first radial profile of oxygen production in current-year stems of *F. ornus* under dark and light conditions. We demonstrate that stem chloroplasts are coherently optimized to the prevailing micro-environmental conditions at the bark and wood level, and are fully photosynthetically active. Taking together all data, our findings may be used to optimize further study focused on the role of stem photosynthesis.



## FUNCTIONAL CHARACTERIZATION OF THE CALCIUM-BINDING PROTEIN BONZAI1 IN *ARABIDOPSIS THALIANA*

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*Arabidopsis* BONZAI1 (BON1) is a calcium-binding protein belonging to the copines family and it is characterized by two N-terminal domains C2A, C2B that bind Ca<sup>2+</sup> and a C-terminal vWA. BON1 is localized at the plasma membrane, and it is involved in the plant immunity response as well as in promoting stomatal closure. In standard growth conditions, at 22°C, bon1 mutants show stunted growth, a phenotype which can be partially reverted by growing plants at 28°C. Our analyses on two independent bon1 homozygous mutants show a drastically reduced adult plant size and defects in phyllotaxis that start to be visible three weeks after germination and reveal that BON1 has a role during the progression from vegetative to reproductive growth, the so-called floral transition. Interestingly, the vWA domain of BON1 is predicted to interact with the N-terminal autoinhibitory domain of 2 plasma membrane Ca<sup>2+</sup>-ATPases, ACA8 and ACA10, to possibly regulate their activity. The putative BON1-Ca<sup>2+</sup>-ATPase interaction caught our attention and pushed us to investigate if the developmental defects of bon1 can be ascribed to impaired regulation of Ca<sup>2+</sup>-ATPase activity, hence, in the regulation of cytosolic Ca<sup>2+</sup> homeostasis. To do so, we are following a multidisciplinary approach which includes phenotypic, molecular, biochemical and Ca<sup>2+</sup> imaging analyses. Confocal microscopy analyses have been used in combination with quantitative RT-PCR to study the expression pattern of BON1 and its potential interactor ACA8 throughout the plant development. Our results highlight that BON1 and ACA8 are co-expressed in the shoot and root apical meristem as well as in the inflorescence meristem and in guard cells. In order to study in planta the dynamics of BON1-ACA8 interaction, FRET-FLIM analyses are ongoing. bon1 and aca8 mutants expressing the ratiometric Cameleon NES-YC3.6 have been obtained, and the analyses of cytosolic Ca<sup>2+</sup> transients in the tissues where the two proteins are co-expressed reveals that Ca<sup>2+</sup> dynamics in response to extracellular ATP administration are altered. To elucidate the relevance of the BON1/ACA8 interaction, we examined its effect on ACA8 activity. Preliminary results show that the hydrolytic activity of ACA8 was significantly stimulated by BON1 in a concentration-dependent manner demonstrating that BON1 regulates ACA8 by activating the pump.

## **NUTRITIONAL QUALITY OF TOMATO FRUITS GROWN UNDER DIFFERENT AGRONOMIC CONDITIONS**

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The increasing use of alternative agronomic strategies that rely on organically grown foods has renewed interest in the relationship between agricultural methods and food quality. This study aims to evaluate whether the use of pesticides in conventional agriculture can alter the nutritional and organoleptic properties of food products with possible repercussions on human health.

For this purpose, a comparison of the nutritional quality of organic and conventional crops was performed in tomato fruits. At the ripe fruit development stage, commercial tomatoes cultivated and commercialized in Italy have been analyzed for their content of lycopene, major hydrophilic antioxidant compounds, total phenolic compounds, and antioxidant capacity. The concentrations of lycopene and total phenolic compounds, as well as the antioxidant activity, is significantly different among organic and conventional tomatoes, while ascorbate and glutathione content, as well as their relative redox state, does not change in relation to the used agronomical strategy. Further activities are required to assess if the application of pesticides have the potential to alter the nutrient quality, taste, and overall plant health of edible crops as a consequence of eventual disruptions in biochemical pathways associated with fruit development and ripening.

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# **ABSTRACTS**

## **Session 4**

### **FROM SEED TO SEED: PLANT PHYSIOLOGY AND DEVELOPMENT**

## WHAT'S NEW IN ARABIDOPSIS THALIANA NITROSYLATION CATABOLISM: AKR (ALDO-KETO REDUCTASE) ARE CAPABLE TO CATALYZE THE REDUCTION OF NITROSILATED SPECIES

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Nitric oxide (NO) is a gaseous signaling molecule that is involved in the control of multiple plant physiological and cellular processes. In cells, the main NO reservoir comes from S-nitrosoglutathione (GSNO), which is catabolized by S-nitrosoglutathione (GSNOR) in an NADH-dependent manner.

To identify new possible enzymes involved in nitrosothiol metabolism, we performed a proteomic analysis examining changes in the total leaf proteome of an *Arabidopsis thaliana* GSNOR-null mutant (hot5-2/gsnor1-3). We identified a significant increase in proteins that belong to the aldo-keto reductase (AKR) protein superfamily, namely AKR4C8 and 9. We expressed and purified four members of *A. thaliana* AKR subfamily 4C (AKR4C8, 9, 10, and 11) and determined that they exhibit NADPH-dependent activity with regards to GSNO and S-nitroso-coenzyme A (SNO-CoA), a metabolite ignored by GSNOR.

Through phylogenetic analysis of *Arabidopsis* proteome, other AKRs have been identified as possible GSNO-degrading enzymes, namely AtAKR4C18, AtAKR4B9, and AtAKR2A4. At present, related AKRs have been expressed and purified and preliminary experiments revealed that they can variably catalyze the degradation of GSNO using NADPH as source of reducing equivalents. Intriguingly, structural comparison shows that the related AKRs possess little homology with AtAKR4Cs and retain few crucial features for nitrosothiol catabolism.

Taken together, these results provide relevant insights into functional properties of AtAKRs related to GSNO and other nitrosothiols, opening the way to search for previously ignored nitrosylation-mediated cell signaling pathways in plants.

## GLUTATHIONYLATION AS DETERMINANT OF STOMATA OPENING AND STARCH DEGRADATION IN GUARD CELLS

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Starch is mainly known as temporary carbon storage in leaves and as long-term storage in source tissues. Beyond the storage function, in the last 10 years starch acquired a clear role in stomata movement. In guard cells, several phenomena including starch degradation direct stomata opening. Molecular players in guard cell starch degradation have been described and the central role of  $\alpha$ -amylase 1 (BAM1) in *A. thaliana* has emerged. The action of BAM1 is completed by  $\gamma$ -amylase 3 (AMY3). Together these enzymes represent the main starch degrading enzymes in guard cells. Both are known to be uniquely activated by thioredoxins (TRX) reduction and so potentially active in the light thanks to photosynthetic electron transport. Redox regulation of BAM1 and AMY3 was further explored and found that both are target of glutathionylation. Glutathione is the main low molecular weight thiol in plant cells and contributes to redox buffering. Moreover, glutathione can react with protein thiols (i.e. cysteine) resulting in glutathionylation. Exposure to oxidized glutathione or  $H_2O_2$  and reduced glutathione led to BAM1 and AMY3 inhibition. While rescue of activity relies only on TRX system for BAM1, AMY3 is activated by both TRX and glutaredoxin, underling some biochemical differences in the mechanism of regulation of these two enzymes. The role of glutathionylation in stomatal opening and starch degradation in guard cells has been investigated by analyzing the behavior of mutant plants depleted of chloroplast glutathione reductase (i.e., plant with high levels of oxidized glutathione). Interestingly, phenotype of guard cells was affected similarly to mutant plants lacking BAM1. These data support the role of BAM1 in stomata movement and suggest glutathionylation of specific enzymes as a novel strategy in stomata control.

## CA<sup>2+</sup> LEVELS IN CYTOSOL AND TONOPLAST DURING STOMATAL OPENING

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Mainly localized in leaves epidermis of land plants, stomata regulate the exchange of gases between tissues and the atmosphere. The evapotranspiration in leaves and the simultaneous CO<sub>2</sub> uptake for photosynthesis are guided by stomatal movements. Stomata are formed by a pair of specialized guard cells which, by modifying their turgor pressure, can open and close the pore. The change in the turgor pressure is dependent on several endogenous and exogenous stimuli. As an example, the perception of blue light stimulates the opening of stomata through the activation of the plasma membrane H<sup>+</sup>-ATPase. This activation results in H<sup>+</sup> extrusion and cytosolic alkalinization. In turn, ions and other osmolytes accumulate in the cytosol and vacuole leading to an increased osmotic pressure and consequent water influx.

Whereas the role of Ca<sup>2+</sup> in the regulation of stomatal closure is well established, its role in the control of stomatal aperture is not well defined.

Here we investigated the role of Ca<sup>2+</sup> during stomatal aperture by using *Arabidopsis thaliana* plants expressing genetically encoded sensors in guard cells localized in the cytosol and in the tonoplast microdomain. To reliably induce the stomatal aperture, we used the fungal phytotoxin Fusaric acid which rapidly stabilizes the H<sup>+</sup>-ATPase in its active form and we monitored the Ca<sup>2+</sup> levels in the two compartments.

## THE INVOLVEMENT OF DNA DAMAGE RESPONSE IN PLANT STRESS: A SNAPSHOT INTO THE ARABIDOPSIS TDP1 GENES FUNCTION

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One of the challenges that living organisms face is to respond promptly to genotoxic stress to avoid DNA damage. The signalling cascade of DNA damage response (DDR) represents an evolutionary conserved network responsible for maintaining genome integrity, still scanty investigated in plants. DDR activation relies on ATM and ATR, two protein kinases that activate downstream components responding to double-strand (DSBs) and single-strand breaks (SSBs). Both ATM and ATR signalling converge to the SOG1 effector, controlling the expression of hundreds of genes involved in cell cycle regulation, cell death control, and DNA repair. Recent studies revealed the occurrence of SOG1-independent pathways, which rely on E2F family. DDR downstream pathways include the tyrosyl-DNA phosphodiesterase1 (TDP1) enzyme that hydrolyses the phosphodiester bond between the tyrosine of topoisomerase I (TopI) and 3'-phosphate of DNA. Due to its function, it is involved in different DNA repair pathways. A small TDP1 gene subfamily, composed by TDP1 $\alpha$  and TDP1 $\beta$ , has been identified in plants. While TDP1 $\alpha$  gene has been demonstrated to be involved in DNA repair and genome stability, TDP1 $\beta$  gene has still unknown functions. In this work, a collection of Arabidopsis DDR mutants was investigated in relation to genotoxic agents like camptothecin (CPT, inhibitor of TopI) and NSC120686 (inhibitor of hTDP1). The results indicate that while TDP1 $\alpha$  gene is mainly involved in the repair of DSBs and SSBs, TDP1 $\beta$  function is more related to replication stress. A possible E2FB-dependent regulation of TDP1 $\beta$  could be hypothesized, together with its role in the cell response to replicative stress.

# THE MOLECULAR PHENOTYPING OF ARABIDOPSIS THALIANA MUTANTS SUGGESTS A ROLE FOR CYTOCHROMES b561 IN THE REGULATION OF INTRACELLULAR ASCORBATE REDOX HOMEOSTASIS AND ROS-MEDIATED SIGNALLING

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Acting mainly as an antioxidant or substrate for several enzymes, ascorbate (ASC) is an important molecule for plant cells and can be found at relatively high concentrations in any cellular subcompartment, mainly in the reduced state. However, the enzymes that reduce its oxidized forms monodehydroascorbate (MDHA) reductase and dehydroascorbate (DHA) reductase seem to be absent in acidic cellular subcompartments, such as the apoplast and vacuoles.

Cytochromes b561 (CYB561s) are potential candidates to perform MDHA reduction in acidic compartments. CYB561s constitute a ubiquitous protein family whose members share a highly conserved structure: they are transmembrane proteins that catalyze a bidirectional one-electronic transfer across the membranes in which they are located. Thus, CYB561s can consume ASC on one side of the membrane (i.e. cytoplasmic compartment) to reduce MDHA on the other side (i.e.. vacuolar compartment).

The *Arabidopsis thaliana* genome contains 4 genes encoding CYB561s. Using an ex vivo electrophysiological approach, we recently demonstrated that the isoform A (CYB561-A) is the main ASC-dependent electron transporter across the tonoplast of mesophyll cells.

To better understand the still unclear physiological role of these proteins in vivo, single T-DNA mutant lines were isolated, and a molecular phenotyping study was initiated in both young and flowering plants. Mutants with a lower CYB561-A expression showed phenotypic traits that suggest an alteration in the intracellular ASC redox homeostasis and ROS-mediated signalling (i.e. increase in total ascorbate peroxidase activity, decrease in ROS content, delay of flowering onset). Preliminary results for the mutants lacking the other isoforms (CYB561-B, CYB561-C and CYB561-D) suggest overlapping functions between these proteins.



SI RINGRAZIANO PER IL LORO CONTRIBUTO

**eppendorf**

