

# ABSTRACT BOOK



PLANT BIOLOGY EUROPE **2021**



**28<sup>th</sup> June - 01<sup>st</sup> July 2021**  
Turin, Italy

jointly organized by:



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JOINTLY ORGANIZED BY:

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WITH THE SUPPORT OF:

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# WELCOME TO THE PLANT BIOLOGY EUROPE 2021

**Andrea Schubert**  
**Convener**



Plant biology is a blooming scientific area where novel developments continuously arise, opening new avenues of research and empowering innovation for agriculture and for food and non-food applications. In the frame of a well-established tradition of biennial congresses jointly organized by the Federation of European Societies of Plant Biology (FESPB) and the European Plant Science Organization (EPSO), the Plant Biology Europe (PBE) Congress was originally planned for June 2020 in Turin, but organization was abruptly halted by the Covid pandemic.

However, capitalizing on the subalpine spirit of this city, longtime trained to resilience and understatement, work was continued through the pandemic with the goal of delivering PBE a year later, performing the event from 28 June to 1 July 2021 with help from advanced tools of online conferencing.

PBE2021 focuses on delivering the latest scientific developments in plant biology, following an inclusive approach where long-standing research sectors and novel breakthrough topics are showcased, including aspects of science policy and ethics, and fostering participation of young scientists.

We regret that you will not be with us in Turin, experiencing our historical setting and the Italian way of life, but I am confident you will feel some Italian atmosphere even in the apparently aseptic frame of an online event. I wish to thank all those that contributed to the success of this Congress, in particular the Scientific Organizing Committee, the Local Organizing Committee, the Turin Task Force, and the Congress Secretariat.

I warmly welcome plant scientists from and outside Europe to present their work and results at PBE2021, sharing a vibrant and successful Congress!

**PBE**   
**2021**

## Laura De Gara FESPB Secretary General



Dear colleagues and friends, dear students,  
I am very pleased to welcome you, also on behalf of the other members of the Executive committee of the Federation of European Societies of Plant Biology, to the 2021 Plant Europe Congress.

As many of you probably know, in the last year, the FESPB renewed its Executive Committee with the election of new members: Christian Zörb as treasurer, Maria Isabel Diaz Rodriguez as Chair of the Awards Committee, Jana Albrechtová as Chair of the Grants committee and János Györgyey as Chair of the Publication Committee (and web media). Also for me, this has been the first year of my mandate as Secretary General.

At the beginning of this congress, I would like to thank the old members of the Executive Committee: Christin Foyer, Heinz Rennenberg and Maria Dolores Rodriguez. Each of them has made a significant contribution to our Federation, really supporting its development with their competences but also with their enthusiasm and generosity. I really thank all of them for their support to the federation and for their friendship during the year in which I had the pleasure to work with them.

As you know, the aims of FESPB are to advance research, education, and the exchange of information amongst plant researchers within Europe and beyond, and to support the publication of the results of research through the affiliated international journals. This international Congress is an example of the activity of exchange and dissemination of scientific knowledge. I hereby would like to thank the President of the FESPB and the Congress Andrea Schubert and all the colleagues involved in the congress organization for their effort (to organize an international congress in a pandemic period is really a big challenge!). I would also like to mention the positive collaboration with EPSO in organizing this congress. The **collaboration** between the two institutions allows the co-organized events to comprise all the aspects of research in plant science, from the fundamental to the very applicative aspects, from the theoretical one to social implications and policies strategies.

Finally, I would like to thank the editors of the affiliated journals for their support, in different ways, to our activities. I hope that the collaboration with the affiliated journals will further increase. I invite the editors participating in the Congress to join the meeting we have organized in these days in order to identify new activity of collaboration.

I would also like to mention the FESPB Council, the key point of FESPB's life. The Council is composed of one delegate for each member scientific society. I really hope that also the collaboration with the delegate will further increase. Their role in giving new ideas, improving the traditional activities and helping the Executive Committee in carrying them out is crucial. I thank the delegates for their last job: the selection of the winners of the grants allowing almost 70 students to participate in the congress, the identification of the evaluation committee for the selection of the two FESPB Awards and the selection of the best posters that will be shown by students during the congress.

Another issue I would like to address and make more known to this research community is our last activity approved by the Council: FESPB annually offers some travel grants to young scientists for spending a short period in other laboratories in Europe. We started this activity just before the lockdown imposed because of the pandemic situation, for this reasons very few students could take advantage of this opportunity, but we are confident that, in a near future, this initiative could help many of our students to improve their scientific expertise and knowledge.

Who is interested in knowing better the initiatives of FESPB can find more information on our website (<https://fespb.org>).

Again, I wish you very interesting scientific days, rich of excellent research and stimulating interaction with other colleagues!



## Alan Schulman

**EPSO President**

Dear colleagues, it is a pleasure to join you as EPSO President in our virtual PBE2021. This pandemic year has been one of big challenges for all of us, both personal and professional. However, the rapid development of vaccines is an example of what science, with the right tools and priorities, is able to deliver in the public service. Likewise, plant science today has the potential to address the primary production side of the many pressing problems in delivering sufficient, sustainable, and secure food while preserving biological diversity in the face of climate change. EPSO has been working hard to extend accurate and cogent advice to policy makers regarding orientation, research areas, and topics in the formation of Horizon Europe. A major EPSO focus, especially of our AgTec Working Group, has been on the current fettered status of NBTs in Europe under the ECJ 2018 decision, which is undermining plant research, development and application. We have been interacting on multiple occasions with those making and implementing policy from Member States within the context of the EC NBT study that was published in April. We have also been very concerned with the ongoing consideration of the position of Digital Sequence Information within the Nagoya Protocol and its implications for R & D. Beyond EPSO's interactions with public servants on policy and EU funding instruments, we have strived to build networks of interactions between scientists. Our Working Groups have been very active in this regard. These WGs, in turn, provide a path for delivering science information and advice to those beyond the walls of research institutions. As widespread adoption of online meeting tools during the pandemic gave EPSO an opportunity to initiate a Zoom-based Plant Science Seminar Series, which is being held on the third Thursday of each month at 3 PM CET. Each has centred on one theme and featured three speakers. Our public outreach through Fascination of Plants Day in May went virtual this year. As we look forward to end of the pandemic and its restrictions and the emergence of a new normal, we welcome all of you to be active EPSO members and participants. We as an organisation and as a discipline are as strong and dynamic as our collective creative energy. I wish you all an inspiring meeting.

## Francesco Loreto

**Chair, PBE2021 Scientific Organizing Committee**



When I accepted chairing the Scientific Organizing Committee of Plant Biology Europe 2021, I was afraid that the task would have been overwhelming. Then the COVID-19 pandemics came in to make our endeavor even more difficult. Defying all incumbent and unfavorable circumstances, and giving one of the best proof of "resilience" I have seen so far, one year after the set congress date, we are finally here with the usual outstanding program. This exceptional result would not have been possible without the strong will and dedication of all members of the SOC, the local organizers, and the funding organizations. They all deserve my deepest acknowledgments for having brought to completion such a "mission impossible". Our work has also highlighted that plant biology is alive and kicking despite the pandemics. If experimental work has often and unavoidably been restrained to comply with the strict regulations set up to combat COVID-19 worldwide, we have seen theoretical, in-silico, and modeling work flourishing with new and important ideas. Minds flew over the pandemics, which gave us an opportunity to rest and think. There is now growing awareness of the multi-faceted and increasingly important role of plants. Plants have created the world as we see it (plant power came first), and plants will help us fight the problems and address the grand challenges of our time: overpopulation and consequent overexploitation of resources, climate change mitigation, and food security attainment among the main ones. We need to better know plants to let them help us with our quests. We are now ready to hear and learn at the PBE 2021 congress the most brilliant new ideas on how plants will drive our society and our planet toward a more sustainable and better future.

# Scientific Organizing Committee

Isabel Bäurle, Potsdam  
Pilar Cubas, Madrid  
Giulia De Lorenzo, Roma  
Robert Hancock, Dundee  
Michel Havaux, Saint-Paul-lez-Durance  
Dirk Inzé, Ghent  
Martin Lascoux, Uppsala  
Antonio Leyva, Madrid  
Francesco Loreto, Rome  
Jos Schippers, Gatersleben  
Kirk Overmyer, Helsinki  
Eva Stöger, Vienna  
Miroslav Strnad, Olomouc  
Paolo Trost, Bologna  
Przemyslaw Wojtaszek, Poznan

## Local Organizing Committee

### **CHAIR**

Andrea Schubert, University of Turin, FESPB

### **VICE-CHAIR**

Aldo Ceriotti, IBBA-CNR, Milan, EPSO

Roberto Bassi, University of Verona, FESPB  
Maria Concetta De Pinto, University of Bari, FESPB  
Giulia De Lorenzo, Sapienza University of Rome, FESPB  
Stefania Grillo, IBBR-CNR, Naples, EPSO  
Fiorella Lo Schiavo, University of Padua, FESPB  
Massimo Maffei, University of Turin, FESPB  
Pierdomenico Perata, Sant'Anna School of Advanced Studies of Pisa, SIBV  
Francesca Secchi, University of Turin, FESPB  
Chiara Tonelli, University of Milan, EPSO  
Roberto Tuberosa, University of Bologna, EPSO  
Giovanni Vendramin, IBBR-CNR, Florence, EPSO

## Turin Task Force

Andrea Schubert  
Massimo Maffei  
Cinzia Berteà  
Francesca Cardinale  
Claudio Lovisolo  
Francesca Secchi  
Giampiero Vigani





08.00 - 08.30

**Connection time**

08.30 - 09.00

**Opening of the Congress**

Andrea Schubert (Convener)  
Laura De Gara (FESPB Secretary General)  
Alan Schulman (EPSO President)  
Francesco Loreto (Chair, PBE2021 Scientific Organizing Committee)

09.00 - 09.45

**Plenary Lecture Theme 1 - Abiotic stress and plant performance**

Pierdomenico Perata (Pisa, Italy)  
Plants and hypoxia: occurrence, sensing and adaptation  
Chair Kirk Overmyer

09.45 - 10.30

**Plenary Lecture Theme 3 - Plant metabolism and bioactive compounds**

Cathie Martin (Norwich, UK)  
The benefits of a colourful diet  
Chair Robert Hancock

10.30 - 10.50

**Comfort break****Parallel Session 1A - Phenotyping plant performance under abiotic stress**

Chair Kirk Overmyer

10.50 - 11.20

**Keynote lecture**

Francois Tardieu (Bordeaux, France)  
Plant performance under abiotic stress: combining multi scale phenotyping, modelling and genomic prediction

11.20 - 11.40

100 Federico Betti - Arabidopsis ARGONAUTE 1, ARGONAUTE 3, and ARGONAUTE 4 regulate gene expression and hypoxia tolerance.

11.40 - 12.00

189 Sara Cimini - A specific intra-species modulation of redox balancing systems is crucial for the tolerance of Baldo rice cultivar against salt stress

12.00 - 12.20

443 Nadia Bazihizina -The characterisation of ion transport in stalk cells reveals their role in salt sequestration in quinoa epidermal bladder cells

**Parallel Session 2A - Cell signaling in plants**

Chair Pilar Cubas

10.50 - 11.20

**Keynote lecture**

Michel Hothorn (Geneva, Switzerland)  
Plant signal transduction cascades - from atoms to phenotypes and back

11.20 - 11.40

149 Attila Fehér - ROP GTPase-activated kinase signaling in Arabidopsis

11.40 - 12.00

529 Martín Guiomar - Contribution of alternative splicing in plants and animals in response to different types of stimuli

12.00 - 12.20

136 Benoit Menand - A pharmacogenetic approach to decipher the role of the TOR signaling pathway in plant growth and development



12.20 - 12.40

**Parallel Session 5B - Plant evolution and development**

Chair Dirk Inzè

10.50 - 11.20

**Keynote lecture**

Sabine Zachgo (Osnabrück, Germany)

Evolution of plant cell proliferation control: Redox matters

11.20 - 11.40

163 Marco Maccaferri - Durum wheat pan-transcriptome as a bridge to unravel tetraploid and hexaploid wheat gene function and evolution

11.40 - 12.00

252 Gergo Palfalvi - Evolution and development of carnivorous plant leaves

12.00 - 12.20

444 Tomás Werner - Studying the evolution of gene networks through Marchantia polymorpha

12.20 - 12.50

**Plenary FESPB Young Scientist Award lecture 1**

Matouš Glanc

Heads and tails? Cell polarity and cell division in the context of each other

Chair Laura De Gara

12.50 - 14.00

**Lunch break**

14.00 - 14.45

**Plenary Lecture Theme 2****Signaling at cell and plant level**

Anna Stepanova (Raleigh, USA)

Building a SynBio toolbox to monitor and control plant hormone activity

Chair Pilar Cubas

14.45 - 15.30

**Plenary Lecture Theme 4****Plant and ecosystem adaptation to environmental change**

Ülo Niinemets (Tartu, Estonia)

Plant adaptation to environmental change: potentials, limits and feedbacks to global change

Chair Francesco Loreto

**Parallel Session 4B - Plants in extreme environments**

Chair Francesco Loreto

15.30 - 16.00

**Keynote lecture**

Xavier Picò (Sevilla, Spain)

Understanding the ecology and genetics of local adaptation in plants: lessons from natural Arabidopsis thaliana populations along wide environmental gradients

16.00 - 16.20

166 Laura Bertini - Global warming: friend or foe for the survival of the C. quitensis antarctic ecotype?

16.20 - 16.40

436 Payel Bhattacharjee - Exploring differential sensitivity to gamma radiation in plants: A systematic approach using growth studies, histology, and molecular biology tools.

16.40 - 17.00

499 Ilaria Colzi - Do Ni-hyperaccumulators manage the high amount of metal in their shoots without affecting the photosynthetic activity?

**Parallel Session 3 - Plant metabolism and bioactive compounds - part 1**

Chair Robert Hancock



15.30 - 16.00

**Keynote lecture**

Lazaro Pereira Peres (Piracicaba, Brasil)

Crop improvement for healthy diet. Tomato as a model system

16.00 - 16.20

191 Carpaneto Armando - I Tonoplast cytochrome b-561 controls ascorbate homeostasis in Arabidopsis plants exposed to high light

16.20 - 16.40

240 Cohen Hagai - The multifaceted networks regulating suberin metabolism in plants

**Parallel Session 10 - Plant nutrition**

Chair Antonio Leyva

15.30 - 16.00

**Keynote lecture**

Hatem Rouached (East Lansing, USA)

Getting to the root of plant mineral nutrition: system genetics to study how plants make sense of various nutrient signals

16.00 - 16.20

187 Grmay Lilay - F-bZIPs - the Sensors and Molecular Switches for Plant Zinc Acquisition

16.20 - 16.40

547 Valéria Custódio - Uncovering factors that modulate the microbiota assembly in Zea mays

16.40 - 17.00

483 Esther Riemer - ITPK1-dependent generation of inositol pyrophosphates is required for systemic regulation of phosphorus homeostasis

28<sup>th</sup> June

08.30 - 09.00

**Connection time**

09.00 - 09.45

**Plenary Lecture - Theme 5 - Plant development and flowering**

Miltos Tsiantis (Köln, Germany)

The genetic basis for diversification of leaf form: from understanding to reconstructing

Chair Jos Schippers

09.45 - 10.30

**Plenary Lecture - Theme 6 - Protein modifications and trafficking**

Eugenia Russinova (Gent, Belgium)

Cell biology meets development: endocytic regulation of signalling pathways in plants

Chair Eva Stöger

10.30 - 11.00

**Plenary EPSO Young Plant Scientist Award on applied science**

Ann-Katrin Beuel (Aachen, Germany)

LEDitGROW - Lighting Systems to Optimize the Secondary Metabolite Content of Plant Cell Cultures Chair Ernst van den Ende

11.00 - 11.20

**Comfort break****Parallel Session 6A - Trafficking and transport in plant cells**

Chair Przemyslaw Wojaszek

11.20 - 11.50

**Keynote lecture**

Viktor Zarsky (Prague, Czech Republic)

Exocyst complex functions in plant secretory pathway

11.50 - 12.10

63 Mathieu Bruggeman - Functional Interactions of Nuclear RNase P in Arabidopsis

12.10 - 12.30

133 Mirko Zaffagnini - Glutathionylation of plant glyceraldehyde-3-phosphate dehydrogenase triggers late and irreversible collapse into insoluble aggregates

12.30 - 12.50

426 Agata Cieřła - The post-translational modifications are crucial for Arabidopsis type III ACC synthases regulation

**Parallel Session 12B - Translating plant research from lab to field**

Chair Eva Stöger

11.20 - 11.50

**Keynote lecture**

Hilde Nelissen (Gent, Belgium)

Translating plant organ growth from model to crop

11.50 - 12.10

115 Martina Huber - Rice growing in high density: Elucidating the genetic networks of weed-competitive rice architectures

12.10 - 12.30

427 Joseph Swift - Single-nuclei sequencing reveals drought's impact on hormone activity and leaf development

12.30 - 12.50

457 Shimizu Kentaro - The pattern of genome-wide polymorphisms in natural and crop polyploid species

**Parallel Session 5A - Plant development and flowering**

Chair Jos Schippers

11.20 - 11.50

**Keynote lecture**

Kerstin Kaufmann (Berlin, Germany)

Mechanisms underlying cellular differentiation in flowers

- 11.50 - 12.10 80 Alexis Porcher - Light-dependent H<sub>2</sub>O<sub>2</sub> scavenging as a critical process in the photo control of axillary bud outgrowth?
- 12.10 - 12.30 429 Alice Pajoro - The anti-florigen TERMINAL FLOWER 1 orchestrate plant architecture coordinating floral transition and vascular bundle development
- 12.30 - 13.30 **Lunch break**
- 13.30 - 14.15 **Plenary lecture - Theme 7 - Molecular and cellular organization of the photosynthetic system**  
Roberta Croce (Amsterdam, The Netherlands)  
Harvesting the sun...safely and efficiently  
Chair Michel Havaux
- 14.15 - 15.00 **Plenary lecture - Theme 12 - Genomics and genome editing for crop design**  
Zachary Lippman (Long Island, USA)  
Revealing cis-regulatory complexity and the principles of quantitative trait variation  
Chair Dirk Inzé
- Parallel Session 7A - Molecular and cellular organization of the photosynthetic system**  
Chair Paolo Trost
- 15.00 - 15.30 **Keynote lecture**  
Donald Ort (Urbana, USA)  
Improving photosynthetic efficiency for increased yield
- 15.30 - 15.50 17 Cristina Pagliano - Molecular determinants of grana stacking in plant thylakoid membranes
- 15.50 - 16.10 111 Radoslaw Mazur - The influence of LHClI complex composition on the grana structural regularity in Arabidopsis thaliana plants
- 16.10 - 16.30 181 Pedro Carvalho - Novel function for an iron regulator: OsbHLH60 activates a C4 PEPC1 promoter
- Parallel Session 12A - Genomics and genome editing for crop design**  
Chair Dirk Inzé
- 15.00 - 15.15 **Samplix Company talk**  
Peter Mouritzen - Xdrop®: targeted enrichment to overcome challenges in long- and short-read sequencing of large and complex plant genomes
- 15.15 - 15.40 **Keynote lecture**  
Catherine Feuillet (Cambridge, USA)  
Seeds for a changing planet
- 15.40 - 16.00 247 Marie Pfeiffer - Overcoming genetic redundancy and lethality: Novel CRISPR tools for plant loss-of-function studies
- 16.00 - 16.20 72 Andrea Moglia - CRISPR/Cas9-Based Mutagenesis of PPO genes in eggplant for the improvement of the berry quality
- 16.20 - 16.40 279 Enikő Lörincz-Besenyi - Developing a viral based genome editing tool for plant editing

## Parallel Session 1C - Stress resilience in horticultural and fruit crops

Chair Isabel Bäurle

15.00 - 15.30

### Keynote lecture

Francesca Cardinale (Turin, Italy)

A tale of plant hormones: how strigolactones cross-talk with ABA to set drought responses in tomato

15.30 - 15.50

539 Andrea Schrader - Characteristics of a salt stress resilient transcriptome - splice variants in tomato roots

15.00 - 17.00

### The Global Plant Council (GPC) Science Communication workshop

Michele Catanzaro, Isabel Mendoza

An interactive, hands-on workshop to provide tools for communicating research based on presentation and discussion of selected press releases.

17.00 - 18.30

### GPC-EPSO panel discussion on access to genetic resources

Roslyn Gleadow, Jens Sundström: Introduction

Jane Anderson: What we are talking about

Nils Stein: A researcher's perspective

KC Bansal: Potential conflicts related to DSI

Amber Hartman Scholz: What to do now



29<sup>th</sup> June

08.30 - 09.00

**Connection time**

09.00 - 09.45

**Plenary lecture - Theme 8 - Carbon fixation and plant productivity**

Spencer Whitney (Canberra, Australia)  
Improving RuBisCO function and plant growth  
Chair Francesco Loreto

09.45 - 10.30

**Plenary lecture - Theme 9 - From plant defence to plant immunity**

Roberto Solano (Madrid, Spain)  
Evolution of jasmonates in land plants and their role in thermotolerance  
Chair Giulia De Lorenzo

10.30 - 10.50

**Comfort break****Parallel Session 6B - Seeds of tomorrow**

Chair Eva Stöger

10.50 - 11.20

**Keynote lecture**

Alessandro Vitale (Milan, Italy)  
How to make a protein body: a cell biologist's evolutionary view

11.20 - 11.40

402 Davide Gerna - Seed physical state critically affects the influence of oxygen on seed deterioration.

11.40 - 12.00

106 Rocío Soledad Tognacca - A novel role for athb2/hat4 as a regulator of germination in *Arabidopsis thaliana* seeds

12.00 - 12.20

535 Elsa Arcalis - Multiscale imaging reveals novel trafficking routes and novel roles for the storage vacuole in maize endosperm

**Parallel Session 9B - Priming and memory of stress - from model to crop**

Chair Miroslav Strnad

10.50 - 11.20

**Keynote lecture**

José Gutierrez Marcos (Warwick, UK)  
Molecular changes induced by stress and developmental reprogramming in plants

11.20 - 11.40

48 Simone Ferrari - The *Arabidopsis thaliana* LysM-containing receptor-like kinase AtLYK2 is required for elicitor-induced priming of defenses against fungal infection independently of chitin perception

11.40 - 12.00

222 Ivan Visentin - From tomato to *Arabidopsis* and back: role of strigolactones in the stomatal memory of drought stress

**Parallel Session 7B - Chloroplast biology**

Chair Michel Havaux

10.50 - 11.20

**Keynote lecture**

Chanhong Kim (Shanghai, China)  
From singlet oxygen signalling to chloroplast protein import pathways:  
An unforeseen adventure

11.20 - 11.40

105 Stefano D'Alessandro - Detox them all! Promiscuous detoxification contributes to plant stress tolerance

11.40 - 12.00

477 Matteo Ballottari - LPA2 protein is involved in Photosystem II assembly in *Chlamydomonas reinhardtii*

- 12.00 - 12.20 486 Luca Tadini - GUN1 promotes the accumulation of NEP-dependent transcripts and chloroplast protein import upon perturbation of plastid protein homeostasis in Arabidopsis cotyledons
- 12.20 - 13.00 **Lunch Break**
- 13.00 - 14.30 **EPSO Plenary Science Policy Session - Contributions of plant science to the European Green Deal and the UN Sustainable Development Goals (SDGs) and the role of the Horizon Europe R&I Programme**  
Opening speaker:  
Dorothee André, Head Plant Health, European Commission, DG SANTE  
Brussels, Belgium  
Panel experts:  
Jingyuan Xia, Director of Plant Production and Protection Division, FAO Rome, Italy  
Raffaele Maiorano Chair, FAO Global Forum on Agricultural Research and Innovation (GFAR), Rome, Italy  
Ulrich Schurr, EPSO Vice-President, Jülich, Germany  
Alan Schulman, EPSO President, Helsinki, Finland  
Massimiliano Giansanti, President Confagricoltura Farmers Association, Rome, Italy
- Parallel Session 8 - Carbon fixation and plant productivity - part 1**  
Chair Michel Havaux
- 14.30 - 15.00 **Keynote lecture**  
Tomas Morosinotto (Padova, Italy)  
Lessons from evolution to improve photosynthetic productivity
- 15.00 - 15.20 93 Stefan Schillberg - Two strategies for promoting carbon fixation in plants to increase biomass production
- 15.20 - 15.40 281 Nelson Saibo - Unveiling the regulatory networks underlying C4 photosynthesis
- Parallel Session 9A - From plant defence to plant immunity**  
Chair Andrea Schubert
- 14.30 - 15.00 **Keynote lecture**  
Giulia De Lorenzo (Roma)  
Plant cell-wall derived DAMPs in immunity and development
- 15.00 - 15.20 220 Tetiana Kalachova - Disrupted actin cytoskeleton : a switch from immunity to senescence
- 15.20 - 15.40 64 Lucia Marti - Orchestration of the oxidative burst in elicitor-induced immunity requires the multiple organelle-targeted Arabidopsis NPK1-related protein kinases (ANPs)
- 15.40 - 16.00 263 Roslyn Gleadow - The end of the trade wars: a new paradigm in plant defence theory
- Parallel Session 1B - Plant adaptation to climate change stress**  
Chair Przemyslaw Wojaszek
- 14.30 - 15.00 **Keynote lecture**  
Julio Salinas (Madrid, Spain)  
Unveiling a new plant molecule involved in tolerance to abiotic stress
- 15.00 - 15.20 481 Melo Fredilson - Functional characterization of drought-responsive RING E3-Ubiquitin ligases in rice



15.20 - 15.40

498 Anna Johanna - Wiese Arabidopsis bZIP18 and bZIP52 accumulate in nuclei following heat stress where they regulate the expression of a similar set of genes.

15.40 - 16.20

**Comfort break**

16.20 - 16.50

**Plenary EPSO Young Plant Scientist Award on Fundamental Science**

Apolonio Huerta

Resistance to Fusarium oxysporum 1: A novel cell wall Integrity sensor required for resistance against F. oxysporum

Chair Alan Schulman

17.00 - 18.30

**Plenary ERC Session - The ERC Funding For Frontier Research In Plant Science**

Dirk Inzè, Miina Rautiainen, Jean-Luc Khalfaoui, Alessandra Ferrari



30<sup>th</sup> June



08.30 - 09.00

## Connection time

09.00 - 09.45

## Plenary lecture - Theme 10 - Plant nutrition and beneficial interactions

Caroline Gutjahr (Munich, Germany)

Arbuscular mycorrhiza development and function

Chair Antonio Leyva

09.45 - 10.30

## Plenary lecture - Theme 11 - Plant epigenetics

Vincent Colot (Paris, France)

Transposable element mobilization in *Arabidopsis thaliana*: highly deleterious yet a major force of local adaptation

Chair Isabel Bäurle

10.30 - 10.50

## Comfort break

## Parallel Session 4A - Plant ecosystems under environmental change

Chair Martin Lascoux

10.50 - 11.20

## Keynote lecture

Giorgio Matteucci (Firenze, Italy)

Plant ecosystems and environmental change: extreme events, resilience and adaptation mechanisms in nature

11.20 - 11.40

129 Fanny Petibon - Leaf pigmentomics - a new approach for better understanding of seasonal pigment dynamics?

## Parallel Session 11 - Plant epigenetics - part 1

Chair Isabel Bäurle

10.50 - 11.20

## Keynote lecture

Franziska Turck (Köln, Germany)

Pinpointing regulation in epigenetic gene regulation

11.20 - 11.40

15 Stéphane Maury - Evolutionary and functional impact of epigenetic variations in forest trees facing climate change

11.40 - 12.00

175 Alba Rodriguez Diez - Loss of function of an *Arabidopsis* ortholog of the mammalian MRG15 adaptor protein connecting splicing to chromatin leads to defective abscisic acid signalling

## Parallel Session 2B - Long-distance messages in plants

Chair Miroslav Strnad

10.50 - 11.20

## Keynote lecture

Sabrina Sabatini (Rome, Italy)

Developmental boundaries: choosing between division and differentiation

11.20 - 11.40

113 Rana Surbhi - Local and systemic effects of brassinosteroid perception in developing phloem

11.40 - 12.00

52 Giovanna Frugis - Dissecting the cytokinin genetic pathway and the main gene regulatory networks in *Cichorium endivia* leaves: fundamental biology in leafy crops

12.00 - 12.20

182 Julia Santiago - Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth

**Parallel Session 3 - Plant metabolism and bioactive compounds - part 2**

Chair Paolo Trost

- 10.50 - 11.10 22 Raimund Tenhaken Why are some sugars toxic to plants?
- 11.10 - 11.30 213 Richard Macknight - Vitamin C biofortification: Overcoming the complexity of metabolic pathway regulation
- 11.30 - 11.50 527 Lena Hunt - Localization of phenolic compounds in barley leaves can be modulated by irradiance and CO<sub>2</sub>

**12.20 - 12.50 Plenary FESPB Young Scientist Award 2**

Sara Izquierdo Zandalinas

ROS-mediated systemic signalling during acclimation to environmental stress conditions

Chair Isabel Diaz-Rodriguez

**12.50 - 14.00 Lunch break****Parallel Session 10B - The plant microbiome and new strategies for biofertilization**

Chair Antonio Leyva

- 14.10 - 14.30 469 Guido Domingo - Proteomic analysis reveals how pairing of a Mycorrhizal Fungus with Plant Growth-Promoting Bacteria modulates growth and defense in wheat
- 14.30 - 14.50 531 Bradley Dotson - Breeding for Plant-Trichoderma compatibility

**Parallel Session 11A - Plant epigenetics - part 2**

Chair Pilar Cubas

**14.00 - 14.30 Keynote lecture**

Martin Crespi (Paris, France)

Plant non-coding RNAs in chromatin regulation

- 14.30 - 14.50 471 Francesca Lopez - Gene dosage compensation of rRNA transcript levels in Arabidopsis thaliana lines with reduced ribosomal gene copy number

**Parallel Session 8 - Carbon fixation and plant productivity - part 2**

Chair Francesco Loreto

**14.00 - 14.30 Keynote lecture**

Alexandra Baekelandt (Gent, Belgium)

CropBooster-P: a roadmap for future European plant research

- 14.30 - 14.50 532 Sina Schultes - Linking root carbon partitioning to inter-kingdom microbial variation in the maize rhizosphere
- 14.50 - 15.10 410 Nicole Salvatori - Dynamic photosynthesis in two soybean varieties: short and long term acclimation to fluctuating light conditions

**15.10 - 16.00 Closing of the Congress**

# Extended Elevator Pitches

(10 minutes, available on the Congress platform throughout the Congress)



To this aim, the Congress will present 12 Plenary lectures each focusing on a theme, and parallel sessions that will explore different angles of the theme itself. Besides keynote speakers, the SOC selected among the more than 500 contributions sent to the Congress oral communications to complete each session. However, as the number of very well rated abstracts was way higher than the available slots, the SOC also selected a number of them to be presented as 10 min EEP that will be available on demand during the Congress.

## Topic 1A - Phenotyping plant performance under abiotic stress

- 406 **Roel Lammerant** - Impact of experimental soil moisture manipulation on tropical tree seedling demographic fates and functional traits
- 424 **Cristian Mateo** - Arsenite provides a selective signal that coordinates arsenate uptake and detoxification in Arabidopsis
- 439 **Sivakumar Krishnamoorthy** - Identification of downstream targets of MAPKKK17/18 cascade in Arabidopsis
- 447 **Micaela Andrea Navarro Correa** - Regulatory interplay between root developmental programs and the arsenic response in Arabidopsis
- 514 **Maria Fitzner** - Influence of salinity and different light regimes on growth and metabolite profiles of halophytes
- 517 **Shaaban Basel** - Molecular analysis of abiotic stress response in different Capsicum genotypes
- 525 **Yuri Luca Negroni** - The fundamental role of plant mitochondria in stress responses is linked to the functionality of the mitochondrial nucleoid binding protein WHIRLY2
- 526 **Ralf Metzner** - In vivo imaging and quantification of carbon tracer dynamics in nodulated root systems of pea plants
- 540 **Andrej Frolov** - Does glycation of plant proteins impact on ageing and response to environmental stress?

## Topic 1C - Stress resilience in horticultural and fruit crops

- 58 **Matteo Chialva** - Comparative transcriptomics between Solanum lycopersicum and S. pennellii sheds light into adaptation to arbuscular mycorrhizal symbiosis and combined stress resilience
- 539 **Andrea Schrader** - Contrasting responses of two grapevine cultivar with different hydraulic behaviour to drought: the role of non-structural carbohydrates in xylem embolism

## Topic 2A - Cell signaling in plants

- 415 **Monika Chodasiewicz** - Stress Granules as novel mechanism for stress signaling
- 445 **Francesca Resentini** - Simultaneous imaging of ER and cytosolic Ca<sup>2+</sup> dynamics reveals long distance ER Ca<sup>2+</sup> waves in plants
- 473 **Catarina Campos** - Involvement of small RNAs in long- and short-term heat stress response in mycorrhizal grapevine plants
- Topic 2B - Long-distance messages in plants
- 463 **Matteo Grenzi** - At the edge of the gate: discerning the GLUTAMATE RECEPTOR-LIKE ligand-binding role in plant systemic responses

## Topic 3A - Plant metabolism and bioactive compounds

- 184 **Katarina Šoln** - Inside of allelopathy of invasive Japanese and Bohemian knotweed: rhizome extracts stimulate programmed cell death and affect cell structure and function in young radish roots
- 452 **Matteo Pivato** - Heterologous expression of cyanobacterial Orange Carotenoid Protein (OCP2) as a soluble carrier of ketocarotenoids in Chlamydomonas reinhardtii
- 524 **Julie Buges** - Metabolic engineering of BAHD acyltransferases involved in the production of an unusual chemical signature in Asteraceae pollen coat

# Extended Elevator Pitches

(10 minutes, available on the Congress platform throughout the Congress)



## Topic 4A - Plant ecosystems under environmental change

- 139 **Cecilia Brunetti** - Widespread holm oak dieback in Mediterranean forests: the roles of carbon stress and hydraulic failure under recurrent drought events  
282 **Norul Sobuj** - Effects of lateral bud removal in growth and phenolics in male and female saplings of *Populus tremula* (L.) under simulated climate change  
285 **Francesca Secchi** - Inhibition of xylem cellular activity blocks recovery from drought induced embolism in poplar - insights from micro-CT analysis  
521 **Oksana Lastochkina** - The role of endogenous salicylic acid in endophytic bacterium *Bacillus subtilis*-mediated drought tolerance in wheat plants

## Session 4B - Plants in extreme environments

- 225 - Chiara Puciariello - Auxin is required for the long coleoptile trait in rice germination under submergence

## Session 5A - Plant development and flowering

- 108 **Francesca Giaume** - A triple florigen system is essential for flowering and panicle architecture in rice  
134 **Maurizio Di Marzo** - SEEDSTICK controls Arabidopsis fruit size by regulating cytokinin levels and FRUITFULL  
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322 **Jose Muino** - Understanding meristem differentiation at single cell level during flower development  
433 **Pedro Barras** - Conserved regulatory networks acting during phellogen development in Arabidopsis and cork oak roots  
464 **Ana Marques** - Functional analysis of AtDRIF genes during the development of Arabidopsis thaliana seedlings  
488 **Luis Andrade** - The evening complex is necessary for rice flowering activation  
522 **Tomáš Takáč** - FSD1: a plastidial, nuclear and cytoplasmic enzyme with antioxidant, osmoprotective and developmental functions

## Session 5B - Plant evolution and development

- 92 **Martin Bayer** - Parental conflict or cell polarity establishment? Mechanistic insight in MAP kinase signaling in the plant embryo

## Session 7A - Molecular and cellular organization of the photosynthetic system

- 476 **Federica Perozeni** - Toward an effective use of microalgae by disentangle LHCSR role on non photochemical quenching (NPQ) in *Chlamydomonas reinhardtii*

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479 **Inês Luis** - A novel threonyl-phosphorylation regulates the activity of maize C4-enzyme phosphoenolpyruvate carboxylase  
544 **James Bunce** - Carboxylation capacity limits photosynthesis at elevated CO<sub>2</sub> throughout diurnal cycles

# Extended Elevator Pitches

(10 minutes, available on the Congress platform throughout the Congress)



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146 **Kriton Kalantidis** - Host factors involved in viroid and satellite RNA infections

173 **Anna Philippova** - Immune signaling peptides in early land plants

413 **Erika Sabella** - Xylella fastidiosa in olive tree: physiological evidences correlated to the resistance

419 **Gaia Salvatore Falconieri** - GLYI4: a potential key hub of primary metabolism and hormone signaling pathway in Arabidopsis thaliana

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441 **Valentino Casolo** - Understanding the response to water stress in poplars: can the balance between non-structural carbohydrates metabolism and growth prime recovery after stress relief?

461 **Moira Giovannoni** - The Plasma Membrane-Associated  $\text{Ca}^{2+}$ -binding protein PCaP1 is required for oligogalacturonide and flagellin-induced priming and immunity

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458 **Francisca Reis** - Cork oak forests plant growth promoting rhizobacteria (PGPR): key partners to prevent drought stress

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## Plenary Lecture

**Transposable element mobilization in *Arabidopsis thaliana*: highly deleterious yet a major force of local adaptation**

**Vincent Colot**

**Institut de Biologie de l'Ecole normale supérieure (IBENS), Paris, France**

Transposable elements (TEs) are potentially powerful endogenous mutagens. Their mobilization can disrupt genes in many ways and their propagation across the genome provides increased opportunities for chromosomal rearrangements, notably through ectopic recombination. Comparative genomics has confirmed that TE sequences are not only the main component of many eukaryotic genomes, including those of mammals and most plants, but also major contributors to the evolution of genome structure and function across species. Yet, we still know little about TE mobilization and its impact within species. Here, I will report the results of our recent study of natural transposition in *Arabidopsis thaliana* (Baduel et al, Genome Biology, 2021), where we found that TE insertions occur at rates of the same order of magnitude as single base substitutions (SNPs), but with much more dramatic consequences. Indeed, while most SNPs are neutral and tend to accumulate over time, over 99% of TE insertions are purged by natural selection within a few thousand years because of their strong and mainly deleterious effects on gene expression. Nonetheless, we find that higher transposition rates are favored at the borders of the species range, due to a combination of environmental and genetic factors that influence the epigenetic control of TEs. Moreover, we find that environmentally-responsive genes, including the main gene controlling flowering time, *FLC*, are recurrent TE targets in nature. I will discuss the implications of these and other findings, notably in light of the increasing need for species to rely on new mutations for their rapid adaption to climate change.



## Plenary Lecture

### Harvesting the sun.....safely and efficiently

**Roberta Croce** <sup>(1)</sup>

**Vrije Universiteit Amsterdam, Faculty of Science, Amsterdam, Netherlands** <sup>(1)</sup>

Photosynthesis sustains nearly all life on earth, but the simultaneous presence of excitation energy and molecular oxygen in the membrane makes it a hazardous business. If excitation energy is not immediately used for charge separation and the subsequent enzymatic reactions, it can lead to the formation of reactive oxygen species that damage the photosynthetic apparatus. Over-excitation happens quite often: oxygenic organisms are exposed to (drastic) changes in environmental conditions (light intensity, light quality and temperature), which influence the physical (light-harvesting) and chemical (enzymatic reactions) parts of the photosynthetic process to a different extent, leading to severe imbalances. To avoid photodamage while keeping the process working efficiently, plants regulate the amount of excitation energy in the photosynthetic membrane by switching from a light-harvesting state, in which most of the photons absorbed are used for photosynthesis, to a quenched state, in which the excess absorbed energy is dissipated as heat. How does it happen? In this presentation, I will discuss our recent *in silico*, *in vitro* and *in vivo* data focusing on the location and mechanism of quenching in plants.

## Plenary Lecture

### Arbuscular mycorrhiza development and function

**Caroline Gutjahr** <sup>(1)</sup>

**Technical University of Munich (TUM), Plant Genetics, Freising, Germany** <sup>(1)</sup>

Arbuscular mycorrhiza (AM) is an ancient symbiosis between plants and glomeromycotan fungi that is extremely widespread in the plant kingdom and is based on nutritional benefits to both symbiotic partners. The plant receives mineral nutrients, especially phosphate from the fungus and in return provides the fungus with carbohydrates and lipids. As a result, AM can significantly increase plant growth in low nutrient soils. For symbiosis establishment AM fungi colonize the root interior. This involves distinct and genetically separable developmental steps that are largely under plant control. They include drastic plant cell rearrangements that precede differentiation of fungal hyphae into particular shapes inside plant cells. We are interested in the plant molecular mechanisms required for initiation of AM symbiosis and for the rearrangement of root cortex cells allowing formation of highly-branched fungal arbuscules, which mediate nutrient exchange. In addition, we work towards elucidating the genetic determinants of AM-mediated plant growth increase. Recent progress in our understanding of AM development and function will be presented.

## Plenary Lecture

### **Tuning Genes, Genomes, and Traits in Plants and Agriculture**

**Zachary Lippman**

**Howard Hughes Medical Institute, Cold Spring Harbor Laboratory**

Genome editing is being lauded as a revolutionary technology that will invigorate plant breeding. However, crop domestication and improvement is founded on genetic complexity that goes well beyond single gene mutations with dramatic phenotypic effects, which have dominated plant genome editing thus far. To advance to the next stage, for both agricultural application and to address fundamental questions, we are dissecting the mechanisms and principles underlying quantitative trait variation. Our early focus was on epistasis, which revealed that interacting genes are highly dose-sensitive systems that can be exploited to create continuums of quantitative variation. More recently we have applied genome editing to investigate the genetic architecture of cis-regulatory regions that control transcriptional and phenotypic outputs from key developmental genes. We have found that gene promoters are also highly dose-sensitive and can serve as “tunable” transcriptional control regions, which can be manipulated to create novel alleles and quantitative variation that goes beyond what nature has provided. Open chromatin and evolutionary conserved non-coding sequences in these promoters may predict functionally important cis-regulatory regions, but hidden epistasis and tissue-specific regulatory elements exist and can be resolved empirically. I will present several case studies that open the debate on whether “rules” can be established to achieve predictable outcomes from genome editing.

## Plenary Lecture

### **The benefits of a colourful diet**

**Professor Cathie Martin MBE, FRS,**

**John Innes Centre, Norwich Research Park, Norwich, UK**

The appreciation of the challenges of achieving global food security has matured to include nutritional security, as scientists have realised that not only calorie content but also food composition impact our health and well-being, dramatically. The ways that the nutrients we consume affect our health are highly complex due to the diversity of what we eat, the varying digestibility of what we eat, the changing composition and functioning of each individual's gut microbiota, the differences in absorption and bioavailability of the nutrients we eat, the differences in responses between individuals to what they eat and the multi-fold mechanisms of action that nutrients have on our health. However, one generic conclusion is possible: it has been accepted for more than 50 years that diets rich in plants, particularly fruit and vegetables, protect health. Yet over this same period diets have declined, with lower fruit and vegetable content replaced by more cheap, sugary, oily processed foods. These dietary shifts are having a marked impact on the incidence of chronic diseases; obesity, metabolic diseases, type2 diabetes and cardiovascular diseases.

By understanding which nutrients from plants confer greatest benefits and how they protect against specific diseases (a process termed comparative nutrition) we hope to achieve dietary improvements at all levels in society. I hope to illustrate the potential of dietary improvement using plant-based foods to improve our health and quality of life and to reduce the economic burden on our health-care systems.

## Plenary Lecture

### **Plant adaptation to environmental change: potentials, limits and feedbacks to global change**

**Ülo Niinemets** <sup>(1)</sup>

**Chair of Crop Science and Plant Biology, Estonian University of Life Sciences, Tartu, Estonia** <sup>(1)</sup>

Global change involves simultaneous alteration of multiple environmental drivers, creating novel environments and increasing the frequency and severity of stress episodes. Plant adaptation to environmental alterations improves plant performance under changed environmental conditions, but there is a large variation in the rate of adaptation and overall adaptability among different species and in dependence on the rate and severity of environmental change. Furthermore, plants can themselves alter their environment via production of volatile organic compounds, emissions of which are particularly strongly enhanced under different abiotic and biotic stresses. Understanding the factors controlling plant adaptation capacity to environmental perturbations is of utmost importance for predicting future changes in vegetation productivity, and release of biogenic volatile compounds and vegetation role in global biosphere-atmosphere interactions.

## Plenary Lecture

**Plants and hypoxia: occurrence, sensing and adaptation**

**Pierdomenico Perata**

**Sant'Anna School of Advanced Studies, Pisa, Italy**

Plant life is greatly impaired under conditions of oxygen deficit. When the supply of oxygen is hampered, a variety of acclimation responses is activated to reduce detrimental effects of energy depletion. The most recent discoveries in the field of oxygen sensing will be presented.



## Plenary Lecture

### Endocytic regulation of signaling in plants

**Eugenia Russinova** <sup>(1)</sup>

**Center for Plant Systems Biology, VIB-UGent, Gent, Belgium** <sup>(1)</sup>

Plants deploy numerous plasma membrane receptors to sense and rapidly react to environmental changes. Correct localization and adequate protein levels of the cell-surface receptors are critical for signaling. Receptor-mediated endocytosis is an integral part of signal transduction. On the one hand, endocytosis attenuates signaling by removal of activated receptors and their bound ligands from the cell surface. On the other hand, receptor internalization allows the spatial and temporal regulation of the signaling outputs from the endosomes. Crucial for understanding the interplay between endocytosis and signaling of plant receptor kinases is the development of imaging tools to visualize active ligand-bound receptor at a high spatiotemporal resolution. We visualized endocytosis of different receptor kinases in living *Arabidopsis thaliana* cells using fluorescent small-molecule and peptide ligands. The bioactive fluorescent probes together with genetic, biochemical and pharmacological analyses revealed distinct dynamics of endocytosis and differences in regulation of signaling outputs. In my talk, I will summarize the up-to-date knowledge of receptor complex endocytosis and its effect on the signaling outcome, in the context of plant development and immunity.

## Plenary Lecture

### Evolution of jasmonates in land plants and their role in thermotolerance

**Roberto Solano** <sup>(1)</sup>

**CNB-CSIC, Plant Mol Genetics, Madrid, Spain** <sup>(1)</sup>

Jasmonate-Isoleucine (JA-Ile) is a fatty acid-derived phytohormone structurally similar to metazoan prostaglandins, and essential for plant defense and development. The SCF<sup>COI1</sup> E3-ubiquitin ligase is the JA-Ile receptor. JA-Ile triggers the interaction of SCF<sup>COI1</sup> with its targets, the JAZ co-receptors, which are repressors of downstream activators of jasmonate responses. This interaction leads to ubiquitination and proteasomal degradation of JAZ repressors.

*A. thaliana* has been an excellent model system in identifying the bioactive hormone and elucidating its signal transduction pathway in eudicots. Nonetheless, information in *A. thaliana* unlikely represents the diversity of this pathway in other plant lineages.

Bryophyte genomes contain conserved sequences for all JA-Ile signaling components, but in contrast to higher plants, lack JA-Ile. I will introduce a new plant model system, the liverwort *Marchantia polymorpha*, and explain its enormous advantages for research in Plant Molecular Biology. I will also discuss that in spite of 450 million years of independent evolution, the JA-Ile co-receptor COI1/JAZ is functionally conserved between the bryophyte *Marchantia polymorpha* and *A. thaliana*. However, this co-receptor perceives and responds to different ligands in each species. Instead of JA-Ile, the ligand of *Marchantia* MpCOI1 is the JA-Ile precursor dinor-OPDA. Our analysis of the biosynthetic pathway for dinor-OPDA uncovered an ancient OPR3-independent pathway for JA biosynthesis that is widely distributed from charophycean algae to eudicots. Moreover, we discovered that dinor-OPDA has a COI1-independent function regulating thermotolerance in all land plants. The evolutionary implications of these discoveries will be discussed during the talk.

## Plenary Lecture

### Building a SynBio toolbox to monitor and control plant hormone activity

**Anna Stepanova** <sup>(1)</sup>

**North Carolina State University, Department of Plant and Microbial Biology, Raleigh, United States** <sup>(1)</sup>

Phytohormones are critical regulators of plant development and environmental responses. In the past three decades, the molecular pathways that govern hormone biosynthesis, signaling, and catabolism have been largely mapped out using a combination of genetics, molecular biology, biochemistry, and cell biology approaches. Despite the major progress, our ability to monitor and precisely control hormone action remains limited. With the development of inexpensive DNA synthesis technologies and the rise of synthetic biology as a new discipline at the intersection of molecular genetics and engineering, new molecular tools can now be built to enable hormone tracking and targeted hormone manipulation. We have generated a synthetic biology toolbox that allows rapid construction of multi-hormone transcriptional reporters. In addition, we are building CRISPR-based logic gate devices to confer novel, highly restricted patterns of expression to any genes of interest using a limited set of available native and synthetic drivers. The latter technology can be employed to tune the expression levels and subtract undesired domains of expression from existing drivers to precisely control output genes of interest, such as hormone biosynthesis, signaling, or catabolism genes, to regulate plant architecture, responses to stress, and other traits of interest. By combining multi-hormone reporters and genetic logic devices, we aim to shed fresh light on the mechanistic role of hormones in orchestrating plant development and stress physiology. That knowledge can then be relied upon to develop resilient next-generation crops.

## Plenary Lecture

### The genetic basis for diversification of leaf form: from understanding to reconstructing

#### Miltos Tsiantis

A key challenge in biology is to understand how diversity in organismal form is generated. While key regulators that shape the body plans of model organisms have been identified, less is known about how the balance of conservation versus divergence of relevant developmental pathways influences cell growth to generate morphological diversity. To help address this issue, we developed the *Arabidopsis thaliana* relative *Cardamine hirsuta* into a versatile system for studying morphological evolution. We use a combination of genetics, advanced imaging and computational modelling to understand the mechanisms through which leaf morphology evolved in these species, resulting in simple leaves in *A. thaliana* and complex leaves with leaflets in *C. hirsuta*. This presentation will discuss our findings on identifying such mechanisms and in conceptualizing how they regulate the number, position and timing of leaflet production. It will also consider progress towards understanding the basis for interspecific variation in *C. hirsuta* leaf form and its physiological significance.

## Plenary Lecture

### Improving Rubisco function and plant growth

Spencer Whitney <sup>(1)</sup>

ARC Centre of Excellence for Translational Photosynthesis, Australian National University, Canberra, Australia <sup>(1)</sup>

Simulations of natural Rubisco kinetic diversity has identified variants beneficial to C<sub>3</sub>-photosynthesis. In this talk I will examine whether this diversity is sufficient to alter plant productivity and discuss the feasibility of directed evolution as a more feasible pathway towards generating the step change in Rubisco performance needed to visibly improve higher rates of leaf photosynthesis and plant growth.

## **FESPB Young Scientist Award lecture 1**

**Heads and tails? Cell polarity and cell division in the context of each other**

**Matouš Glanc**

**Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium**

**VIB Center for Plant Systems Biology, 9052 Ghent**

Eukaryotic cells are polarized. Polarity, or structured and asymmetric distribution of components in space, enables cells to exchange directional signals with each other and the environment, and as such is a key prerequisite for the development and coordinated functioning of complex multicellular organisms. All cells are formed from other cells, either by cell fusion or, much more often, by cell division. Because somatic plant cells cannot migrate within tissues, they must precisely position the division plane according to polarity cues during cell division to ensure correct patterning and morphogenesis. On the other hand, as plant cytokinesis is executed by transient re-routing of most vesicle trafficking routes to the cell plate, the asymmetric subcellular localization of polarity landmarks must be correctly (re)-established in the daughter cells after each cell division event. These features imply that in plants, cell polarity and cell division are very tightly connected and interdependent phenomena. In spite of their developmental importance, the cellular determinants of polarity, within and beyond the context of cell division, are only beginning to emerge. I will discuss the efforts I have been involved in that aim to shed light on this fascinating, yet still enigmatic area at the interface of plant cell and developmental biology.

## FESPB Young Scientist Award lecture 2

### ROS-mediated systemic signalling during acclimation to environmental stress conditions

Sara Izquierdo Zandalinas & Ron Mittler<sup>1</sup>

The Division of Plant Sciences, College of Agriculture, Food and Natural Resources, Christopher S. Bond Life Sciences Center, University of Missouri, 1201 Rollins St, Columbia, MO, 65201

Stress-induced systemic signaling and systemic acquired acclimation (SAA) play a key role in optimizing growth and preventing damages associated with changes in the plant environment. To be effective, SAA has to occur at a fast rate utilizing rapid signaling pathways that transmit signals from affected tissues to all parts of the plant. Each leaf therefore rapidly acclimates to the naturally occurring fluctuations in environmental conditions (e.g., light intensity, temperature and/or mechanical injury), and different leaves of the same plant coordinate their responses with each other via rapid systemic signals. Our studies reveal that the systemic stomatal and transcriptomic responses of plants to excess light, wounding and/or heat stress are dependent on the function of the reactive oxygen species (ROS) wave, and the respiratory burst oxidase homolog D (RBOHD) protein. We further determine that specific plant tissues, such as the phloem, mesophyll and xylem parenchyma tissues are involved in mediating rapid systemic signals. Furthermore, we reveal that different stresses simultaneously impacting the same plant can trigger stress-specific rapid systemic responses, and that the rapid systemic signals generated by the simultaneously occurring stresses are integrated in plants to induce SAA. Plant can therefore mount a rapid and dynamic response to different stresses, even if they occur together within the plant environment. Because global warming and climate change are increasing the number of co-occurring stresses in the plant biosphere, we also begun a study of plant responses to multifactorial stress combinations. Our findings reveal that as the number and complexity of multifactorial stress combination increase, plant growth and survival decrease. This finding should serve as a dire warning to our society! Further polluting our environment could result in even higher complexities of multifactorial stress combinations that in turn would drive a critical decline in plant growth, soil conditions and overall agricultural productivity.

## Plenary EPSO Young Plant Scientist Award on applied science

### **LEDitGROW - Lighting Systems to Optimize the Secondary Metabolite Content of Plant Cell Cultures – AWARD 3**

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I won the EPSO Young Plant Scientist Award (YPSA) 2020 for applied sciences with LEDitGROW:

Plant secondary metabolites (PSM) are important ingredients for the food, cosmetic and pharmaceutical industries. They can be produced in plant cell cultures under sterile conditions, thereby eliminating the risk of contamination with bacterial endotoxins. Moreover there is no need to use herbicides, further contributing to the high and consistent quality of the PSM. Additionally, plant cells can be cultured independent on seasonal effects, location or geopolitical issues and enable easy harvesting and processing.

However, yields and quality strongly depend on the cultivation conditions, including optimal illumination. Light influences photosynthesis, biomass production, plant morphology and the production of PSM. But current shaking incubators do not allow different light wavelengths, intensities and photoperiods to be tested in parallel.

We therefore developed LEDitGROW, a modular lighting system with six different LEDs (red, green, blue, white, farred, UV), allowing the screening of multiple lighting conditions simultaneously. The system is suitable for plants, plant callus cultures (LEDitREST) and plant suspension cultures (LEDitSHAKE). LEDitSHAKE offers the possibility to test up to 12 different lighting conditions within one shaking incubator at the same time, which was not realizable before.

As a proof of principle, we used LEDitSHAKE to optimize anthocyanin production in grapevine cell suspension cultures. We determined the effect of 24 different light compositions on the total anthocyanin content of grapevine cell suspension cultures with a Design of Experiments approach. The optimal lighting conditions for the upregulation and downregulation of 30 anthocyanins were predicted and we found that long-wavelength light (red) decreased the concentration of most anthocyanins, whereas short-wavelength light (blue, UV) had the opposite effect. Based on these results we concluded that LEDitSHAKE is suitable to study the influence of lighting conditions for the optimization of plant cell cultures.



## Plenary EPSO Young Plant Scientist Award on Fundamental Science

**RESISTANCE TO FUSARIUM OXYSPORUM 1: A novel Cell Wall Integrity sensor required for resistance against *F. oxysporum*. -AWARD 4**

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*Fusarium oxysporum* is one of the most important fungal plant pathogens, infecting over 100 different crop varieties. During infection, these fungi squeeze between cells, loosening and deforming plant cell walls – rigid cellular scaffolds made up of cross-linked polysaccharides and proteins, such as cellulose, pectin, lignin and arabinogalactan proteins. For plants to perceive and launch an attack against *F. oxysporum*, they must first be able to detect molecules derived from the fungus (e.g. chitin) and/or molecules released from the plant cell wall during infection. Both strategies employ extra- and intracellular receptors. Although much is known about the perception of fungal derived molecules, little evidence exists which directly links cell wall-derived signals with plant immunity.

Here we aim to characterize the *Arabidopsis* plasma membrane receptor, RESISTANCE TO FUSARIUM OXYSPORUM 1 (RFO1) as a putative cell wall integrity (CWI) sensor acting during plant defense against *F. oxysporum*. Previous work identified RFO1 as a WALL-ASSOCIATED RECEPTOR KINASE-LIKE (WAKL) protein necessary for full resistance to *F. oxysporum* infection. However, the molecular mechanism of RFO1-mediated defense, including what it perceives and how it acts during fungal attack is still unknown. We have confirmed that RFO1 mediates plant responses to genetic and pharmacological modification of pectin at the cell wall. Furthermore, the transiently expressed RFO1 ectodomain strongly localizes to plant cell walls upon plasmolysis. Finally, preliminary bimolecular fluorescence complementation (BIFC), biochemical, structural, and proteomic data provide further evidence that RFO1 acts to detect changes in pectin integrity and shed light on the mechanisms used to activate downstream defense responses during *F. oxysporum* infection. Overall, our data implicate RFO1 as a novel CWI sensor that acts against *F. oxysporum* infection through its perception of changes in pectin structure at the cell wall.

**Keywords:** Cell wall integrity signalling, plant innate immunity, live-cell imaging, pectin, receptor kinase.

## TOPIC:

### Carbon fixation and plant productivity

#### **Keynote Lecture**

##### **Lessons from evolution to improve photosynthetic productivity**

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Life on earth depends on photosynthetic organisms capable of exploiting sunlight to fix carbon dioxide. Natural environmental conditions are highly dynamic and photosynthesis requires continuous modulation to maintain the balance between light availability and metabolic demands. Photosynthetic organisms evolved multiple mechanisms to modulate the flow of excitation energy and electrons according to metabolic constraints and environmental cues.

Even if photosynthesis is finely regulated in all organisms, the molecular mechanisms are generally not conserved and changed during evolution following adaptation to new ecological niches.

Considering the major impact of those regulatory mechanisms have on growth, a deeper understanding of their role opens the possibility of improving biomass productivity in plants and algae.

## TOPIC:

### Carbon fixation and plant productivity

#### Oral Communications

##### **93 - Two strategies for promoting carbon fixation in plants to increase biomass production**

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We have manipulated photorespiration and integrated a CO<sub>2</sub>-concentrating mechanism to significantly increase photosynthetic efficiency and growth in C<sub>3</sub> plants. A photorespiratory bypass has been engineered in potato plants by expressing a polyprotein (DEFp) comprising all three subunits (D, E and F) of *Escherichia coli* glycolate dehydrogenase (GlcDH). Transgenic potato plants accumulated functional DEFp in the plastids, reducing photorespiration and improving CO<sub>2</sub> uptake with a significant impact on carbon metabolism. Transgenic lines with the highest DEFp levels and GlcDH activity produced significantly higher levels of glucose (5.8-fold), fructose (3.8-fold), sucrose (1.6-fold) and transitory starch (threefold), resulting in a substantial increase in shoot and leaf biomass. The higher carbohydrate levels produced in potato leaves were utilized by the sink capacity of the tubers, increasing the tuber yield by 2.3-fold in greenhouse experiments and by 29% in semi-field trials. Comparable phenotypic effects were also observed when tobacco plants overexpressing DEFp were grown under normal and nitrogen-restricted conditions. In addition to the higher shoot and root biomass, the transgenic lines also suffered less chlorosis under nitrogen-restricted conditions.

Alternatively, we increased photosynthetic efficiency and biomass of tobacco plants by expressing individual components of the *Chlamydomonas reinhardtii* carbon concentration mechanism (CCM). Independent transgenic lines accumulating carbonic anhydrases CAH1 or CAH3, or bicarbonate transporters LCIA or LCIB, respectively, showed enhanced CO<sub>2</sub> uptake rates (up to 15%), increased photosystem II efficiency (by up to 18%), and chlorophyll content (up to 19%). Transgenic lines produced more shoot biomass than wild-type and azygous controls, and accumulated more carbohydrate and amino acids, reflecting the higher rate of photosynthetic CO<sub>2</sub> fixation. These data demonstrate that individual algal CCM components can be integrated into C<sub>3</sub> plants to increase biomass, suggesting that transgenic lines combining multiple CCM components plus the DEFp polyprotein could further increase the productivity and yield of C<sub>3</sub> crops.

## 281 - Unveiling the regulatory networks underlying C4 photosynthesis

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One of the greatest scientific challenges in the coming decades is to enhance crop productivity. It is well known that C4 photosynthesis is much more efficient than C3 photosynthesis and many efforts have been undertaken to implement the C4 metabolism in C3 crops. However, among others, the little knowledge regarding the regulation of the C4 photosynthesis has impaired this challenge. During the last years, our lab has contributed to better understand the molecular mechanisms underpinning the regulation of the C4 photosynthesis. Since the C4 metabolism is highly dependent on the compartmentation between mesophyll and bundle sheath cells, it is fundamental to unveil the molecular networks regulating the differential accumulation of key photosynthetic proteins in these specialized cells. Using different approaches, we have identified and characterized a number of transcription factors and cis-acting regulatory elements involved in the cell-specific expression of the PEPC1 and NADP-ME maize genes. We have identified, among others, two bHLH transcription factors that bind to the ZmPEPC1 promoter, act antagonistically, and thus contribute to regulate PEPC1 cell-specific gene expression in maize. Given that C4 PEPC1 promoters can drive cell-specific gene expression in rice, we have also identified and characterized rice transcription factors underpinning this regulation. In addition, we have identified and characterized novel bHLH TFs binding to the ZmNADP-ME gene promoter as well as their binding cis-elements. We showed that each of these TFs interact synergistically with two cis-regulatory elements present in the ZmNADP-ME gene promoter and these interactions seem to be based on an ancient code found in the ancestral C3 state. In order to identify new C4 regulators, we have lately performed a 24 h time-course analysis in maize bundle sheath and mesophyll cells (separately) followed by RNA-seq. We will present and discuss our last results and future perspectives.

#### **410 - Dynamic photosynthesis in two Soybean varieties: short and long term acclimation to fluctuating light conditions**

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It has been widely recognized the need to consider some photosynthetic processes in their transient states since those are more representative of the natural environment. Recently, findings on physiological and phenotypical acclimation to fluctuating light assisted selection for more productive varieties and understanding of natural ecosystem functioning. Here we present two experiments with our newly constructed system which measures canopy gas exchange in 12 growth chambers in a controlled environment. The fully randomized design includes light treatments - diurnal light trend and fluctuating light in which we added oscillations every 2 minutes - and two Soybean varieties (Eiko and Minngold). The two separate experiments had different maximum intensity of light and different amplitude of oscillations (high light and low light).

Minngold has a modified Mg-chelatase enzyme which entails lower total chlorophyll and carotenoids, but higher chl a/b ratio, higher PSII/PSI ratio and a reduced WUE. We hypothesized that the two varieties would respond differently to fluctuations of light, but this was only confirmed in the high light treatment. Then, we looked at phenotypical adaptation of photosynthesis (Ac) at both canopy and leaf level during short term fluctuations. Eiko and Minngold had similar steady state Ac but different photosynthetic induction rates. Furthermore, once steady state is reached, but the light is still fluctuating, the two varieties have an interesting response in Ac, i.e. carbon uptake is faster in Minngold than in Eiko. Finally, we present a dynamic process-based photosynthetic model at leaf level focused on these fast dynamics to explain the differences observed by few state variables and to test different hypothesis on the relation among CO<sub>2</sub> uptake, Rubisco activity, electron flux, and NPQ.

### 532 - Linking root carbon partitioning to inter-kingdom microbial variation in the maize rhizosphere

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As much as 20% of a crop's photosynthetically fixed carbon is transported belowground where it is used for root growth, respired or released into the rhizosphere. The excretion of plant derived carbon compounds into the rhizosphere is a substantial source of soil organic carbon. It supports the development of rhizosphere microorganisms and can thereby benefit plant performance. Meanwhile, little is known about the temporal and spatial distribution patterns of recently fixed carbon in roots and how it links to the rhizosphere microbial community structure. To address this point, we employed a combination of the two non-invasive imaging techniques magnetic resonance imaging (MRI) and positron emission tomography (PET) to visualize root carbon allocation over time. MRI allows 3D monitoring of root growth in soil, while PET uses the short-lived radioactive <sup>11</sup>CO<sub>2</sub> to trace recently fixed carbon within the root system. Maize plants were grown in a sandy loam for three weeks. Roots were scanned using MRI and PET at day 6, 13 and 21 after sowing. Monitoring of root growth and tracer allocation revealed an increased accumulation of recently assimilated carbon at root tips, particularly at young crown root tips. On day 21 after sowing, image-guided sampling based on co-registration of PET and MRI scans allowed us to sample the rhizosphere at high spatial resolution, whilst targeting areas with distinct patterns of recently assimilated carbon. We furthermore distinguished between all relevant root types and age classes to document small-scale differences in microbial community structure. Amplicon sequencing revealed that the community composition of bacteria, fungi and protists was significantly influenced by both, root carbon partitioning and the associated root type. During the congress, findings of bacterial, fungal and protist community analysis will be discussed, along with the associated tracer allocation patterns obtained by MRI/PET.

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## TOPIC:

### Carbon fixation and plant productivity

#### **Extended Elevator Pitches**

##### **131 - Molecular clues from the redox regulation of Calvin-Benson cycle**

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Photosynthetic conversion of CO<sub>2</sub> in organic compounds (fixation) supports all life on Earth and understanding its regulation is essential for optimizing the production of food, fuels and chemicals by crops and algae. In oxygenic photosynthetic organisms, carbon fixation is operated by the Calvin-Benson (CB) cycle, a complex metabolic pathway catalysed by 11 enzymes that are differentially regulated to coordinate whole photosynthetic process in a continuously changing light environment.

Thioredoxins (TRXs) are small ubiquitous proteins having the function of reducing disulfide bonds in target enzymes thereby modulating their activity. During the day, photosynthetic reducing equivalents are transferred from photosystem I to TRXs which in turn reduce key enzymes of the CB cycle. At night, electrons are drawn off by the recently discovered TRX-peroxiredoxin system.

In either plants or algae, the activities of five out of eleven enzymes of the CB cycle are modulated by light via TRXs. We focused on phosphoribulokinase (PRK), whose redox regulation is conserved in both oxygenic photosynthetic prokaryotes and eukaryotes, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which evolved a TRX-dependent isoform in land plants (GAP-B) that can form inactive hexadecameric complexes. Moreover PRK and GAPDH may also form a different type of supramolecular complex assembled by CP12, a redox-sensitive disordered protein, in which both enzyme are temporarily inactivated. Recently we obtained the first crystal structure of PRK from two oxygenic photosynthetic organisms (*Arabidopsis thaliana* and *Chlamydomonas reinhardtii*) providing fundamental insights into the regulatory mechanisms in which PRK is involved. Moreover, we obtained the first cryoEM structure of hexadecameric GAPDH, the last still unresolved element of the CB-cycle redox regulation puzzle.

Altogether our investigations shed new light on the fine regulation of the main CO<sub>2</sub> fixation pathway on Earth.

## **274 - Source-sink carbon movements in grapevine under drought stress and following rehydration.**

**Davide L. Patono**<sup>(1)</sup> - **Daniel Said Pullicino**<sup>(1)</sup> - **Leandro Eloi Alcatrao**<sup>(1)</sup> - **Giorgio Ivaldi**<sup>(1)</sup> - **Andrea Firbus**<sup>(1)</sup> - **Giorgio Gambino**<sup>(2)</sup> - **Irene Perrone**<sup>(2)</sup> - **Walter Chitarra**<sup>(2)</sup> - **Davide Ricauda Aimonino**<sup>(1)</sup> - **Luisella Celi**<sup>(1)</sup> - **Claudio Lovisolo**<sup>(1)</sup>

**DISAFA, University of Turin, Grugliasco, Italy**<sup>(1)</sup> - **Institute for Sustainable Plant Protection, CNR, Torino, Italy**<sup>(2)</sup>

Allocation kinetics of carbon in the different sinks competing in drought stressed and rehydrated grapevines have been investigated.

A plant growth chamber for stable isotope labeling has been set in an environmental control system, basing on pulse-chasing isotopic strategy to trace carbon phloem flows.

In addition, an open-air plant/soil growth system consisting in twelve independent plant/pot balloons with computing-adjustable air flows allowing continuous gas exchange detection between plants / soil and atmosphere has been set.

Water stress led to a drastic decrease in the photosynthesis rate and a decrease in the respiration rate of the soil by about 50%; after rehydration the plants fully recovered the photosynthetic capacity in the morning, while the photosynthetic capacity in the afternoon remained compromised. Sugar accumulation in berries decreased in plants subjected to continuous stress, while the acidity was higher for both plants subjected to continuous stress and rehydrated plants. Grape production was lower in plants subjected to continuous stress.

Plants under water stress had a low and constant microbial biomass throughout the season, while irrigated and rehydrated plants remained similar in the first days of the experiment, but an explosion of microbial biomass was recorded in plants rehydrated 15 days after rehydration. This may indicate a greater contribution of carbon allocated by the rehydrated plant to the microbial mass of the rhizosphere, thanks to an increase in root respiration.

Delivery of labeled carbon in different sinks is discussed in parallel with the expression of genes involved in carbohydrate transport. Genes encoding proteins that regulate the delivery of sucrose to the sinks and which catalyze the hydrolysis of the sucrose discharged to trigger respiration or carbon storage are analyzed.

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#### **479 - A novel threonyl-phosphorylation regulates the activity of maize C4-enzyme phosphoenolpyruvate carboxylase**

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C4-photosynthesis evolved by establishing a mechanism to concentrate CO<sub>2</sub> around RuBisCO. A key player of the C4-photosynthesis is the enzyme phosphoenolpyruvate carboxylase (C4-PEPC). C4-PEPC locates in the mesophyll cells and uses the atmospheric CO<sub>2</sub> to produce oxaloacetate, which is converted to malate. The latter accumulates in the mesophyll cells to diffuse into the bundle sheath cells, where the CO<sub>2</sub> is released to be fixed by RuBisCO. But, C4-PEPC is allosterically inhibited by malate. And, to maintain activity during the photosynthetic process, C4-PEPC inhibition is alleviated by a conserved seryl-phosphorylation at the enzyme's N-terminal. However, this posttranslational modification (PTM) was abolished in the C4-plant *Flaveria bidentis* and no alterations in carbon assimilation were found. Therefore, we used the C4-plant *Zea mays* and an LC-MS approach to study the proteome of photosynthetically active leaves and search for additional PTMs regulating C4-PEPC activity in vivo. We found 15 new PTMs occurring on C4-PEPC, 8 of which are phosphorylations. To dissect the functional role of these phosphorylation sites we produced phosphor-mimic and -null proteoforms for each residue with amino acid substitutions to Asp and Ala residues, respectively. Then, *E. coli* strain PCR1 ( $\Delta$ ppc-2) was complemented with the mutant proteoforms to evaluate the effect of the phosphorylation. *E. coli* protein extracts were used to in-depth characterize both the kinetics and the allosteric regulation of the mutant enzymes. Results revealed the role of a novel threonyl-phosphorylation in the regulation of the catalytic mechanism of maize C4-PEPC.

## 544 - Carboxylation capacity limits photosynthesis at elevated CO<sub>2</sub> throughout diurnal cycles

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The response of carbon fixation in C<sub>3</sub> plants to elevated CO<sub>2</sub> is relatively larger when photosynthesis is limited by carboxylation capacity ( $V_c$ ) than when limited by electron transport ( $J$ ). Recent experiments under controlled, steady-state conditions have shown that photosynthesis at elevated CO<sub>2</sub> may be limited by  $V_c$  even at limiting PPFD. These new experiments were designed to test whether this also occurs in dynamic field environments. Leaf gas exchange was recorded every 5 minutes using two identical instruments both attached to the same leaf. The CO<sub>2</sub> concentration in one instrument was controlled at 400  $\mu\text{mol mol}^{-1}$  and one at 600  $\mu\text{mol mol}^{-1}$ . Leaves were exposed to ambient sunlight outdoors, and cuvette air temperatures tracked ambient outside air temperature. The water content of air in the leaf cuvettes was kept close to that of the ambient air. These measurements were conducted on multiple, mostly clear days for each of three species, Glycine max, Lablab purpureus, and Hemerocallis fulva. The results indicated that in all species, photosynthesis was limited by  $V_c$  rather than  $J$  at both ambient and elevated CO<sub>2</sub> both at high midday PPFDs and also at limiting PPFDs in the early morning and late afternoon. During brief reductions in PPFD due to midday clouds, photosynthesis became limited by  $J$ , indicating that it may take several minutes for the RuBisCo activation state to decrease at low PPFD. The net result of the apparent deactivation of Rubisco at low PPFD was that the relative stimulation of diurnal carbon fixation at elevated CO<sub>2</sub> was larger than would be predicted when assuming limitation of photosynthesis by  $J$  at low PPFD.

## TOPIC:

### Carbon fixation and plant productivity

#### Posters

**672 - *Hirschfeldia incana*, an attractive model to explore the physiology and genetics of high photosynthesis**

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Not all plants have the same overall efficiency of photosynthesis, the biological process that sustains most life on our planet. *Hirschfeldia incana* is a member of the Brassicaceae family that can achieve very high photosynthesis rates at high irradiances ( $>1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) compared to many other species with C3 or even C4 photosynthesis. This translates into high rates of CO<sub>2</sub> assimilation at high irradiances. Unravelling the genetic basis of the high photosynthetic light use efficiency of *H. incana* will open a new avenue for understanding the mechanistic basis of high light use efficiency in plant photosynthesis, and an important resource for future crop yield improvements. To achieve this, we use an interdisciplinary approach combining physiology, genetics and bioinformatics. We verified the high CO<sub>2</sub> assimilation rates of *H. incana* relative to close relatives *Brassica nigra* and *Brassica rapa*, and the more distantly related *Arabidopsis thaliana*. We modeled and compared parameters of the photosynthetic metabolism of *H. incana* and *B. nigra*, highlighting differences that may be essential in achieving higher photosynthetic light-use efficiency. We present the first reference genome assembly for *H. incana* and analyzed the genome sequence for preferential maintenance or loss of genes upon an ancient whole genome triplication. Genes associated with photosynthesis appeared to be preferentially maintained in *H. incana*, compared to *B. nigra*, *B. rapa*, and *A. thaliana*. The first preliminary investigations revealed several of them to be highly expressed upon high light treatment in *H. incana*. With the results obtained so far, we propose the use of *H. incana* as a preferred model for the study of high photosynthetic light-use efficiency at high irradiance.

## 8 - Exploiting Increased Atmospheric Carbon Dioxide Concentrations to Enhance Crop Yields

**Bruce Osborne** <sup>(1)</sup> - Charilaos Yiotis <sup>(1)</sup>

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Increasing crop productivity to feed an ever ever-increasing population remains a major challenge. Whilst a range of strategies have been proposed for enhancing crop productivity, many recent studies have focussed primarily on increasing leaf photosynthetic rate under current atmospheric CO<sub>2</sub> concentrations. Given that the atmospheric CO<sub>2</sub> concentration is likely to increase significantly in the foreseeable future an alternative/complementary strategy might be to exploit the well-known fact that many crop plants, at least in the short term, show significant increases in growth/yield and leaf photosynthesis at elevated CO<sub>2</sub> concentrations. In order to exploit this, we need to know what genetic variability there is in the response of plants to elevated CO<sub>2</sub> and what trait(s) underlie any differences. To examine this we assessed the response of a wide range of ryegrass genotypes, comprising cultivars differing in their year of introduction, as well as wild and semi-natural material with contrasting geographical origins, to ambient (400 ppm) and elevated (800ppm) CO<sub>2</sub> concentration. We show large (~8 fold) intraspecific variations in above-ground biomass productivity among the genotypes at both elevated and ambient CO<sub>2</sub>. Examination of the reason for these intraspecific differences showed that this was related largely to variations in tillering/leaf area, with only small differences associated with leaf photosynthetic rates. As expected, however, whole-plant photosynthesis, approximated as the product of leaf photosynthesis and total leaf area, was strongly correlated with plant productivity. Our results suggest that greater yield gains are likely through the exploitation of genetic differences in tillering and leaf area to exploit future increases in CO<sub>2</sub> concentration, rather than solely focussing on leaf photosynthesis. These results also indicate that the practical realisation of any intraspecific differences in productivity will depend on a better understanding of tiller formation and growth and how this information can be utilised under field conditions.

**275 - Effects of light spectral quality on the photosynthetic activity and biomass production in lettuce plants**

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A series of experiments with lettuce, *Lactuca sativa* L., plants using artificial lighting based on narrow-band LEDs was carried out in controlled environment in a special multifunctional photobiological research unit. Experimental design provided conditions for the studies on plant responses to the exclusion of certain spectral ranges of light in the region of photosynthetically active radiation; in comparison, the responses to quasi-monochromatic radiation in the red and blue regions were studied. The data on plant phenotyping, photosynthetic pigment accumulation, photosynthetic activity determination, and PAM-fluorometry, indicating plant functional activity and stress responses to anomalous light environments are presented. The study of carbon isotopic composition of photoassimilates in the diel cycle made it possible to characterize the balance of carboxylation and photorespiration processes in the leaves, using the previously developed oscillatory model of photosynthesis. Thus, the share of photorespiration increased in response to red light action, while blue light accelerated carboxylation. However, the lowest water use efficiency was observed in the last case. These data were supported by the observations from the light environments missing distinct PAR spectrum regions. The fact that light of different wavelengths affects the isotopic composition of total carbon allows us to elucidate the nature of its action on the organization of metabolism in plants. Further study of these photo-dependent mechanisms should help in the development of ways of fine regulation of physiological processes during plant cultivation with artificial lighting, e.g., in plant factories. This research was supported by the RSF grant No. 19-16-00078.

**276 - Studies on light-dependent processes in Indian mustard (*Brassica juncea*) plants using PAR quasimonochromatic spectra and distinct spectrum regions knockout**

**Ivan Tarakanov <sup>(1)</sup> - Maxim Lomakin <sup>(1)</sup> - Alexander Shmarev <sup>(2)</sup> - Nikolay Sleptsov <sup>(1)</sup> - Vladimir Litvinskiy <sup>(3)</sup> - Alexander Ivlev <sup>(1)</sup>**

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To explore the action mode of different light spectrum regions, various experimental approaches are used. Experimental set up can include studies on the effects of the quasimonochromatic irradiation. Also, plant responses to PAR missing distinct spectrum regions can be investigated. In our studies with Indian mustard, *Brassica juncea* (L.) Coss., we have used both screens. Plants were grown in special light modules with tunable light-emitting diodes (LEDs) varying in the wavelength and spectral composition of the emitted light. Four types of high-performance narrow-band LEDs were used: short-wave red (640 nm), long-wave red (660 nm), far-red (730 nm), and blue (460 nm). Plant responses were investigated under various light quality treatments. The control variant included all 4 types of LEDs, in each of the other regimes one of them was excluded in order to elucidate the wavelength that affect distinct crop physiological processes. Besides, plant responses to quasimonochromatic red and blue light were studied. The earliest transition to flowering was observed in control and 660 nm band missing treatments; absence of 730 nm and monochromatic blue and especially red light (660 nm) delayed bolting. The dependence of various functional processes on the presence of a certain light wavelength in the spectrum of optical radiation was examined in the course of complex studies. The data on plant phenotyping, photosynthetic pigment accumulation, gas exchange (determination of the intensity of photosynthesis, respiration, transpiration, stomatal conductivity) are presented. Studies on the carbon isotopic composition of photoassimilates from donor leaves and acceptor organs included water-soluble and water-insoluble fractions of organic matter determination using mass spectrometric analysis. This made it possible to characterize more precisely the balance of carboxylation and photorespiration processes within different examples of source–sink relations, using previously developed oscillatory model of photosynthesis.

This research was supported by the RSF grant No. 19-16-00078.

### 533 - A Protein Contact Network approach applied on L8S8 plant Rubisco

**Matteo Gionfriddo**<sup>(1)</sup> - **Sara Cimini**<sup>(1)</sup> - **Hamid Hadi-Alijanvand**<sup>(2)</sup> - **Francesco Loreto**<sup>(3)</sup> - **Luisa Di Paola**<sup>(4)</sup> - **Laura De Gara**<sup>(1)</sup>

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Plants fix atmospheric CO<sub>2</sub> in organic molecules using ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) within the photosynthetic Calvin-Benson-Bassham cycle. In higher plants, a complex of eight large subunits (L) and eight small subunits (S) forms the L<sub>8</sub>S<sub>8</sub> Rubisco structure. For catalysis, optimal complementarity between the L and S subunits is required. Rubisco is indispensable for plant survival and growth, but photosynthesis is often limited by Rubisco low efficiency. Besides being a very slow enzyme, catalyzing 3-4 reactions per second in higher plants, Rubisco poorly discriminates between fixing CO<sub>2</sub> and O<sub>2</sub> as substrates, the oxygenation reaction leading to the energy consuming and CO<sub>2</sub>-releasing process of photorespiration. Improving the carboxylation properties of Rubisco has therefore long been as a main target for improving plant photosynthesis and growth. Structural and topological differences among selected Rubisco proteins crystallized in *Spinacia oleracea* have been identified by means of the emerging Protein Contact network (PCN) technology. PCN is based on the formalization of 3D structures as contact networks among amino-acidic residues. We aimed to reconstruct the already known functional domains and to identify significant amino acid regions involved in the interactions between chaperonin complexes and large/small (L/S) subunits. Here we presented preliminary results showing a topological signature (graph energy) of the different affinity of the enzymes towards different substrates and inhibitors. This allowed the identification of regions/specific amino acids more involved in the enzymatic regulation. By using two more tools, Affinity by Flexibility (SEPAS) and Perturbation Response Scanning (PRS) based on elastic network modes on the selected Rubisco structures, the dynamics and properties of binding interfaces between Rubisco subunits and protein interactors has also been studied. The obtained results help to decipher the systemic regulation of Rubisco as a complex protein network.

## 643 - Maximizing glycolate production in *Chlamydomonas reinhardtii* mutants

Sonja Rössler<sup>(1)</sup> - Christian Wilhelm<sup>(1)</sup>

Leipzig University, Department Algal biotechnology, Leipzig, Germany<sup>(1)</sup>

The goal of the concept of “New Green Chemistry” is the excretion of glycolate by green algae in order to replace biomass for starch production. In this alternate approach biomass production is prevented while glycolate production needs to be increased as far as possible. Ideally, harvesting and refinement can be avoided using the glycolate enriched medium directly in downstream processing (e.g. fermentation). In this study *Chlamydomonas reinhardtii* cells are converted into continuous glycolate producing factories. An increase in glycolate production can be achieved by exploiting the oxygenase function of Rubisco. This procedure requires three steps. Firstly, an adjustment of the CO<sub>2</sub> / O<sub>2</sub> ratio. Secondly, it calls for the inactivation of the Carbon Concentrating Mechanism (CCM) and lastly the inhibition of the Glycolatedehydrogenase (GYD), the key enzyme in glycolate metabolization. This can be done e.g. by adding an inhibitor such as Ethoxzolamide (EZA). However, EZA disturbs the subsequent usage of the glycolate enriched medium for direct fermentation or chemical conversion. Therefore, process optimization asks for genetic modification of the cell. To show the biotechnological potential of such modified cells, double mutants with functional defects in the CCM master regulator gene (*cia 5*) and in the gene coding for GYD were used for screening. Glycolate production process optimization on physiological and biotechnological levels are presented.

### Guidelines for abstract submission

- All abstracts must be submitted in English and in Word format following the template provided.
- Abstract cannot exceed a limit of 300 words (excluding title, authors and affiliations).
- Standard abbreviations do not need explanation. Otherwise abbreviations must be defined in brackets after when first used in the text.
- Do not include citations of published work in the abstract.
- Each author may be the presenting author of only one abstract.
- The abstract should be submitted by the presenting author only. The presenting author's name will appear in underlined text. The presenting author is the corresponding author who will receive all communications, including acceptance and scheduling notifications.
- The abstract submission deadline is **15 January 2020** to be considered for a short talk and grant application (insert link alla pagina di questo sito); and **30 April 2020** for poster only.
- The abstract will be automatically withdrawn in case the presenter has not completed registration by **April 30, 2020**.

The Scientific Committee will review the received abstracts for oral or poster presentations. Participants will be notified of the abstract selection results for oral presentations by **February 15 2020**.



## TOPIC:

### Carbon fixation and plant productivity 2

#### Keynote Lecture

##### **CropBooster-P: A roadmap for future plant research in Europe**

**Alexandra Baekelandt**<sup>(1)</sup> - **Martin Parry**<sup>(2)</sup> - **Vandasue Lily Rodrigues Saltenis**<sup>(3)</sup> - **Philippe Nacry**<sup>(4)</sup> - **Mathias Pribil**<sup>(3)</sup> - **Aleksandra Malyska**<sup>(5)</sup> - **Sam Taylor**<sup>(6)</sup> - **Xinyou Yin**<sup>(7)</sup> - **Erik Murchie**<sup>(6)</sup> - **Amrit K. Nanda**<sup>(5)</sup> - **Jessica Davies**<sup>(2)</sup> - **Ralf Wilhelm**<sup>(8)</sup> - **Norbert Rolland**<sup>(9)</sup> - **Jeremy Harbinson**<sup>(10)</sup> - **Dirk Inzé**<sup>(1)</sup> - **René Klein Lankhorst**<sup>(11)</sup>

**VIB-UGent, Center for Plant Systems Biology, Gent, Belgium**<sup>(1)</sup> - **Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom**<sup>(2)</sup> - **Copenhagen Plant Science Centre, Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark**<sup>(3)</sup> - **BPMP, Univ Montpellier, INRAE, CNRS, Montpellier SupAgro, Montpellier, France**<sup>(4)</sup> - **European Technology Platform 'Plants for the Future', Plant ETP, Brussels, Belgium**<sup>(5)</sup> - **Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington campus, Nottingham, United Kingdom**<sup>(6)</sup> - **Centre for Crop Systems Analysis, Department of Plant Sciences, Wageningen University & Research, Wageningen, Netherlands**<sup>(7)</sup> - **Institute for Biosafety in Plant Biotechnology, Julius Kühn-Institut - Federal Research Centre for Cultivated Plants, Quedlinburg, Germany**<sup>(8)</sup> - **LPCV, Univ. Grenoble Alpes, INRAE, CNRS, CEA, Grenoble, France**<sup>(9)</sup> - **Laboratory of Biophysics, Wageningen University & Research, Wageningen, Netherlands**<sup>(10)</sup> - **Wageningen Plant Research, Wageningen University & Research, Wageningen, Netherlands**<sup>(11)</sup>

To achieve food and nutrition security and to meet the demands of a future bio-economy, a doubling of global crop production is required by 2050, while mitigating the effects of global climate change. To future-proof European agriculture, the H2020 CropBooster-P project aims to draft a roadmap to re-design our current crops, accompanied with the broadest societal support. For this, we carried out a scenario building analysis in which a multitude of trends and key uncertainties were considered. This resulted in the development of four contrasting scenarios depicting potential future socio-economic developments: 'Bio-Innovation', 'My Choice', 'Food Emergency' and 'REJECTech'. In parallel, a database was developed capturing information on plant traits, technologies, genes and methods exploitable to sustainably improve crop productivity and nutritional and/or industrial quality in a wide range of crops. Based on the database, potential routes to improve crops were identified, focusing on their genetic basis and delivered through conventional breeding and/or more advanced biotechnological methods. Modelling approaches were also used with photosynthetic traits as an example to demonstrate their potential in improving crop biomass and yield across Europe for current and projected environments.

In this way, CropBooster-P will define (1) which land-based and aquatic production systems should be in focus, (2) which crop traits can be engineered to produce high quality biomass in a sustainable manner for food, feed and non-food purposes, and (3) which methods will be available and/or should be considered to meet the future needs. The option space was further developed with relevant stakeholders, involving consumers, farmers, industry, policy-makers and networks of EU scientists, and the implications of the different scenarios on Europe's options to future-proof its crops were interrogated. Altogether, this will result in a roadmap that sets out the plant science and multi-actor systems research required to enable future European agriculture while ensuring the broadest societal support and benefits.

## TOPIC:

### Carbon fixation and plant productivity 2

#### **External Elevator Pitches**

##### **398 - The potential of transgenic *Chlamydomonas reinhardtii* in the recycling of dairy wastes**

**Manuel Benedetti** <sup>(1)</sup> - **Giovanna Gramegna** <sup>(1)</sup> - **Anna Scortica** <sup>(1)</sup> - **Valentina Scafati** <sup>(1)</sup> - **Luca Dall'Osto** <sup>(2)</sup> - **Roberto Bassi** <sup>(2)</sup> - **Benedetta Mattei** <sup>(1)</sup>

**University of L'Aquila, Department of Life, Health and Environmental Sciences, L'Aquila, Italy** <sup>(1)</sup> - **University of Verona, Department of Biotechnology, Verona, Italy** <sup>(2)</sup>

The Dairy sector produces the 16% of the total organic carbon waste from food and feed industry, producing 200 million cubic meters of wastewaters per year. This requires a correct waste management process in order to mitigate pollution. Application of microalgae culturing to wastewater treatments offers the opportunity of producing valuable biomass containing enzymatic activity for lignocellulose degradation and biofuel production. Here, we investigated the potential of transgenic *Chlamydomonas reinhardtii* to support the mixotrophic growth of the oleaginous microalga *Chlorella vulgaris* in dairy waste-based media despite lactose, the most abundant carbohydrate in such wastes, cannot be metabolized by either microalgal species. The transplastomic *C. reinhardtii* strain accumulating the  $\beta$ -galactosidase from *Pyrococcus furiosus* grew in a dairy waste-based medium with a productivity of 0.28 g dry weight (DW) day<sup>-1</sup>L<sup>-1</sup>. Subsequently, the heat treatment of the exhausted growth medium with the transplastomic *C. reinhardtii* cell extract converted the residual lactose into glucose and galactose, allowing for assimilation by *Chlorella vulgaris*. Following this two-phase mixotrophic growth, microalgae reached a total biomass yield greater than 4 g DW L<sup>-1</sup> upon 12 days, by concomitantly cleaning the dairy waste-based medium that, at the end of the growth, met the analytical parameters for its release in the environment.

## TOPIC:

### Carbon fixation and plant productivity 2

#### Posters

#### **195 - Performance and lateral mobility of photosynthetic complexes in grana membranes of Arabidopsis and barley mutants lacking chlorophyll b**

**Elena Tyutereva**<sup>(1)</sup> - **Svetlana Senik**<sup>(1)</sup> - **Anastasiya Maksimova**<sup>(1)</sup> - **Alexandra Ivanova**<sup>(1)</sup> - **Ekaterina Kotlova**<sup>(1)</sup> - **Olga Voitsekhovskaja**<sup>(1)</sup>

**Komarov Botanical Institute, ul. Professora Popova, 2, Saint-Petersburg, Russian Federation**<sup>(1)</sup>

The lateral mobility of integral components of thylakoid membranes, such as plastoquinone, xanthophylls and pigment-protein complexes, is critical for maintenance of efficient light harvesting, linear electron transport and successful repair of damaged photosystem II (PSII). The packaging of the photosynthetic complexes in the membrane depends on their size and form, which in turn depend on their composition. Chlorophyll b (Chlb) is an important regulator of antenna size and composition. In this study, the lateral mobility of pigment-protein complexes and lipids was analyzed in grana membranes of chlorina mutants of Arabidopsis (ch1-3) and barley (chlorina f2-3613) lacking Chlb.

The sizes of mobile fractions and the rates of diffusion of membrane components were determined by Fluorescence Recovery After Photobleaching (FRAP). The composition of PSII antennae was analyzed by immunoblotting. The sizes of the total PQ-pool vs. the reduced PQ-pool under high light, as well as functional antenna size, were determined using PAM fluorimetry. Production of reactive oxygen species (ROS) was estimated using ROS-sensitive dyes (the singlet oxygen-specific dye SOSG and the general ROS indicator CM-H2DCFDA). In the barley chlorina f2-3613 mutant, lipid profiles of the photosynthetic membranes were examined using HPTLC (for lipid classes) and GC-MS methods (for fatty acids), and the distribution of photosynthetic complexes in grana membranes was analyzed after their isolation using differential centrifugation by means of SEM and TEM.

The data will be discussed in the context of protein composition of antennae, thylakoid lipid composition, characteristics of the plastoquinone pool and production of reactive oxygen species in leaves of chlorina mutants.

The study was supported by RSF projects No. 14-16-00120 and No. 14-16-00120-P.

## 232 - The potential of transgenic *Chlamydomonas reinhardtii* in the recycling of dairy wastes

**Manuel Benedetti** <sup>(1)</sup> - **Giovanna Gramegna** <sup>(1)</sup> - **Anna Scortica** <sup>(1)</sup> - **Valentina Scafati** <sup>(1)</sup> - **Luca Dall'Osto** <sup>(2)</sup> - **Roberto Bassi** <sup>(2)</sup> - **Maria Benedetta Mattei** <sup>(1)</sup>

**University of L'Aquila, Department of Life, Health and Environmental Sciences, L'Aquila, Italy** <sup>(1)</sup> - **University of Verona, Department of Biotechnology, Verona, Italy** <sup>(2)</sup>

The Dairy sector produces the 16% of the total organic carbon waste from food and feed industry, producing 200 million cubic meters of wastewaters per year. This requires a correct waste management process in order to mitigate pollution. Application of microalgae culturing to wastewater treatments offers the opportunity of producing valuable biomass containing enzymatic activity for lignocellulose degradation and biofuel production. Here, we investigated the potential of transgenic *Chlamydomonas reinhardtii* to support the mixotrophic growth of the oleaginous microalga *Chlorella vulgaris* in dairy waste-based media despite lactose, the most abundant carbohydrate in such wastes, cannot be metabolized by either microalgal species. The transplastomic *C. reinhardtii* strain accumulating the  $\beta$ -galactosidase from *Pyrococcus furiosus* grew in a dairy waste-based medium with a productivity of 0.28 g dry weight (DW) day<sup>-1</sup>L<sup>-1</sup>. Subsequently, the heat treatment of the exhausted growth medium with the transplastomic *C. reinhardtii* cell extract converted the residual lactose into glucose and galactose, allowing for assimilation by *Chlorella vulgaris*. Following this two-phase mixotrophic growth, microalgae reached a total biomass yield greater than 4 g DW L<sup>-1</sup> upon 12 days, by concomitantly cleaning the dairy waste-based medium that, at the end of the growth, met the analytical parameters for its release in the environment.

**351 - Directed evolution of Chlorella strains at reduced cell wall robustness for efficient lipid and metabolite extraction.**

**Anna Scortica** <sup>(1)</sup> - **Giovanna Gramegna** <sup>(1)</sup> - **Maira Giovannoni** <sup>(1)</sup> - **Valentina Scafati** <sup>(1)</sup> - **Manuel Benedetti** <sup>(1)</sup> - **Benedetta Mattei** <sup>(1)</sup>

**University of L'Aquila, Dept. of Life, Health and Environmental Sciences, L'Aquila, Italy** <sup>(1)</sup>

Chlorella is a genus of microalgae with application as bioenergetic feedstock due to the high level of lipid accumulation and the relatively fast growth rate in waste-based growth media. However, exploitation of Chlorella species in biofuel industry is still limited by the low extractability of intracellular compounds, the latter mainly due to the intrinsic robustness of Chlorella cell-wall. Here we report on the generation of Chlorella strains with reduced cell wall robustness by directed evolution. Genetic variability was randomly induced by EMS-mediated mutagenesis and the putative cell wall deficient mutants were selected by differential sedimentation using a Percoll-based gradient. Part of the microalgae mutants characterized by a lower cell density, were also characterized by an increased permeability to Nile-Red dye, a molecule that binds the intracellular lipids with high affinity. Notably, the isolated mutants released more proteins than wild-type strain upon disruption through mild-extractive procedures. The next step will be the evaluation of lipid extraction yield from these mutants upon growth in waste-based media. Thus, the reduced cell-wall recalcitrance of these Chlorella mutants can allow their exploitation also in other important fields ranging from the cosmetic to nutraceutical due to the high metabolic versatility of microalgae.

### **360 - Characterization of an *Aspergillus* isolate with degrading activity towards green microalgae cell wall**

**Valentina Scafati** <sup>(1)</sup> - **Maira Giovannoni** <sup>(1)</sup> - **Anna Scortica** <sup>(2)</sup> - **Giovanna Gramegna** <sup>(1)</sup> - **Manuel Benedetti** <sup>(1)</sup> - **Maria Benedetta Mattei** <sup>(1)</sup>

**University of L'Aquila, Department of Health, Life and Environmental Sciences, L'Aquila, Italy** <sup>(1)</sup> - **University of L'Aquila, Department of Health, Life and Environmental Sciences, L'Aquila, Italy** <sup>(2)</sup>

Microalgae are currently considered an attractive source of lipids and high-value chemicals for biofuel production and nutraceutical and pharmaceutical industries. Moreover, *Chlorella* species have the ability to remove nutrients rich in nitrogen and phosphorous, making them a good candidate for wastewater bioremediation

The main limitation in the industrial exploitation of microalgae is the recalcitrance of their cell walls consisting of a polysaccharide and glycoprotein matrix characterized by a high resistance to degradation. Technologies to disrupt microalgae cells wall are needed to gain access to their proteins and lipids and to improve their exploitation as biomass for biofuel production.

Here we report on the isolation of an *Aspergillus* specie capable of disrupting *Chlamydomonas reinhardtii* and *Chlorella vulgaris* microalgae in dark condition. The analyses of fungal medium revealed the presence of CWDEs enzymes whit pectinase,  $\beta$ -glucosidase and cellulolytic activities. Cell disruption induced by enzymatic treatment was assessed by measuring the reducing sugars and the oligosaccharides profile. The release of lipids and proteins was assessed in both supernatant and residue after incubation with the enzymatic mixture. Whole genome sequencing and comparative genomic analysis of the fungus combined with secretome proteomic analysis were conducted to identify cell wall degrading enzymes positively impacting the extraction of useful metabolites from these algal species.

## TOPIC:

### Cell signaling in plants

#### **Keynote Lecture**

##### **Plant membrane & nutrient signal transduction**

**Hothorn Michael** <sup>(1)</sup>

**University of Geneva, Department of Botany and Plant Biology, Geneva, Svizzera** <sup>(1)</sup>

Plants have evolved unique signal transduction cascades to coordinate their growth and development and to interact with their environment. I will present how plant unique membrane receptor kinases with leucine-rich repeat ectodomains sense small molecule and different peptide ligands to regulate various aspects of plant development. Receptor activation of these membrane proteins relies on the interplay of shape-complementary co-receptor kinases and receptor pseudokinases. Their signaling mechanisms can be exploited to constitutively activate membrane receptor kinase signaling cascades in plants.

Next, I will discuss a nutrient signaling pathway that enables plants to maintain sufficient amounts of cellular phosphate, and to take up more phosphate when needed. The pathway is based on inositol pyrophosphate nutrient messengers that are perceived by cellular receptors termed SPX domains. SPX domains can engage in protein – protein interactions, for example with phosphate starvation response transcription factors. I will describe how inositol pyrophosphate level change in response to changes in nutrient availability control protein – protein interactions, resulting in specific signal transduction events.

## TOPIC:

### Cell signaling in plants

#### Oral Communications

##### 113 - Local and systemic effects of brassinosteroid perception in developing phloem

**Surbhi Rana**<sup>(1)</sup> - **Moritz Graeff**<sup>(1)</sup> - **Petra Marhava**<sup>(1)</sup> - **Bernard Moret**<sup>(1)</sup> - **Christian S Hardtke**<sup>(1)</sup>

**University of Lausanne, Department of Plant Molecular Biology, Lausanne, Switzerland**<sup>(1)</sup>

The plant vasculature is an essential adaptation to terrestrial growth. Its phloem component permits efficient transfer of photosynthates between source and sink organs, but also transports signals that systemically coordinate physiology and development. Here we provide evidence that developing phloem orchestrates cellular behaviour of adjacent tissues in the growth apices of plants, the meristems. *Arabidopsis thaliana* plants that lack the three receptor kinases BRASSINOSTEROID INSENSITIVE 1 (BRI1), BRI1-LIKE 1 (BRL1) and BRL3 (“bri<sup>3</sup>” mutants) can no longer sense brassinosteroid phytohormones and display severe dwarfism as well as patterning and differentiation defects, including disturbed phloem development. We found that despite the ubiquitous expression of brassinosteroid receptors in growing plant tissues, exclusive expression of the BRI1 receptor in developing phloem is sufficient to systemically correct cellular growth and patterning defects that underlie the bri<sup>3</sup> phenotype. Although this effect is brassinosteroid-dependent, it cannot be reproduced with dominant versions of known downstream effectors of BRI1 signaling and therefore possibly involves a non-canonical signaling output. Interestingly, the rescue of bri<sup>3</sup> by phloem-specific BRI1 expression is associated with antagonism towards phloem-specific CLAVATA3/EMBRYO SURROUNDING REGION-RELATED 45 (CLE45) peptide signaling in roots. Hyperactive CLE45 signaling causes phloem sieve element differentiation defects, and consistently, knock-out of CLE45 perception in bri<sup>3</sup> background restores proper phloem development. However, bri<sup>3</sup> dwarfism is retained in such lines. Our results thus reveal local and systemic effects of brassinosteroid perception in the phloem: it locally antagonizes CLE45 signaling to permit phloem differentiation, while it systemically instructs plant organ formation via a phloem-derived, non-cell autonomous signal.



### **136 - A Pharmacogenetic Approach to Decipher the Role of the TOR Signaling Pathway in Plant Growth and Development**

**Adam Barrada <sup>(1)</sup> - Romain Perdoux <sup>(1)</sup> - Christophe Robaglia <sup>(1)</sup> - Marie-Hélène Montané <sup>(1)</sup> - Benoit Menand <sup>(1)</sup>**

**Aix Marseille Univ, CEA, CNRS, UMR7265, Biosciences and biotechnologies institute of Aix-Marseille, marseille, France <sup>(1)</sup>**

Target of Rapamycin (TOR) protein is the central component of the TOR signaling pathway which regulates cell growth and metabolism in response to environmental cues in eukaryotes. Our long-term goal is to understand how this conserved central regulator of eukaryotic growth has integrated new functions through evolution of photosynthetic organisms. In order to decipher new aspects of the plant TOR signaling pathway, we have performed a screen of *Arabidopsis thaliana* ethyl methanesulfonate (EMS) mutants having different sensitivity to ATP-competitive TOR inhibitors. We will present recent advances on the use of this screen to discover new members of the plant TOR pathway, such as the DYRK kinase YAK1 (Barrada et al 2019 Development: [dev.171298](https://doi.org/10.1242/dev.171298)), and to help understand how the TOR pathway regulates plant growth and development.

#### 149 - ROP GTPase-activated kinase signaling in Arabidopsis

Valkai Ildikó <sup>(1)</sup> - Lajkó Dézi B. <sup>(1)</sup> - Kenesi Bettina <sup>(1)</sup> - Ménesi Dalma <sup>(1)</sup> - Borbély Péter <sup>(1)</sup> - Bodai László <sup>(2)</sup> - Durr Julius <sup>(3)</sup> - Fehér Attila <sup>(1)</sup>

Biological Research Center, Institute of Plant Biology, Szeged, Hungary <sup>(1)</sup> - University of Szeged, Dept. of Biochemistry and Molecular Biology, Szeged, Hungary <sup>(2)</sup> - University of Warwick, School of Life Sciences, Warwick, United Kingdom <sup>(3)</sup>

Receptor-like cytoplasmic kinases (RLCKs) are related to plant receptor kinases (PRKs) but have neither transmembrane nor extracellular domains. The Arabidopsis genome codes for almost two hundred of these cytoplasmic kinases that were classified into several families. The functions of most of these plant-specific kinases are unknown. In our laboratory, we characterize those members of the RLCK Class VI family (RLCK VI\_A) that were shown to have ROP G-protein-dependent in vitro activity. One of these kinases, RLCK VI\_A2 was investigated in detail using loss-of-function and gain-of-function transgenic plants, biochemical and molecular biology tools. Seedlings of the T-DNA insertion mutant rlck VI-a2 exhibited short hypocotyls and small cotyledons, while the adult plants had small rosettes. Cellular investigations indicated that the above phenotypes are associated with impaired cell elongation (epidermal cell length/shape was measured) in the loss-of-function mutant. Exogenous gibberellic application could restore normal seedling/plant growth. Since gibberellic acid synthesis was found to be unaffected in the mutant, the kinase may affect gibberellic acid sensitivity. Transcriptomic data supported the role of the kinase in the above pathways. Yeast two-hybrid screening completed with in vitro kinase assays as well as phospho-proteomic characterization of the mutant was used to identify potential substrates. A chromatin-remodelling ATPase, involved in similar processes as the kinase, was identified as a potential target of the RLCK VI\_A2 signaling pathway.

This research was supported by NKFI “OTKA” grants (K 124828, K132486) and a grant from the Hungarian Ministry for National Economy (GINOP-2.3.2-15-2016-00001).

## 182 - Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth

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Plant reproduction relies on the highly regulated growth of the pollen tube for sperm delivery. This process is controlled by secreted RALF signaling peptides, which have previously been shown to be perceived by *Catharanthus roseus* RLK1-like (CrRLK1Ls) membrane receptor-kinases/LORELEI-like GLYCOLPHOSPHATIDYLINOSITOL (GPI)-ANCHORED PROTEINS (LLG) complexes, or by leucine-rich repeat (LRR) extensin proteins (LRXs). Here, we demonstrate that RALF peptides fold into bioactive, disulfide bond-stabilized proteins that bind the LRR domain of LRX proteins with low nanomolar affinity. Crystal structures of LRX2-RALF4 and LRX8-RALF4 complexes at 3.2 and 3.9 Å resolution, respectively, reveal a dimeric arrangement of LRX proteins, with each monomer binding one folded RALF peptide. Structure-based mutations targeting the LRX-RALF4 complex interface or the RALF4 fold reduce RALF4 binding to LRX8 in vitro and RALF4 function in growing pollen tubes. Mutants targeting the disulfide-bond stabilized LRX dimer interface fail to rescue *lrx* infertility phenotypes. Quantitative biochemical assays reveal that RALF4 binds LLGs and LRX cell wall modules with drastically different binding affinities, and with distinct and mutually exclusive binding modes. Our biochemical, structural and genetic analyses reveal a complex signaling network by which RALF ligands instruct different signaling proteins using distinct targeting mechanisms.

## **211 - Membrane bound class III peroxidases: Overlooked enzymes in the initial phase of plant stress response**

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Class III peroxidases are haem-containing peroxidases of the secretory pathway. Due to their catalytic cycles these enzymes are involved in production and scavenging of reactive oxygen species (ROS). Only a few studies demonstrated presence of guaiacol peroxidases in plant plasma membranes and some of these data suggest a localisation of these enzymes in microdomains. Although these peroxidases have been characterised biochemically, our knowledge of these enzymes is still in its infancy. After contact with biotic or abiotic stressors a so-called oxidative burst, a massive production of ROS, can be observed within 15 to 30 minutes. Respiratory burst oxidase homologues (Rboh) have been established as the major source of this burst. While up-regulation of several soluble peroxidase isoenzymes have been demonstrated after the initial phase of stress response, membrane-bound peroxidases appeared to be overlooked. Our studies demonstrated not even an increased abundance of plasma membrane-bound peroxidases after treatment with biotic and abiotic stressors, but also up-regulation and higher abundance of specific plasma membrane-bound peroxidases within 15 to 30 minutes by heavy metals and salinity. A hypothetical model will explain possible functions of these enzymes in stress response.

## 529 - Contribution of alternative splicing in plants and animals in response to different types of stimuli

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Plants adapt their development to different environmental and developmental cues by rewiring their transcriptomes and proteomes. During the last decades, much effort has been put into understanding how signaling cascades initiated by multiple stimuli control transcriptomic responses. These research efforts have focused mainly on the role of transcription factors in regulating gene expression. However, recent studies are beginning to uncover a key contribution of alternative splicing, a posttranscriptional mechanism that generates multiple transcripts from the same gene, to the diversification of plant transcriptomes. Despite functional evidence pointing to alternative splicing as a major player in the adaptation of plant development to different external and internal stimuli, little is known about the molecular mode of action of these mechanisms from a genomic point of view. What are the molecular features of genes undergoing splicing in response to different stimuli? Do they have a similar impact on protein levels and on proteome diversification? Are these patterns similar to those of animals? To assess the similarities and differences between the molecular processes controlled by alternative splicing in different multicellular organisms, we have first implemented vast-tools for the model plant *Arabidopsis thaliana*, a computational pipeline to detect and quantify all types of alternative splicing events from RNA-seq data (<https://github.com/vastgroup/vast-tools>). We then used this pipeline to quantify alternative splicing from RNA-seq samples available in the Short Read Archive (SRA) for various environmental conditions and differentiated tissues in *A. thaliana*. We compared the regulatory and functional patterns observed in *A. thaliana* with those of three animals with very distinct exon-intron architectures (humans, fruitflies and round worms), shedding light on the particularities of the mode of action and biological functions of alternative splicing in response to different stimuli in plants.

## TOPIC:

### Cell signaling in plants

#### **Extended Elevator Pitches**

##### **415 - Stress Granules as novel mechanism for stress signaling**

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Stress granules (SGs) are evolutionary conserved membrane-less organelles formed in response to stress. Despite their importance for stress adaptation and tolerance, we lack the knowledge about their assembly and signaling during stress conditions. In this study, we addressed the existing gap. We provide evidence for proteome and metabolome composition, not only for cytosolic SGs (cSGs) but also for plastidial SGs (pSGs). To isolate SGs we used Arabidopsis seedlings expressing green fluorescent protein (GFP) fusion of the SGs marker protein, Rbp47b for cSG and SCO1 for pSGs. We used an experimental protocol combining differential centrifugation with affinity purification (AP). SGs isolates were analyzed using mass spectrometry-based proteomics and metabolomics. In addition, sequestered mRNA was analyzed for pSGs. From cSG proteome, a quarter of the identified proteins constituted known or predicted SG components. Intriguingly, the remaining proteins were enriched in key enzymes and regulators, such as cyclin-dependent kinase A (CDKA), that mediate plant responses to stress. In addition to proteins, nucleotides, amino acids and phospholipids also accumulated in SGs. Also for pSGs proteome showed high diversity and similar metabolites were identified. Taken together, our results indicated the presence of a preexisting SG protein interaction network; an evolutionary conservation of the proteins involved in SG assembly and dynamics; an important role for SGs in moderation of stress responses by selective storage of proteins and metabolites.

#### **445 - Simultaneous imaging of ER and cytosolic Ca<sup>2+</sup> dynamics reveals long distance ER Ca<sup>2+</sup> waves in plants**

**Francesca Resentini <sup>(1)</sup> - Matteo Grenzi <sup>(1)</sup> - Daniele Ancora <sup>(2)</sup> - Mara Cademartori <sup>(1)</sup> - Laura Luoni <sup>(1)</sup> - Marianna Franco <sup>(1)</sup> - Andrea Bassi <sup>(2)</sup> - Maria Cristina Bonza <sup>(1)</sup> - Alex Costa <sup>(1)</sup>**

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Plants are exposed to environmental factors such as fluctuating light, day/night cycle, temperatures, water availability, humidity and interaction with other organisms. Often these variations can affect plant growth, development and, in several cases, causing major yield losses in agriculture. Unlike animals, plants cannot move away from danger to survive and they, thus, need to continuously control, and possibly anticipate, any upcoming stress.

Plants early responses to stress often appear in a time frame of seconds or a few minutes in different cell compartments, and in these cases, they mainly rely on quick changes of ions concentrations (e.g. Ca<sup>2+</sup>, H<sup>+</sup>, K<sup>+</sup>, NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, etc) which are dependent by their movements across membranes.

In plants, a plethora of environmental and developmental stimuli promote specific Ca<sup>2+</sup> increases in cytosol as well as in different cellular compartments including the endoplasmic reticulum (ER). The ER represents an intracellular Ca<sup>2+</sup> store that actively accumulates Ca<sup>2+</sup> taken up from the cytosol.

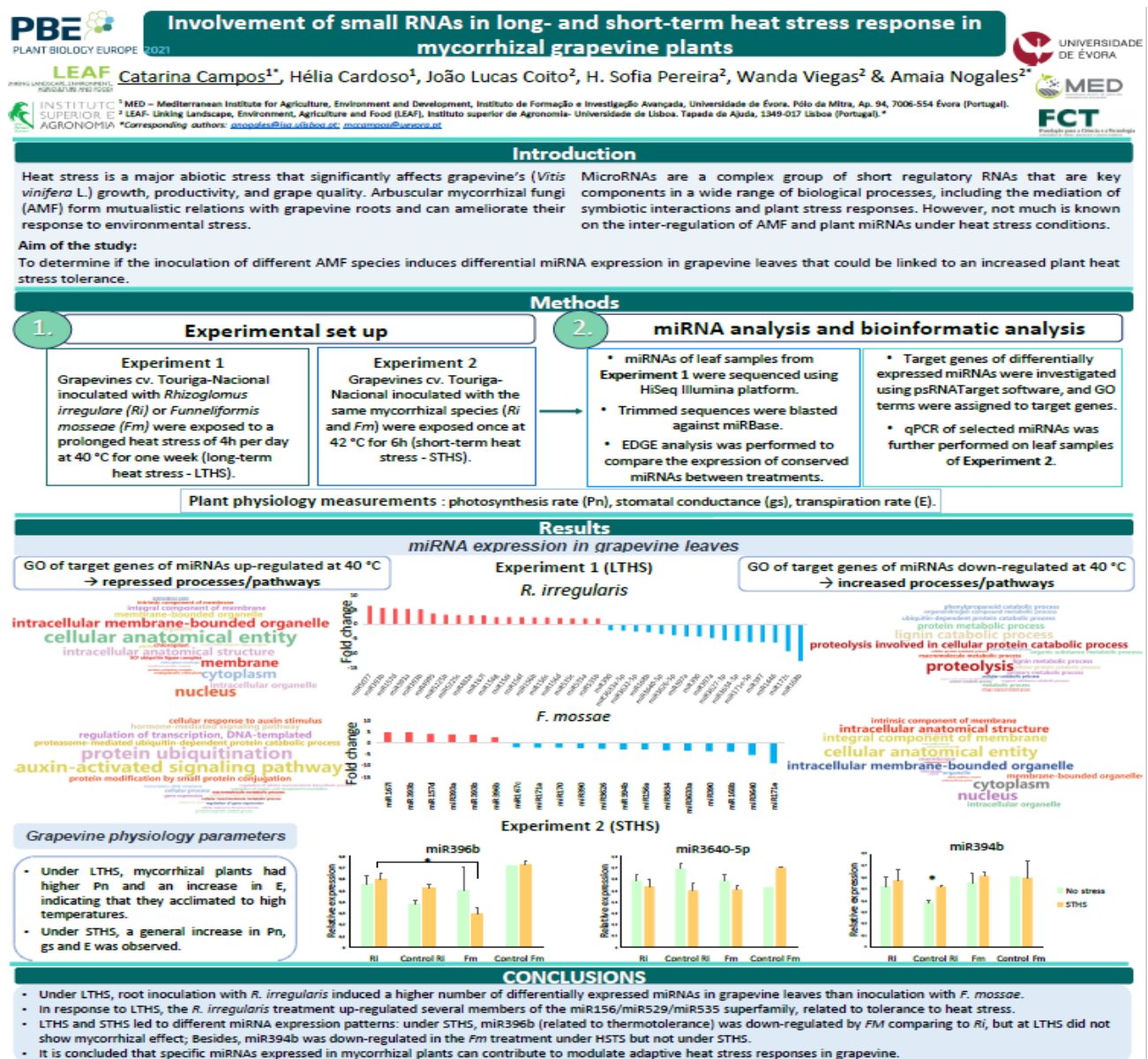
By exploiting state of the art genetically encoded Ca<sup>2+</sup> indicators (GECIs), we describe the generation and characterization of a new *Arabidopsis thaliana* line that allows for simultaneous imaging of Ca<sup>2+</sup> dynamics in both the ER and cytosol, at different spatial scales. By performing analyses in single cells, we accurately quantify the time required by ER to import Ca<sup>2+</sup> from the cytosol into the lumen. Furthermore, the imaging of mature and soil-grown plants, revealed the existence of a wounding-induced long distance ER Ca<sup>2+</sup> wave propagating in the injured and systemic rosette leaf.

This technology boosts high-resolution analyses of intracellular Ca<sup>2+</sup> dynamics at cellular level and in adult organisms, and paves the way for new methodologies aimed at defining the contribution of subcellular compartments in Ca<sup>2+</sup> homeostasis and signaling.

## 473 - Involvement of small RNAs in long- and short-term heat stress response in mycorrhizal grapevine plants.

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## TOPIC:

### Cell signaling in plants

#### Posters

**611 - ERAD-mediated maturation of the regulatory protein of plant meristematic cells CLAVATA 3 emerged during evolution from algae to higher plants.**

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The indefinite growth capacity of plants is guaranteed by meristems, where totipotent cells are in continuous division and differentiation. The activity of these undifferentiated cells is under control of the negative feedback between two genes WUSCHEL (WUS) and CLAVATA3 (CLV3) (Brand et al. 2000). CLV3 gene encodes a small secretory protein that undergoes proteolytic maturation to release an active dodecapeptide that binds to a receptor complex (CLV1/2), but the actual maturation mechanism is still unknown (Kondo et al. 2006). The current model predicts CLV3 secretion in the extracellular space and maturation mediated by specific proteases, with the enzymes that carry out this proteolysis not yet identified. A recent study suggested that CLV3 maturation in higher plants occurred in the endoplasmic reticulum via a process mediated by Endoplasmic Reticulum Associated Degradation (ERAD), and that CLV3-GFP expressed in transgenic tobacco was biologically active because only the CLV3 peptide, and not the entire fusion protein, was present in the extracellular medium (De Marchis et al. 2018). Our idea is that the maturation mechanism of CLV3 emerged when photosynthetic organisms evolved into vascular plants, therefore unicellular algae should have not developed this mechanism. To prove our hypothesis, we transformed cells of *C. reinhardtii* to express CLV3-GFP. Secretion of the intact CLV3-GFP protein in the algal growth medium was detected, corroborating our hypothesis. Moreover we show , by using a physiological assay based on root growth inhibition, that the production of the active 12 amino acid peptide is absent in algae of the genus *Chlamydomonas*. In the future, we will try to discover which genes are involved in the maturation mechanism of CLV3.

#### **644 - A fungal sRNA may guide the silencing of host plant genes in the arbuscular mycorrhizal symbiosis**

**William Ledford** <sup>(1)</sup> - **Alessandro Silvestri** <sup>(1)</sup> - **Valentina Fiorilli** <sup>(1)</sup> - **Massimo Turina** <sup>(2)</sup> - **Raffaella Balestrini** <sup>(2)</sup>  
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Small RNAs (sRNAs) are short non-coding RNA molecules that regulate gene expression in eukaryotes via RNA interference (RNAi) (1). Interest in sRNAs has grown as we have discovered the fundamental roles they play in a wide variety of biological processes, such as developmental regulation and stress responses. sRNAs are also emerging as important signalling molecules in different inter-species, and even inter-kingdom, interactions (2). Indeed, mobile sRNAs can be transferred from “donor” to “receiver” organisms, where they can regulate host gene expression by exploiting the host’s molecular RNAi machinery. While this process, cross-kingdom RNAi, has been principally studied in plant-pathogen interactions, increasing evidence points towards mobile sRNAs as potential contributors to the inter-species molecular dialogue in plant mutualistic associations, including the arbuscular mycorrhizal (AM) symbiosis.

Previously, we characterized the sRNA population of the AM fungus *Rhizophagus irregularis* during the symbiosis with the host plant *Medicago truncatula* and we reported, based on in silico data, that dozens of fungal sRNAs have potential for host gene regulation through cross-kingdom RNAi (3). We chose to further characterize the fungal sRNA Rir-2216 in view of its high number of in silico predicted *M. truncatula* target genes. Utilizing *Agrobacterium*-mediated transient co-expression assays in tobacco leaves, we proved that Rir-2216 can silence two of the predicted plant targets: a WRKY transcription factor and a Lipid Transfer Protein. Moreover, by exploiting laser microdissection coupled to qRT-PCR assays, we observed that some Rir-2216 plant targets, including WRKY, showed a reduced transcript accumulation in specific populations of root cortical cells colonized by *R. irregularis* compared to corresponding cells from non-colonized roots. We are currently characterizing the targets of Rir-2216, but the data already obtained suggest that fungal sRNAs can silence host plant gene expression in the AM symbiosis. These results represent an important step towards understanding the sRNA-mediated dialogue in this mutualistic interaction.

### 13 - PII signal transduction proteins in plants: conservation and evolution of structure and sensory properties.

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The PII signal-transduction superfamily is widespread in all domains of the life, representing one of the largest and most ancient families of signaling proteins in nature. Signaling proteins of the PII superfamily are characterized by their highly conserved trimeric structure. Canonical PII proteins sense the cellular energy state through competitive binding of ATP and ADP and sense the C/N balance through 2-oxoglutarate binding (2-OG). The ancestor of Archaeplastida inherited the PII signal transduction protein from the ancient cyanobacterial endosymbiont. In plants, the major interaction partner of PII is the controlling enzyme of arginine synthesis, N-acetyl-L-glutamate kinase (NAGK). The plant PII proteins are not covalently modified and its mode of action has been shown to require direct binding of effector molecules. The results suggest that PII signalling system of red algae represents an intermediary state between Cyanobacteria and Chlorophyta. To our data, in the course of evolution, plant PII proteins acquired a glutamine-sensing C-terminal extension, present in all Chloroplastida PII proteins (except Brassicaceae family). Sensing glutamine as the primary product of nitrogen assimilation indicates that in the Chloroplastida, PII became specialized to respond to the nitrogen status. In agreement with glutamine becoming the dominant signal for plant PII proteins, the sensory properties of PII proteins that were determined from different Chlorophyta towards the ATP/ADP status or towards 2-OG are quite variable. Among these organisms, the colorless alga *Polytomella parva* is a special case, as its the PII-NAGK system has lost the ability to estimate the cellular energy and carbon status but has specialized to provide an entirely glutamine-dependent arginine-feedback control. The observed differences towards the effector molecules between PII proteins from different representatives of Chlorophyta and higher plants demonstrate the evolutionary plasticity of PII signaling system. (This research was funded by Russian Science Foundation, N 16-14-10004, and DFG, Fo195/9-2, Fo195/13-1).

## 29 - Novel results of application of methylglyoxal for priming crop plants

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Many abiotic and biotic stress lead to the generation of reactive oxygene species (ROS) and lipid peroxidation, the subsequent damage of membrane lipids. Beside ROS, certain lipid peroxidation breakdown products, the reactive aldehydes (RA) also contribute to the cellular damage. In nature, many effective defence mechanisms have evolved for RA-detoxification in the cell, including the glyoxalase system, glutathione-S-transferase, alkenal/alkenone reductases, aldehyde dehydrogenases and aldo-keto reductases. However to an extent, the increased ROS and RA level can still be considered physiological, acting as signal to activate stress-tolerance mechanisms. The signal can also be added from outside. Appropriate level of chemical hardening can facilitate growth and develop stress tolerance. Our aims were to find the possibility of priming, the optimum level of exogenously applied RA, named methylglyoxal (MG).

In the frame of our work, we found the adequate way of treatment and MG concentration which enhanced frost-hardiness of wheat in the absence of prior cold-acclimation from less than 10% to more than 30%. The role of several polyols and RA-scavenging enzymes, including glyoxalases and tau class glutathione S-transferases were found to be responsible for the frost-tolerance. On the other hand, exogenous MG facilitated the growth and assimilation of young maize seedlings at low temperature. The hormonal and transcriptome sequencing analysis revealed gene expressional changes which explain the differences in growth. Since maize is a basically cold-sensitive organism, our results open new perspectives to enhance cold-tolerance and extend the growth period of maize via chemical hardening.

### 31 - How does Arabidopsis escape from vegetative shade?

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**(2)** - **Jorge José Casal**<sup>(3)</sup> - **Christian Fankhauser**<sup>(1)</sup>

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In nature, light conditions can be highly dynamic and heterogeneous. Plants can use light as a signal to obtain information from the environment and adapt their growth. In dense vegetation, the far red (FR) light is reflected by leaves decreasing the red (R)/FR ratio. In a more dramatic situation, called canopy shade, not only the R/FR ratio decrease, but light is also filtered by leaves creating an environment with low PAR, low R and blue light. The shade-intolerant species *Arabidopsis thaliana* perceives these conditions as a signal of competition for light, which triggers several responses, such as hypocotyl elongation, leaves elevation, flowering acceleration, etc...

When a canopy shade situation is combined with a directional blue light signal, also phototropism is improved to position the photosynthetic organs towards the best source of light. Phototropism is mainly controlled by the blue light photoreceptors phototropins, which sense the direction of light and drive plant curvature. This peculiar response is due to asymmetrical cell growth, as a consequence of differential auxin distribution across the hypocotyl. In canopy shade, the availability of auxin in the seedlings increases because of new auxin biosynthesis, improving plants' growth towards light. Here we show that also cryptochromes, a class of photoreceptors sensing the intensity of blue light in the environment, modulate phototropism. If seedlings are in the presence of directional blue light in a full sunlight condition, cry1 avoids phototropism balancing the levels of the transcription factor PIF4. On the contrary, in presence of the same directional blue light signal but in a canopy shade situation, the inhibitory effect of cry1 is relieved, triggering the accumulation of PIF4, which positively regulates the hypocotyl re-orientation towards the upcoming blue light. These results highlight the capacity of plants to integrate different light stimuli, in order to adopt the best growth solution.

#### **44 - New insights into stomatal immunity and CO<sub>2</sub> responses revealed by guard cell 'omics**

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Human population is expected to reach 9 billion by 2050, and global crop productivity needs to increase by 70% to feed the growing population. Unfortunately, pathogen infection and other adverse environmental conditions have posed grand challenges to crop yield and food security. Stomatal pores are major entry points of bacteria pathogens. How stomatal guard cells respond to pathogen invasion and other environmental factors (e.g., rising CO<sub>2</sub> levels) is an important and interesting question. Recently, we have reported a new redox proteomics method called cystTMTRAQ that combines two types of isobaric tags, isobaric tag for relative and absolute quantification (iTRAQ) and cysteine tandem mass tag (cysTMT) in one experiment. The method not only enables simultaneous analysis of cysteine redox changes and total protein level changes, but also allows determination of bona fide redox modified cysteines in proteins through correction of protein turnover. This technology has recently been applied to discover potential redox proteins in stomatal guard cells in response to the flagellin's N-terminal domain's 22-aa peptide (flg22) of *Pseudomonas syringae* pv. tomato str. DC3000 (PstDC3000). Stomatal closure was observed within 5 minutes of the flg22 treatment and became significant after 15 minutes of treatment. Reactive oxygen species (ROS) levels increased throughout the time course of treatment, and reached the peak at 15 minutes. Based on these results, three time points (15, 30 and 60 minutes) were selected for the cystTMTRAQ experiments. A total of 2144 proteins were identified, 677 contained cysteines with cysTMT labels, and 57 showed significant redox changes ( $q < 0.05$ ) after flg22 treatment. Here I report the functional characterization of a lipid transfer protein in guard cell innate immunity. As CO<sub>2</sub> levels affect stomatal immunity and stomatal movement, we studied CO<sub>2</sub> signaling using hyphenated metabolomics technologies. A new signaling pathway involving jasmonic acid was discovered. Future directions in signal crosstalk and data integration will be discussed.

## 50 - Plastid and bacterial stringent response in plant-microbe interplay

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Signaling pathway of prokaryotic type named stringent response (SR) is activated in plant chloroplasts under abiotic and biotic stress. In bacteria and plants, the SR mediator is ppGpp, a specific signaling molecule that modulates the expression of a number of genes encoding defense factors such as bacterial stress sigma factor RpoS and plant salicylic acid signaling. Since the internal environment of the host organism is stressful for microorganisms, we assumed that under infection SR is activated both in the plant-host and in the pathogen. In our work we have studied coupled SR in bacterial pathogen *Pectobacterium atrosepticum* and in specific plant-host potato *Solanum tuberosum* or nonspecific plant-host tobacco *Nicotiana tabacum* under infection (in terms of gene expression).

Bacterial SpoT-dependent SR was activated in *Pectobacterium* when pathogen infected potato as well as tobacco plants. Plastid SR was not activated in potato plants under infection. At that time the induction of salicylic acid signaling pathway occurred and transcripts of key gene PR-1 accumulated in potato leaves. Symptoms of the disease developed slowly and only 53% of plants died after 25 days of inoculation. Pectobacterial infection induced SR in tobacco plants with the increase in the expression level of Ntrsh2 gene encoding ppGpp synthase. Jasmonate signal pathway was activated in tobacco leaves: expression of allene oxide cyclase and lipoxygenase genes increased by more than 27 and 360 times respectively. Rapidly progressive disease led to death 100% tobacco plants during 3 days of inoculation.

Thus, under infection SR is always activated in bacteria, but not always in plants. The lack of activation of SR in potato plants probably ensures a more prolonged coexistence of the phytopathogen with its host, which can be considered as a more perfect form of plant-microbe interactions developed during co-evolution.

This study was supported by RFBR 20-34-70043.

## 82 - H<sub>2</sub>O<sub>2</sub> and Ca<sup>2+</sup> interplay in conferring salt tolerance to rice

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Salinity tolerance is a complex trait and, despite many efforts to obtain crop plants resistant to the salt, few results have been achieved and a deeper understanding of the tolerance mechanisms is needed. By studying two Italian rice varieties with contrasting salt response, we found that salt tolerant plants adopted morphological, hormonal, and gene regulation changes in order to achieve the survival and resuming of growth. Calcium and ROS are well-known signal molecules in the transduction of external stimuli, like salt stress. By using Ca<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> fluorescent probes we observed peculiar dynamics of the two signaling molecules in the cytosol of salt-tolerant plants. In response to salt stress, an early and narrow H<sub>2</sub>O<sub>2</sub> burst was detected in the roots of the salt-tolerant rice variety while, in the sensitive variety, a later and long lasting H<sub>2</sub>O<sub>2</sub> production was observed. These different H<sub>2</sub>O<sub>2</sub> profiles were associated with a dissimilar regulation of genes involved in signal transduction, oxidative stress and ion homeostasis recovery thus leading to different fates: survival or death. The comparison between Ca<sup>2+</sup> dynamics in the cytosol of salt-tolerant and -sensitive cells showed a different response upon salt and H<sub>2</sub>O<sub>2</sub> application. Differences are related to H<sub>2</sub>O<sub>2</sub> signals and gene expression between the two rice varieties. Our results suggest that the acclimation mechanism adopted by tolerant plants is regulated by specific H<sub>2</sub>O<sub>2</sub> responses, putatively triggered by specific Ca<sup>2+</sup> signatures, that lead to the osmotic compensation, photosynthesis preservation and phenotypic plasticity needed for the survival of plants.

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## 107 - Early response in salicylic acid-treated roots of *Arabidopsis* glutathione transferase (Atgstf8 and Atgstu19) mutants

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Glutathione transferases (GSTs) play crucial role in detoxification processes due to their glutathione (GSH) conjugating activity, and through glutathione peroxidase or dehydroascorbate reductase activities they influence the redox state of GSH and ascorbate. Thiol redox biochemistry is considered to have pivotal role in cellular processes, thus maintaining equilibrium through antioxidant mechanisms is crucial. However, it is still an outstanding question what are the detailed mechanisms by which redox regulation occurs and what is the significance of GSTs in this process.

Seven-day-old *Arabidopsis thaliana* L. wild type (Col-0), Atgstf8 and Atgstu19 mutant seedlings were used to investigate the role of AtGSTF8 and AtGSTU19 in early salicylic acid (SA) responses of roots. Vitality and H<sub>2</sub>O<sub>2</sub> levels were measured with fluorescent microscopy. Redox state of GSH was estimated using redox-sensitive green fluorescent protein. Gene expression of selected GSH related sequences, AtGST genes and transcription factors were studied using high throughput quantitative real-time PCR.

Atgst mutants had more positive GSH redox potential (E<sub>GSH</sub>) compared to Col-0 throughout the experiment. E<sub>GSH</sub> values became more positive in the Col-0 plant after SA treatment however, it shifted toward more negative values in the mutants especially after 1 and 24 hours of treatments. The vitality was lower and H<sub>2</sub>O<sub>2</sub> levels were higher in the mutants after SA treatment. The expression of several investigated genes was induced in all lines, but there were only slight differences among the Col-0 and mutant plants.

According to our results AtGSTF8 and AtGSTU19 may be part of the redox state regulation under control conditions and in SA treatment. However, to reveal the exact molecular mechanisms through which they can act needs further investigations.

Financial support to the project was provided by the Hungarian National Research, Development and Innovation Fund (grant numbers are K 125265, PD 121027, PD 131909 and PD 131884).

**143 - A peptide PSEP1 encoded by a lncRNA regulates growth and response to stress conditions in the moss *Physcomitrella patens***

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The biological functions of peptides or microproteins encoded by short Open Reading Frames (sORFs) in plants are generally unknown. Based on known examples, it can be suggested that these peptides regulate the diverse range of cellular processes in plants, such as cell proliferation (ROT4), nodulation and sugar metabolism (ENOD40), pollen grains germination (Zm908), programmed cell death (KOD) and root growth (PLS). Using a mass-spectrometry approach, we discover a new regulatory 41-aa peptide PSEP1 (*Physcomitrella* sORF-encoded peptide) encoded by lncRNA in the moss *Physcomitrella patens*. The overexpression of PSEP1 increase the moss protonema growth rate but decrease stress tolerance. In particular, PSEP1 overexpressing plants are more sensitive to salt and oxidative stresses. Moreover, these plants were partly insensitive to abscisic acid (ABA). To estimate quantitative changes between wild type and mutant lines, the proteomic profiling analysis with isobaric tags for relative and absolute quantification labeling (iTRAQ) was performed. Overall, we identified 95 differently expressed proteins (DEP) in PSEP1 overexpressing line and 70 DEPs in a psep1 knockout mutant line. Based on proteomic analysis we show that PSEP1 can regulate primary metabolism, photosynthesis, translation and stress responses. To discover the mechanism of action of PSEP1 peptide we used a co-immunoprecipitation assay. We created the *Physcomitrella* mutant lines with overexpression of PSEP1 fused with FLAG tag and use its in further study. The list of possible protein partners of PSEP1 includes calmodulin, pathogen related protein 10 (PR-10) and non-symbiotic hemoglobin class 1. Calmodulin is a small conservative calcium-binding protein which involved in response to phytohormones, light, wounding, biotic and abiotic stresses. Both PR-10 protein and non-symbiotic hemoglobin class 1 takes part in a responses to different stresses. So, all of these proteins can be a link between PSEP1 peptide, ABA signaling and regulation of growth and stress responses.

### 153 - Transcriptomic Analysis of Arabidopsis thaliana plants treated with Cycloastragenol

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Cycloastragenol (CAG), a molecule isolated from 'Astragalus membranaceus', significantly stimulates the telomerase activity and cell proliferation. It is proven that CAG has ability to prevent some diseases in human. In this study, we aimed to figure out the CAG effects on the different signaling mechanisms in plants and to broadly analyze the genome-wide transcriptional responses in order to demonstrate CAG as a new key molecule that can potentially help plants to overcome different environmental stresses. RNA-seq strategy was employed to assess the transcriptional profiles in A. thaliana roots calli. Our work primarily focused an overall study on the transcriptomic responses of Arabidopsis to CAG. A total of 22,593 unigenes have been detected among which 1045 unigenes were differentially expressed. The up-regulated genes are principally involved in cellular and metabolic processes in addition to response to stimulus. The data analysis revealed genes associated with defense signaling pathways such as cytochrome P450s transporter, antioxidant system genes and stress responsive protein families were significantly upregulated. The obtained results can potentially help in better understanding biotic and/or abiotic tolerance mechanisms.

**167 - The mutations of phosphatidylinositol-4-kinases  $\beta 1$  and  $\beta 2$  affect cell division and cell elongation in Arabidopsis roots**

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Phosphoinositides (PIs) are minor structural components of plant cell membranes. They are characterized by negatively charged polar heads. PIs have emerged as important second messengers regulating growth, development and responses to environmental changes. PIs are produced by the phosphorylations of phosphatidylinositol by specific lipid kinases. Phosphatidylinositol 4-kinases (PI4Ks) generate phosphatidylinositol-4-phosphate and have been recently reported to participate in vesicular trafficking. The aim of our work is to decipher the role of PI4Ks and their products in plant morphogenesis, especially in root development. An Arabidopsis thaliana mutant, deficient in two isoforms of PI4Ks ( $pi4k\beta 1\beta 2$ ), shows stunted growth both in rosettes and roots. While the rosette dwarfism is connected with the accumulation of salicylic acid, the root phenotype appears to be independent on it. To reveal the mechanism beyond this phenotype, we analysed root elongation in various conditions.  $pi4k\beta 1\beta 2$  roots showed lower sensitivity to exogenous auxins and impaired cell elongation. Interestingly, while some defects at cytokinesis have been detected in  $pi4k\beta 1\beta 2$  root cells, the sensitivity to cytokinins and the expression of CycB1 were not affected in the double mutant. We found that response to gravistimulation is slower in  $pi4k\beta 1\beta 2$  mutant, however it is not connected with auxin gradient re-establishment. Otherwise, PIN2 internalization in response to light was impaired in  $pi4k\beta 1\beta 2$  mutants. Our work thus opens new insights into the involvement of phosphoinositides in morphogenesis through affecting phytohormonal signaling.

## **212 - HSP90 forms complexes with BRI1 and BAK1 to mediate active BR signalling**

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Heat Shock Protein 90 (HSP90) is a molecular chaperone that is required for the function of various substrate proteins that are collectively referred as clients, is considered also as one of the few well-connected hubs in molecular networks. HSP90 activities through signalling pathways and internal signals are interconnected with abiotic cues. In plants, HSP90 are involved in brassinosteroid (BR), auxin, and jasmonate signalling pathways. Brassinosteroids (BRs), a class of plant steroid hormones, are modulated by light and act synergistically with other hormones to promote development from early stages of embryogenesis to flower pattern formation. BRs bind either to preformed LRR receptor kinase BRI1 (BRASSINOSTEROID INSENSITIVE 1) homodimers which facilitates BRI1 ASSOCIATED RECEPTOR KINASE1 (BAK1) binding, or to a small pool of preformed BRI1-BAK1 heterooligomers.

The signaling pathway results in the activation of BRI1 EMS SUPPRESSOR1 (BES1) and BRASSINAZOLE RESISTANT1 (BZR1), transcriptional factors controlling BR-responsive gene expression via the inactivation of BRASSINOSTEROID INSENSITIVE 2 (BIN2).

Using genetic, fluorescence live cell imaging, molecular and biochemical approaches, such as, co-immunoprecipitation assay, yeast-two hybrid and Bimolecular fluorescence complementation (BiFC), we studied the ability of HSP90 to interact and form complexes with BRI1 and BAK1. The interaction investigated under active BR signaling and under inhibitor of the ATPase activity of HSP90.

## **221 - Transport of L-ascorbic acid across plant membranes: the role of ion channels and involvement to redox signaling, programmed cell death and growth regulation**

**Vadim Demidchik** <sup>(1)</sup>

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L-ascorbic acid is a major antioxidant and one the most abundant carbohydrates in plants. We still poorly understand how this molecule is transported within plants and whether it is involved in signaling and growth control. Here, we tested the hypothesis that plasma membrane anion channels catalyse the efflux of L-ascorbate from plant cells. We also report novel physiological functions of extracellular ascorbate, such as  $\text{Ca}^{2+}$  signaling and control of growth. Intriguingly, patch-clamp electrophysiological analyses demonstrated that efflux currents of L-ascorbate in *Arabidopsis thaliana* roots were comparable with chloride and malate currents. Biophysical properties of ascorbate conductance were similar to those that are mediated by malate-permeable ALMT channels. EPR spectroscopy tests showed that the level of extracellular ascorbyl radicals increased dramatically under salt stress (50-200 mM NaCl), osmotic shock, in the presence of elicitors and heavy metals. This is indicative of massive stress-induced ascorbate efflux. Exogenously added ascorbate (0,05-10 mM) induced transient elevation of cytosolic free  $\text{Ca}^{2+}$  triggering  $\text{Ca}^{2+}$  signaling events. The shape of ascorbate-induced  $\text{Ca}^{2+}$  signals resembled those that are produced by hydroxyl radicals. The ascorbate-induced  $\text{Ca}^{2+}$  elevation was inhibited by cation channel blockers, free radical scavengers and by removal of extracellular  $\text{Ca}^{2+}$ . Moreover, removal of the cell walls (in protoplasts) resulted in disappearance of ascorbate-induced  $\text{Ca}^{2+}$  transients. Copper and iron stimulated ascorbate-induced  $\text{Ca}^{2+}$  elevation while other transition metals, such as nickel and manganese, inhibited this reaction. Phenotyping of ascorbate effects on growth demonstrated that 0,01-0,3 mM L-ascorbate stimulated plants growth while 0,3-10 mM ascorbate inhibited this processes. Ascorbate also triggered programmed cell death and autophagy in the range of species/models and induced changes in *Arabidopsis* proteome demonstrating specific signaling and redox proteome modifications. This work was supported by Pearl River Fellowship to VD and Belarus-China Exchange Project (Ministry of Science of China and Belarusian SCST).

### **233 - The mitochondrial nucleoid WHIRLY2 protein plays a key role in Arabidopsis thaliana during development and stress response**

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WHIRLY2 is a single-stranded DNA binding protein associated with mitochondrial nucleoids. Although the why 2-1 mutant does not show a macroscopic phenotype during vegetative growth, a major proportion of the mutant leaf mitochondria owns an aberrant structure characterized by disorganized nucleoids, reduced abundance of cristae and a low matrix density. These features coincide with impairment in dynamics and functionality of mitochondria.

In contrast to vegetative growth, embryo development and seeds germination are compromised in the why 2-1 mutant. The energy demand due to a greater metabolism rate, required for cell division and differentiation in these early phases of development, could account for the crucial role of WHIRLY2. We observed, indeed, that WHIRLY2 is highly expressed during embryo development and the early stages of seed germination, when compared to other plant life phases..

Experimental evidences show the importance of WHIRLY2 also in plants exposed to abiotic stress. In particular, the absence of WHIRLY2 strongly compromises the ability of mutant plant to build-up an efficient drought stress resistance response.

**248 - PKS4 has a role in the interaction between shade and wounding in the control of leaf positioning**  
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Within dense communities, competition for light resources affects plant growth and leads to the shade-avoidance syndrome (SAS), characterized by elongation of vegetative organs and upward leaf movements (hyponasty) to favor access to unfiltered sunlight. In parallel, attacks from herbivores induce defense mechanisms depending on the jasmonate (JA) pathway and are associated with growth inhibition. Previous studies have shown that shaded plants challenged with herbivores, or more generally with pathogens, prioritize growth over defense. Our aim was to investigate the potential interaction between shade-avoidance and JA pathways on leaf growth and movement, using repetitive wounding of leaf blades to mimic herbivore attacks. Phenotyping experiments with combined treatments on *Arabidopsis thaliana* rosettes revealed that shade inhibits the wound effect on leaf elevation while both stimuli act additively for petiole elongation. Thus the relationship between shade and wounding pathways varies depending on the physiological response.

To identify regulatory genes controlled in opposite ways by these stimuli, we analyzed gene expression patterns of petioles upon single and combined treatments by RNA-sequencing. We chose to focus on genes with expression patterns matching the hyponastic response (opposite regulation by both stimuli, interaction between treatments with shade dominating wound signal). Our hypothesis is that some of these genes may be important for the regulation of leaf movement and the interaction between shade and wounding. Among them, we found PKS4, one of the four members of the PKS (Phytochrome Kinase Substrate) gene family, which was mostly studied for its role in phototropism. Interestingly we observed less suppression of wounding effect by shade in a *pks2pks4* double mutant while a PKS4 overexpressing line showed constitutively elevated leaves and was less sensitive to wounding. This result is in total agreement with our hypothesis, which makes PKS genes good candidates controlling the interaction of shade and wounding regulating leaf hyponasty.



### 395 - Contribution of Alternative Splicing in Plants and Animals in Response to Different Types of Stimuli

**Guiomar Martin** <sup>(1)</sup> - **Yamile Marquez** <sup>(2)</sup> - **Paula Duque** <sup>(1)</sup> - **Manuel Irimia** <sup>(2)</sup>

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Plants adapt their development to different environmental and developmental cues by rewiring their transcriptomes and proteomes. During the last decades, much effort has been put into understanding how signaling cascades initiated by multiple stimuli control transcriptome diversification. These research efforts have focused mainly on the role of transcription factors in regulating gene expression. However, recent studies are beginning to uncover a key contribution of alternative splicing, a posttranscriptional mechanism that generates multiple transcripts from the same gene, to the diversification of plant transcriptomes. Despite functional evidence pointing to alternative splicing as a major player in the adaptation of plant development to different external and internal stimuli, little is known about the molecular mode of action of these mechanisms from a genomic point of view. Does alternative splicing have a similar impact on protein levels and on protein diversity in response to different stimuli? Is this impact comparable to that of animals? And, finally, are the genomic features associated with these responsive alternative splicing events similar between plants and animals? To assess the similarities and differences between the molecular processes controlled by alternative splicing in different multicellular organisms, we have first implemented vast-tools for the model plant *Arabidopsis thaliana*, a computational pipeline to detect and quantify all types of alternative splicing events from RNA-seq data (<https://github.com/vastgroup/vast-tools>). We then used this pipeline to quantify alternative splicing from RNA-seq samples available in the Short Read Archive (SRA) for various environmental conditions and differentiated tissues in *A. thaliana*. We compared the regulatory and functional patterns observed in *A. thaliana* with those of three animals with very distinct exon-intron architectures (humans, fruitflies and round worms), shedding light on the particularities of the mode of action and biological functions of alternative splicing in response to different stimuli in plants.

#### 400 - Investigation of Selenium-Binding Protein 1 interactions

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**NATIONAL & KAPODISTRIAN UNIVERSITY OF ATHENS, DEPARTMENT OF BOTANY, FACULTY OF BIOLOGY, ATHENS, Greece** <sup>(1)</sup>

Selenium-Binding Protein 1 (SBP1) is a highly conserved widespread protein in all life kingdoms. SBPs are not members of the selenoproteins as they don't contain the modified amino acids of selenomethionine and selenocysteine, however they bind selenium in a covalent manner. Moreover, SBP1 binds cadmium. Its human homologue is connected with many malignancies and it has been proposed as a pharmacological target and biomarker for psychotic diseases. Studies in different plant species such as *Oryza sativa*, *Triticum aestivum*, *Theobroma cacao*, *Lotus japonicus* and *Arabidopsis thaliana* indicate that SBP participates in a cadmium / selenium detoxification mechanism. Furthermore, SBP1 enhances tolerance not only against abiotic stress but against pathogenetic fungi and bacteria, as well. In *Arabidopsis thaliana* there is evidence that indicate the participation of SBP1 in a novel protein network, as it is able to interact with a GAPDH and FBA, as well as, with other proteins related to vesicle trafficking, membrane synthesis and redox control of cells. In our study, we have shown that SBP1 interacts in planta with the glutaredoxins AtGRX14 and AtGRX16, the phospholipase A1, AtDALL3 and the papain-like protease AtRD19c. Glutaredoxins are connected to the regulation of the redox state of chloroplasts, while AtDALL3 has crucial role in the biosynthesis of jasmonic acid, a hormone necessary for stress responses. Our lab has also revealed interactions of AtSBP1 with the papain-like cysteine protease, AtRD19c that is probably involved in the activation of programmed cell death. Glutaredoxins and AtDALL3 are localized in chloroplasts with different patterns of expression while AtRD19c is localized probably in vacuoles. The interaction of AtSBP1-AtDALL3 complex is localized in chloroplasts while that with AtRD19c is probably in the vacuoles. Interestingly, the AtSBP1-AtGRX14 interaction takes place in the cytoplasm and nucleus, while the respective SBP1-AtGRX16 only in the cytoplasm.

#### **404 - Hemoglobins 1 and 2 are involved in the acclimation to phosphorus deficiency via control of nitric oxide levels in Chlamydomonas**

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Truncated hemoglobins (trHbs) form a widely distributed family of proteins found in archaea, bacteria, and eukaryotes. Truncated hemoglobin sequences are 20–40 amino acid residues shorter than full-length hemoglobins and form a characteristic helix arrangement folded in a 2-on-2  $\alpha$ -helical sandwich. Accumulating evidence suggests that trHbs may be implicated in functions other than oxygen delivery, but these roles are largely unknown. Characterization of the conditions that affect trHb expression and investigation of their regulatory mechanisms will provide a framework for elucidating the functions of these globins. Two THB family members, THB1 and THB2, were expressed at the highest level. For the first time, we demonstrate the synthesis of nitric oxide (NO) under P-limiting conditions and the production of NO by Chlamydomonas cells via a nitrate reductase-independent pathway. To clarify the functions of THB1 and THB2, we generated and analyzed strains in which these THBs were strongly under-expressed by using an artificial microRNA approach. Similar to THB1 knockdown, the depletion of THB2 led to a decrease in cell size and chlorophyll levels. We provide evidence that the knockdown of THB1 or THB2 enhanced NO production under P deprivation. Moreover, the THB1- and THB2-downregulation led to mis-regulation of P-deprivation induced genes. PSR1 is a well-studied transcription factor that regulates P-metabolism in Chlamydomonas. To our data, the accumulation of PSR1 transcripts were affected in the amiTHB1- and amiTHB2-strains starved for P. Overall, these results demonstrate that THB1 and THB2 are likely to contribute, at least in part, to acclimation responses in P-deprived Chlamydomonas. (This research was funded by Russian Science Foundation, N 21-14-00017).

#### **417 - On the Mechanism of MAPKKK18 degradation by the ubiquitin–proteasome pathway**

**Małgorzata Tajdel-Zielińska<sup>(1)</sup> - Małgorzata Marczak<sup>(1)</sup> - Maciej Janicki<sup>(1)</sup> - Agnieszka Ludwików<sup>(1)</sup>**

**Department of Biotechnology, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland<sup>(1)</sup>**

The MAPKKK18-mediated MAPK module harbouring downstream MAPKK3 and MPK1/2/7/14 is involved in ABA signalling, drought tolerance and senescence in *Arabidopsis thaliana*. Available studies demonstrate that the stability of MAPKKK18 is regulated by the ubiquitin proteasome system (UPS) via the ABA core pathway. However, the tight control of MAPKKK18 protein turnover is still not fully characterized.

Plants utilize UPS to modulate growth and development. The importance of the UPS in plants is exemplified by the fact that nearly 6% of the of the *Arabidopsis thaliana* genome is involved in UPS-related functions. UPS involves the covalent attachment of one or more ubiquitin (Ub) proteins to a specific lysine residue within target proteins through a consecutive action of three enzymes: E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzymes and E3 ubiquitin-protein ligases. Among these enzymes, in response to various cellular cues, a critical role is played by E3 ubiquitin ligases that recognize specific protein substrate and catalyses the transfer of activated ubiquitin to the target protein.

To elucidate the molecular mechanism of MAPKKK18 degradation we use multiple approaches. Using computational methods we selected potential ubiquitination sites, which were further analysed by molecular studies. Here we show that the arginine substitution on lysine 154 (K154) has significant effect on MAPKKK18 stability, compared to the wild-type protein. In addition to that we found that the turnover of ABA-regulated MAPKKK18 is controlled by specific E3 ligases, belonging to HECT and RING family, respectively. Collectively, this study provides a mechanistic understanding of how MAPKKK18 abundance is regulated by the the ubiquitin-proteasome pathway.

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#### **450 - OsPIL15 and OsPIL16 are key inducers of coleoptile growth in rice**

**André Cordeiro <sup>(1)</sup> - Catarina Monteiro <sup>(1)</sup> - Guilherme Leitão <sup>(1)</sup> - João Costa <sup>(1)</sup> - Pedro Barros <sup>(1)</sup> - Tiago Lourenço <sup>(1)</sup> - Nelson Saibo <sup>(1)</sup>**

**Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal**  
**(1)**

Elongation of coleoptile is essential to ensure emergence of rice seedling from soil and is highly regulated by light conditions. Light represses coleoptile growth, being its maximum growth rate and final length observed under dark. However, the regulatory mechanisms underlying this process remains largely unknown. In the plant model *Arabidopsis thaliana*, phytochromes, the Red/Far-Red photoreceptors, are activated by light and induce the degradation of Phytochrome-Interacting Factors (PIFs), a bHLH transcription factor family, by targeting them for degradation. PIFs are therefore more stable under dark, when they promote plant growth. In rice, six PIF-like (OsPIL) are identified and we have produced rice single knockout mutants for each OsPIL using the CRISPR/Cas9 technology. When we analyzed the above ground development of the different mutants grown under dark, we observed that OsPIL15-KO and OsPIL16-KO show shorter coleoptile and longer leaves, as compared to WT, a phenotype that resembles light grown seedlings. In addition, under Red or Far-Red no differences were observed between WT, OsPIL15-KO, and OsPIL16-KO mutants, suggesting that both OsPILs are degraded by Red and Far-Red. We also observed that light-mediated coleoptile repression is dependent on light quality, Red plays a stronger repression than Far-Red, suggesting that other OsPILs might play a minor role in coleoptile elongation under Far-Red.

Overall, our results suggest that both OsPIL15 and OsPIL16 promote coleoptile growth and that the light-induced repression of coleoptile elongation is mediated by the degradation of both OsPIL15 and OsPIL16. We are now analyzing the transcriptome of dark- and light-grown mutants. These data will be shown and discussed in order to understand the molecular mechanisms underlying rice coleoptile elongation. This study will contribute for the identification of candidate genes that in the future can be used in breeding programs to improve seed emergence under adverse conditions, such as low temperature, water deficit, etc.

#### 455 - Fatty acid desaturation as a trigger to grapevine immunity towards downy mildew

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Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ci4ncias da Universidade de Lisboa, Lisboa, Portugal <sup>(1)</sup>

Viticulture has been highly affected by several diseases. Downy mildew, caused by the obligatory biotrophic oomycete *Plasmopara viticola*, is one of the most devastating grapevine (*Vitis vinifera* L.) diseases worldwide. To cope with it, the current strategies rely on the massive use of phytochemical compounds on each cultivation season. Understanding the grapevine defence mechanisms is vital to develop alternative and sustainable disease control approaches. We have provided evidences about the role of fatty acids (FA) and lipid signalling in the establishment of the incompatible grapevine-*Plasmopara viticola* interaction. In response to abiotic and biotic stresses, changes in membrane lipid composition can alter membrane properties. Moreover, membrane lipids and FA can act themselves as signalling molecules or can be channelled to biosynthetic pathways of other signalling molecules, such as jasmonic acid (JA). In a tolerant grapevine genotype (*Vitis vinifera* cultivar Regent) there is an accumulation of JA, activation of its biosynthetic pathway and accumulation of its precursor,  $\alpha$ -linolenic acid (C18:3), a polyunsaturated FA. The generation of unsaturated FA results from the activity of FA desaturases, that catalyse the insertion of double bonds into the FA chains of glycerolipids. Despite being an essential process in lipid signalling events, FA desaturation has not yet been studied in grapevine response to pathogens. Here we characterized for the first time the FA desaturase's gene family in grapevine and demonstrate their involvement in defence response to the downy mildew agent. In the first hours upon pathogen inoculation, Regent showed an increase of the expression of genes encoding FA desaturases acting on galactolipids, the major lipids of chloroplast membranes. Reenforcing these evidences, a progressive FA desaturation in galactolipids was also observed, culminating in an increase in C18:3 content. Our work provides a new insight on the involvement of lipids in grapevine signalling processes in response to *Plasmopara viticola*.

#### 496 - H<sub>2</sub>O<sub>2</sub> and Ca<sup>2+</sup> interplay in conferring salt tolerance to rice

Federica Massa <sup>(1)</sup> - Bushra Ijaz <sup>(2)</sup> - Luca Brunello <sup>(1)</sup> - Piergiorgio Stevanato <sup>(3)</sup> - Elisabetta Barizza <sup>(1)</sup> - Mingxing Cai <sup>(1)</sup> - Michela Zottini <sup>(1)</sup> - Fiorella Lo Schiavo <sup>(1)</sup> - Elide Formentin <sup>(1)</sup>

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Salinity tolerance is a complex trait and, despite many efforts to obtain crop plants resistant to the salt, few results have been achieved and a deeper understanding of the tolerance mechanisms is needed. By studying two Italian rice varieties with contrasting salt response, we found that salt tolerant plants adopted morphological, hormonal, and gene regulation changes in order to achieve the survival and resuming of growth. Calcium and ROS are well-known signal molecules in the transduction of external stimuli, like salt stress. By using Ca<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> fluorescent probes we observed peculiar dynamics of the two signaling molecules in the cytosol of salt-tolerant plants. In response to salt stress, an early and narrow H<sub>2</sub>O<sub>2</sub> burst was detected in the roots of the salt-tolerant rice variety while, in the sensitive variety, a later and long lasting H<sub>2</sub>O<sub>2</sub> production was observed. These different H<sub>2</sub>O<sub>2</sub> profiles were associated with a dissimilar regulation of genes involved in signal transduction, oxidative stress and ion homeostasis recovery thus leading to different fates: survival or death. The comparison between Ca<sup>2+</sup> dynamics in the cytosol of salt-tolerant and -sensitive cells showed a different response upon salt and H<sub>2</sub>O<sub>2</sub> application. Differences are related to H<sub>2</sub>O<sub>2</sub> signals and gene expression between the two rice varieties. Our results suggest that the acclimation mechanism adopted by tolerant plants is regulated by specific H<sub>2</sub>O<sub>2</sub> responses, putatively triggered by specific Ca<sup>2+</sup> signatures, that lead to the osmotic compensation, photosynthesis preservation and phenotypic plasticity needed for the survival of plants.

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## TOPIC:

### Chloroplast biology

#### **Keynote Lecture**

**From singlet oxygen signaling to chloroplast protein import pathways: An unforeseen adventure.**

**Jun Fang, Bingqi Li, Vivek Dogra, and Chanhong Kim**

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Chloroplast-generated singlet oxygen ( $^1\text{O}_2$ ) alters nuclear transcriptome via retrograde signaling pathways, contributing to foliar acclimation and cell death responses. An *Arabidopsis crumpled leaf (crl)* mutant deficient in plastid binary fission is known to express  $^1\text{O}_2$ -responsive nuclear genes constitutively. The *crl* gigantic chloroplasts induce autoimmune responses through lipid peroxidation-dependent retrograde signaling. In the present study, via a forward genetic screen, we unveil dominant gain-of-function TRANSLOCON AT THE INNER ENVELOPE OF CHLOROPLAST (TIC236) mutations, each of which abolishes the autoimmune responses and rescues the plastid-division defect in *crl*. This result indicates the shared functionality between CRL (mainly located in the outer envelope of chloroplast) and TIC236 proteins. Ensuing reverse genetic analyses show CRL's genetic interaction with SP1, a RING-type ubiquitin E3 ligase, and the intrinsic inner envelope membrane FTSH11 protease, whose functions are implicated in TOC and TIC turnover, respectively. Either loss of *SP1* or *FTSH11* rescues *crl* phenotypes in varying degrees due to increased translocon levels. Consistent with the impaired plastid division in both *crl* and *tic236*-knockdown mutants, CRL interacts with the preproteins of plastid-division machinery, and TIC236GF reinforces their import. Last but not least, we found these GF mutations significantly stabilize TIC236 proteins. We collectively shed new light on the probable link between protein import defect and plant innate immunity.

## TOPIC:

### Chloroplast biology

#### **Oral Communications**

##### **105 - Detox them all! Promiscuous detoxification contributes to plant stress tolerance**

**Stefano D'Alessandro** <sup>(1)</sup> - **Brigitte Ksas** <sup>(2)</sup> - **Michel Havaux** <sup>(2)</sup>

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Living organisms are exposed to many potentially toxic compounds, both from ectopic sources, like pollutants, and from endogenous sources. For example, under excessive light, saturation of the photosynthetic electron transport chain leads to a ROS burst generating apocarotenoids and lipid peroxides. These two molecular families have opposite effects:  $\beta$ -cc and dha, two apocarotenoids, can induce stress acclimation, while Reactive Carbonyl Species, such as 4-hydroxynonenal, lead to cell death. We discovered that the protective action of  $\beta$ -cc is mediated by the induction of the SCL14 dependent xenobiotic detoxification response. Consistent with this, mutants of SCL14 show lower stress tolerance and overexpressing SCL14 enhances plant performance under conditions causing photooxidative stress such as excess light and drought. Finally, we show that apocarotenoids generated during photosynthesis signal the oxidative status of the chloroplast and elicit an additional level of photo-protective mechanisms.

#### **477 - LPA2 protein is involved in Photosystem II assembly in *Chlamydomonas reinhardtii***

**Matteo Ballottari**<sup>(1)</sup> - **Michela Cecchin**<sup>(1)</sup> - **Jooyeon Jeong**<sup>(2)</sup> - **Woojae Son**<sup>(3)</sup> - **Minjae Kim**<sup>(2)</sup> - **Seunghye Park**<sup>(2)</sup> - **Luca Zuliani**<sup>(1)</sup> - **Stefano Cazzaniga**<sup>(1)</sup> - **Andrea Pompa**<sup>(4)</sup> - **Chan Young Kang**<sup>(3)</sup> - **Sangsu Bae**<sup>(3)</sup> - **EonSeon Jin**<sup>(2)</sup>

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Light-dependent photosynthesis in photosynthetic eukaryotes requires the proper assembly of photosystem II (PSII). In *Arabidopsis thaliana*, one of the PSII subunits (CP43/PsbC) was suggested to be assembled into the PSII complex via its interaction with an auxiliary protein called Low PSII Accumulation 2 (LPA2). However, the original articles describing the role of LPA2 in PSII assembly in higher plants have been retracted. To investigate the function of LPA2, here we generated in the model organism for green algae, *Chlamydomonas reinhardtii*, knockout *lpa2* mutants by using the CRISPR-Cas9 target-specific genome-editing system. Biochemical analyses revealed the thylakoidal localization of LPA2 protein in the WT while *lpa2* mutants were characterized by a drastic reduction in the level of D1, D2, CP47 and CP43 proteins. Consequently, reduced PSII supercomplex accumulation, chlorophyll content per cell, PSII quantum yield and photosynthetic oxygen evolution were measured in the *lpa2* mutants, leading to an almost impairment of photoautotrophic growth. Pulse-chase experiments demonstrated that the absence of LPA2 protein caused a reduced PSII assembly and reduced PSII turnover. Reduced PSII activity was only partially compensated in *lpa2* mutants by increased cyclic electron transport, and state transition. Taken together, our data indicate that, in *Chlamydomonas reinhardtii*, LPA2 is required for PSII assembly and its proper function.

**486 - GUN1 promotes the accumulation of NEP-dependent transcripts and chloroplast protein import upon perturbation of plastid protein homeostasis in Arabidopsis cotyledons**

**Luca Tadini** <sup>(1)</sup> - **Carlotta Peracchio** <sup>(1)</sup> - **Andrea Trotta** <sup>(2)</sup> - **Monica Colombo** <sup>(3)</sup> - **Ilaria Mancini** <sup>(2)</sup> - **Nicolaj Jeran** <sup>(1)</sup> - **Alex Costa** <sup>(1)</sup> - **Franco Faoro** <sup>(4)</sup> - **Milena Marsoni** <sup>(5)</sup> - **Candida Vannini** <sup>(5)</sup> - **Eva-Mari Aro** <sup>(2)</sup> - **Paolo Pesaresi** <sup>(1)</sup>

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Correct chloroplast development and functioning require the orchestrated expression of plastid and nuclear genes and rely on a complex network of signals that flow from the plastid towards the nucleus. The plastid-located protein GUN1 (genomes uncoupled 1) plays a central role in the chloroplast-to-nucleus communication. GUN1 was found to physically interact with factors involved in chloroplast protein homeostasis and with enzymes involved in tetrapyrrole biosynthesis that act in various retrograde signalling pathways. Although a clear and unified view of GUN1's role is still missing, we recently showed that GUN1 promotes the activity of the Nuclear-Encoded plastid RNA Polymerase (NEP) upon impairment of chloroplast protein homeostasis, having an impact on chloroplast protein import and cytosolic folding stress. Biochemical and genetic evidences suggest that this response becomes critical for the correct proplastid-to-chloroplast differentiation when plastid protein synthesis, folding, import or degradation are perturbed. Here we show and discuss the recently identified connections between plastid RNA metabolism and retrograde signalling, by providing an extended concept of GUN1 activity, which integrates the multitude of functional genetic interactions with its primary role in plastid transcription and transcript editing.

## TOPIC:

### Chloroplast biology

#### **Extended Elevator Pitches**

**198 - Chlorophyll b and far-red light influence permeability of plasmodesmata in leaves of *Arabidopsis thaliana***

**Valeriia Dmitrieva**<sup>(1)</sup> - **Valentina Domashkina**<sup>(1)</sup> - **Elena Tyutereva Tyutereva**<sup>(1)</sup> - **Alexandra Ivanova**<sup>(1)</sup> - **Olga Voitsekhovskaja**<sup>(1)</sup>

**Komarov Botanical Institute of the Russian Academy of Sciences, Laboratory of molecular and ecological physiology, Saint-Petersburg, Russian Federation**<sup>(1)</sup>

Plasmodesmata (PD) allow cell-to-cell exchange of water, ions, nutrients, hormones and informational macromolecules like mRNAs, small RNAs or transcription factors. The ability to adjust the PD size exclusion limit (SEL) is critical for plant performance in any environment at every stage of a plant's life. Photosynthesis regulates a plethora of cell functions via the reduction state of thioredoxins which is controlled by electron supply from PSI. At the same time, photosynthesis can serve as a source of ROS, which act as signal molecules while at high levels damage cells. Both thioredoxins and ROS levels influence the number and permeability of PD.

We investigated the relationship between the functions of PSI and PSII, ROS production and PD permeability using *Arabidopsis* mutants with changed levels of Chlb (ch1-3 lacking Chlb and PhCAO transgenic plants over-accumulating Chlb) as well as *trxm3* and *ntrc* mutants impaired in thioredoxin functions. Numbers of PD were studied by TEM and immunohistochemistry, the functions of PSII and PSI were analyzed using a DUAL-PAM 100 (Walz, Germany), ROS were detected using fluorescent probes with a special attention paid to singlet oxygen production. Callose was quantified using aniline blue staining, and PD permeability was estimated using a symplastic tracer.

The results showed that in *Arabidopsis* leaves, performance of PSI and PSII depended on Chlb-levels and on the presence of *trxm3*. The levels of chlorophyll b inversely correlated with PD SEL. Most interestingly, an increase in the levels of far-red light in the illumination spectrum influenced PD SEL in the mutants via metabolism of PD-associated callose. Altogether, our results suggest that photosynthesis exerts control over PD functions not only via ROS levels and thioredoxins but also via supply of energy to leaf cells.

Supported by the Russian Foundation for Basic Research (project # 18-34-00821) and Russian Science Foundation (project #14-16-00120-P).

### 231 - Chloroplast-localized ion channels in the modulation of stress response in higher plants

**Luca Carraretto** <sup>(1)</sup> - **Enrico Teardo** <sup>(1)</sup> - **Roberto Moscatiello** <sup>(1)</sup> - **Enrico Cortese** <sup>(1)</sup> - **Mattia Vicario** <sup>(1)</sup> - **Margherita Festa** <sup>(1)</sup> - **Lorenzo Maso** <sup>(1)</sup> - **Sara De Bortoli** <sup>(1)</sup> - **Tito Cali** <sup>(2)</sup> - **Andrei Herdean** <sup>(3)</sup> - **Ute C. Vothknecht** <sup>(4)</sup> - **Elide Formentin** <sup>(1)</sup> - **Laura Cendron** <sup>(1)</sup> - **Lorella Navazio** <sup>(1)</sup> - **Cornelia Spetea** <sup>(3)</sup> - **Ildikò Szabó** <sup>(1)</sup>

**Department of Biology, University of Padova, Padova, Italy** <sup>(1)</sup> - **Department of Biomedical Sciences, University of Padova, Padova, Italy** <sup>(2)</sup> - **Department of Biological and Environmental Sciences, University of Göteborg, Göteborg, Sweden** <sup>(3)</sup> - **IZMB – Plant Cell Biology, University of Bonn, Bonn, Germany** <sup>(4)</sup>

Chloroplasts sense biotic and abiotic stress in plants, but their role in transducing  $\text{Ca}^{2+}$ -mediated stress signals is still poorly understood. cMCU, a member of the mitochondrial calcium uniporter (MCU) family, is an ion channel mediating  $\text{Ca}^{2+}$  flux into chloroplast in vivo. Aequorin reporters targeted to chloroplast stroma and the cytosol in wild-type and knock-out lines for cMCU, provided evidence that stress stimulus-specific  $\text{Ca}^{2+}$  dynamics in the chloroplast stroma correlate with expression of the channel. Fast downstream signalling events triggered by osmotic stress, involving activation of the mitogen-activated protein kinases (MAPK) MAPK3 and MAPK6, and also the transcription factors MYB60 and ethylene-response factor 6 (ERF6), are influenced by cMCU activity. Relative to wild-type plants, cMCU knock-out ones display increased resistance to long-term water deficit and improved recovery on re-watering. Modulation of stromal  $\text{Ca}^{2+}$  in specific processing of stress signals identifies cMCU as a component of plant environmental sensing.

$\text{Ca}^{2+}$  represents the most important second messenger in plant cell, but  $\text{Ca}^{2+}$  channels are not the only players of stress response modulation. In particular, a newly-identified  $\text{K}^+$  channel in the chloroplast envelope is indirectly involved in  $\text{Ca}^{2+}$  dynamics regulation during abiotic stress exposure. Aequorins targeted to chloroplast stroma and cytosol revealed a statistically-significant increase in  $\text{Ca}^{2+}$  transients triggered by mannitol (that mimics drought stress) in both compartments of knock-out plants respect to wild-type controls. Further, mass spectrometry analysis indicated also a clear correlation between this  $\text{K}^+$  channel and chlorophyll synthesis; preliminary experiments shown that mutants have a lower chlorophyll content respect to wild-type.

The search for the link between ion channels of plant bioenergetic organelles and stress response (or development) is still pioneering, but the limited knowledge already acquired is very promising. Understanding of plant response mechanisms will open new paths to increase resistance and, therefore, plant productivity, useful for food purposes.

## TOPIC:

### Chloroplast Biology

#### Posters

**62 - GUN1 influences the accumulation of NEP-dependent transcripts and chloroplast protein import in *Arabidopsis thaliana* cotyledons**

Luca Tadini<sup>(1)</sup> - Carlotta Peracchio<sup>(1)</sup> - Andrea Trotta<sup>(2)</sup> - Monica Colombo<sup>(3)</sup> - Ilaria Mancini<sup>(1)</sup> - Nicolaj Jeran<sup>(1)</sup> - Alex Costa<sup>(1)</sup> - Franco Faoro<sup>(4)</sup> - Milena Marsoni<sup>(5)</sup> - Candida Vannini<sup>(5)</sup> - Eva-Mari Aro<sup>(2)</sup> - Paolo Pesaresi<sup>(1)</sup> - University of Milan, Biosciences, Milan, Italy<sup>(1)</sup> - University of Turku, Department of Biochemistry, Turku, Finland<sup>(2)</sup> - Fondazione Edmund Mach, Centro Ricerca e Innovazione, San Michele all'Adige, Italy<sup>(3)</sup> - University of Milan, Dipartimento di Scienze Agrarie e Ambientali, Milan, Italy<sup>(4)</sup> - Università degli Studi dell'Insubria, Dipartimento di Biotecnologie e Scienze della Vita, Varese, Italy<sup>(5)</sup>

Correct chloroplast development and function require coordinated regulation of chloroplast and nuclear gene expression. This is achieved through chloroplast signals that modulate nuclear gene expression in accordance with the chloroplast's needs. Genetic evidence indicates that Genomes Uncoupled 1 (GUN1), a chloroplast-localized pentatricopeptide-repeat (PPR) protein with a C-terminal Small MutS-Related (SMR) domain, is involved in integrating multiple developmental and stress-related signals in both young seedlings and adult leaves. Recently, GUN1 was found to interact physically with factors involved in chloroplast protein homeostasis, and with enzymes of tetrapyrrole biosynthesis in adult leaves that function in various retrograde signaling pathways. Here we show that, following perturbation of chloroplast protein homeostasis i) by growth in lincomycin-containing medium, or ii) in mutants defective in either the FtsH protease complex (ftsH), plastid ribosome activity (prps21-1 and prpl11-1) or plastid protein import and folding (cphsp70-1), GUN1 positively influences the Nuclear Encoded Polymerase (NEP)-dependent transcript accumulation during cotyledon greening and intervenes in chloroplast protein import.

**Key Message:** GUN1 positively modulates plastid NEP-dependent transcription and protein import, upon perturbations of plastid protein homeostasis, and is required for chloroplast-to-nucleus retrograde signaling.

**95 - The chloroplast signaling nucleotide ppGpp - diversity of metabolism and function in photosynthetic eukaryotes.**

Luisana Avilan <sup>(1)</sup> - Seddik Harchouni <sup>(2)</sup> - Adrien Villain <sup>(3)</sup> - Samantha England <sup>(2)</sup> - Julien Vieu <sup>(4)</sup> - Julia Bartoli <sup>(5)</sup> - Carine Puppo <sup>(1)</sup> - Sylvie Citerne <sup>(6)</sup> - Gregory Mouille <sup>(6)</sup> - Emanuelle Bouveret <sup>(7)</sup> - Yonghua Li-Beisson <sup>(2)</sup> - Benoit Menand <sup>(8)</sup> - Brigitte Gontero <sup>(1)</sup> - Ben Field <sup>(8)</sup>

CNRS, Bioénergétique et Ingénierie des Protéines (BIP), IMM, Marseille, France <sup>(1)</sup> - CEA, Bioscience and Biotechnology Institute of Aix-Marseille (BIAM), Marseille, France <sup>(2)</sup> - AMU, Laboratoire Information Génomique et Structurale (IGS), Marseille, France <sup>(3)</sup> - AMU, Bioscience and Biotechnology Institute of Aix-Marseille (BIAM), Marseille, France <sup>(4)</sup> - CNRS, Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM), IMM, Marseille, France <sup>(5)</sup> - INRAE, Institut Jean - Pierre Bourgin, Paris, France <sup>(6)</sup> - Stress Adaptation and Metabolism in Enterobacteriae Unit, Institut Pasteur, Paris, France <sup>(7)</sup> - CNRS, Bioscience and Biotechnology Institute of Aix-Marseille (BIAM), Marseille, France <sup>(8)</sup>

Chloroplasts retain elements of a bacterial stress response pathway that is mediated by the signaling nucleotides guanosine penta- and tetraphosphate (ppGpp). In Arabidopsis, ppGpp can act as a potent regulator of plastid gene expression and influences photosynthesis, plant growth and development. However, little is known about ppGpp metabolism or its evolution in other photosynthetic eukaryotes. Here, we will present recent results on the role of ppGpp in non-vascular plants and algae, highlighting conserved roles, such as the regulation of photosynthesis, and specialised roles, such as in the regulation of growth and stress acclimation. Our findings underline the importance of ppGpp as a regulator of chloroplast function across different domains of life, and lead to new questions about the molecular mechanisms and roles of ppGpp signalling in photosynthetic eukaryotes.



**371 - Determination of bipartite transit peptide of the 23 kDa Oxygen-evolving protein for targeting of proteins to the thylakoid lumen.**

**Jae Sung Shim**<sup>(1)</sup> - Sang Hoon Ma<sup>(1)</sup> - Hyun Min Kim<sup>(1)</sup> - Se Hee Park<sup>(1)</sup> - Seo Young Park<sup>(1)</sup> - Thanh Dat Mai<sup>(1)</sup> - Ju Hui Do<sup>(1)</sup> - Tuan Viet Do<sup>(1)</sup> - Won Choi<sup>(1)</sup> - Hye Min Jang<sup>(1)</sup> - In yeong Na<sup>(1)</sup> - Hyeon Bae Hwang<sup>(1)</sup> - Young Hee Joung<sup>(1)</sup>

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Nuclear-encoded chloroplast proteins require the transit signal peptide for translocation to the chloroplast. Particularly, thylakoid targeted proteins possess bipartite transit peptide. One part of the transit peptide targets protein into the chloroplast and another part targets protein into the thylakoid lumen. To determine the signal peptide for thylakoid targeting, we used the 23kDa Oxygen-Evolving proteins (OE23s), which are involved in photosynthesis in plants. The OE23s contain bipartite transit peptide for translocation to the thylakoid lumen. Four different transit peptides isolated from tobacco (*Nicotiana tabacum* cv. Xanthi NC) OE23s were translationally fused to the green fluorescence protein (GFP), then cloned into the plant expression vector and transformed into tobacco plants. The GFP was localized into the thylakoid lumen and the transit peptide was removed from GFP after the localization. In order to determine transit peptide sequence mediating thylakoid targeting, we generated transgenic tobacco plants expressing the GFP fused with truncated signal peptides. The N-terminal 38 amino acids were sufficient for the translocation of GFP across the two membranes of the chloroplast. The 20 amino acids from the first cleavage site mediated the translocation of GFP into the thylakoid lumen. The peptide contains twin arginine which is known to be required for translocation of proteins into the thylakoid. Interestingly, only 10 amino acids from the cleavage site without twin arginine also mediated translocation of GFP into the thylakoid lumen. Collectively, we determined region of signal peptide required for translocation into the chloroplast and the thylakoid lumen.

## 564 - From singlet oxygen signaling to chloroplast protein import pathways: An unforeseen adventure

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Chloroplast-generated singlet oxygen ( $^1\text{O}_2$ ) alters nuclear transcriptome via retrograde signaling pathways, contributing to foliar acclimation and cell death responses. An Arabidopsis crumpled leaf (crl) mutant deficient in plastid binary fission is known to express  $^1\text{O}_2$ -responsive nuclear genes constitutively. The crl gigantic chloroplasts induce autoimmune responses through lipid peroxidation-dependent retrograde signaling. In the present study, via a forward genetic screen, we unveil dominant gain-of-function TRANSLOCON AT THE INNER ENVELOPE OF CHLOROPLAST (TIC236) mutations, each of which abolishes the autoimmune responses and rescues the plastid-division defect in crl. This result indicates the shared functionality between CRL (mainly located in the outer envelope of chloroplast) and TIC236 proteins. Ensuing reverse genetic analyses show CRL's genetic interaction with SP1, a RING-type ubiquitin E3 ligase, and the intrinsic inner envelope membrane FTSH11 protease, whose functions are implicated in TOC and TIC turnover, respectively. Either loss of SP1 or FTSH11 rescues crl phenotypes in varying degrees due to increased translocon levels. Consistent with the impaired plastid division in both crl and tic236-knockdown mutants, CRL interacts with the preproteins of plastid-division machinery, and TIC236GF reinforces their import. Last but not least, we found these GF mutations significantly stabilize TIC236 proteins. We collectively shed new light on the probable link between protein import defect and plant innate immunity.

## TOPIC:

### Crop improvement for healthy diet

#### Oral Communications

##### 213 - Vitamin C biofortification: Overcoming the complexity of metabolic pathway regulation

**Richard Macknight** <sup>(1)</sup> - **Matthew Chisnall** <sup>(1)</sup> - **Erin Riche** <sup>(1)</sup> - **Sean Bulley** <sup>(2)</sup> - **William Laing** <sup>(2)</sup> - **Roger Hellens** <sup>(3)</sup>

**University of Otago, Department of Biochemistry, Dunedin, New Zealand** <sup>(1)</sup> - **Plant & Food Research, Food Industry Science Centre, Palmerston North, New Zealand** <sup>(2)</sup> - **Centre for Tropical Crop and Biocomodities, Queensland University of Technology, Brisbane, Australia** <sup>(3)</sup>

Ascorbate (or vitamin C) is an essential human micronutrient predominantly obtained from plants. Our primate ancestor lost its ability to synthesis vitamin C at a time when its diet was rich in ascorbate. Today, eating sufficient fruit and vegetables to obtain more than the minimum level of ascorbate consistently is a challenge for many people. While scurvy is uncommon, research is revealing the importance of ascorbate in human health. Since ascorbate plays a crucial role in regulating iron uptake, increasing dietary ascorbate levels should help reduce iron deficiency anaemia, which affects around two billion people worldwide. Ascorbate also acts as a cofactor for enzymes involved in epigenetic programming, providing a possible mechanism by which insufficient ascorbate contributes to the progression of cancer and age-related diseases.

We have been investigating the regulation of ascorbate production and are using this knowledge to generate plants with elevated ascorbate levels. We have previously shown that GDP Galactose Phosphorylase (GGP) mRNA, which encodes the rate-limiting enzyme of ascorbate biosynthesis, contains an upstream open reading frame (uORF) which negatively regulates the translation of GGP. We have used gene-editing to mutated the uORF, and this results in higher ascorbate levels. Overexpressing GGP gene also leads to increased ascorbate but only to modest levels. RNAseq analysis of plants either stably or transiently overexpressing GGP, reveal that transcriptional feedback results in the downregulation in endogenous ascorbate biosynthesis genes and the upregulation of genes that likely function to limit ascorbate accumulation. To reduce the impact of this feedback regulation, we used fruit-ripening specific promoters to increase GGP expression late in tomato fruit development. This resulted in ~5-fold increase in ascorbate (200 mg/100 gFW), the highest recorded ascorbate content for fresh tomato fruit. Overall, this work provides insights into the complexity of ascorbate regulation and ways to generate ascorbate biofortified crops.

## TOPIC:

### Crop improvement for healthy diet

#### **Posters**

##### **245 - Novel candidate genes for grain texture in Russian wheat varieties**

**Antonina Kiseleva**<sup>(1)</sup> - **Irina Leonova**<sup>(1)</sup> - **Tatyana Pshenichnikova**<sup>(1)</sup> - **Elena Salina**<sup>(1)</sup>

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Grain texture is an important milling characteristic of common wheat. Previously, it was shown that the Ha locus on 5DS, containing the puroindoline a, puroindoline b and GSP-1 genes, has a crucial role in endosperm texture. Puroindoline-like genes mapped to chromosome group 7 also contribute to this trait. However, some other loci were detected on different chromosomes, but no specific genes were proposed to be involved in grain hardness organization.

In this study, we used a panel of spring wheat varieties adapted to Western Siberian environments. The varieties were different in their grain texture, ranging from soft to hard. Analysis of the flour particle size and flour particle specific surface of wheat varieties cultivated in different environments demonstrated high heritability (0.75-0.82) and strong repeatability of the traits. Genotyping of wheat varieties with allele-specific markers for the puroindoline-a and puroindoline-b genes demonstrated that both the Pina and Pinb genes were polymorphic in our population. A strong association with grain hardness was shown for allele Pina-D1k, which lacked both Pina and Pinb genes (double null allele). Association analysis with the use of SNP genotyping (Illumina 15K Wheat) and two-year phenotyping confirmed the key role played by puroindoline genes in determining the grain texture of Russian spring wheat varieties. The analysis also detected significant SNPs on the 1B, 3A, 5B, 6A, 6D, 7B, and 7D chromosomes.

The best candidate loci for the grain texture were located on chromosomes 5B and 7B ( $FDR \leq 0.05$ ). We dissected a number of candidate genes and proposed a possible mechanism for their contribution to endosperm texture determination. These genes are involved in the metabolism of galactolipids (DGDG), carbohydrates (1,3- $\beta$ -glucan) and defensins.

This study was supported by the Russian Scientific Foundation (Project No. 16-16-00011-P).

**577 - Synergy between light quality and biostimulant improves the antioxidant capacity and photosynthetic traits in soybean (*Glycine max* L.) sprouts**

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In the last decades, agricultural practices are changing to meet the increasing market demand in response to a growing population's nutritional requirements. This high food production leads to an overexploitation of the resources, especially of the soil, and it is needed to develop new cultivation methods to enhance its production in a sustainable way. This work evaluates the combined effect of light quality regimes and biofertilization treatments on soybean plants (*Glycine max* L.) to improve sprout nutritional value and seedling photosynthetic traits. Germinated seeds pre-treated with different concentrations (0.01%, 0.05%, 0.5%) of an aminoacidic biostimulant were grown for 4 days at the light quality regimens D-dark, white W, full-spectrum FS and red-blue RB to evaluate the variations in antioxidant capacity. Part of the sprouts was grown at W, FS, and RB light regimes for 24 days to assess the possible beneficial effect on the photosynthetic apparatus. The seed priming with all biostimulant concentrations significantly increased sprouts antioxidant compounds, sugar and protein content compared to control. The positive biostimulant effect was improved under FS and RB compared to D and W light regimes. At the seedling stage, 0.05% was the only biostimulant concentration effective in increasing the specific leaf area (SLA) and photosynthetic efficiency. The growth under FS and RB light regimes significantly enhanced the beneficial effect of 0.05% on SLA and photosynthesis compared to W.

The present study provides evidence that the seed priming with proper biostimulant concentration combined with specific light regimes during plant development may be a valuable means to modify the bioactive compound amount and leaf structural and photosynthetic traits.

## **598 - Establishment of Yam as a potential crop in Europe**

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Yam (genus *Dioscorea*) are monocotyledonous perennial climbing vines that can be cultivated as annual plants for tuber production. The Chinese yam *D. polystachya* is an edible yam of Asian origin that can grow in temperate climates and therefore comprises high potential as a crop in Germany and Europe. The delicious tubers of Chinese yam are rich in starch and minerals and contain healthy ingredients that can be applied for treatment of diabetes and hypertension as well as regulation of blood cholesterol levels. Therefore, Chinese yam tubers have a high potential as “functional food”. Unfortunately, cultivation is very labor intensive because the heavy underground tubers reach up to 1.5 meters deep into the ground and have a club like shape with the tuber being thin at the top and getting thicker at the base. This shape makes harvest a challenge as tubers cannot be simply pulled out. We focus our research on this unique growth behavior of Chinese yam tubers, because harvesting the tubers would be much easier if they were shorter and thicker with a more roundish shape at the base. To date tuberization in yam has not been investigated on a molecular level. Our project project aims to identify molecular key regulators of tuber development in Chinese yam to enable future breeding options.

## **620 - Row distance of wheat and barley in field trials and effects on yield and wheat quality in organic agriculture for production of bread and beer**

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Wheat and barley production under low energy input in organic farming was tested in a randomized split plot trial at two experimental field stations of University of Hohenheim with either an organic or conventional farming system. The results showed that the yield of summer wheat and summer barley in organic agriculture was lower compared to conventional conditions. Effects of root densities (different row distance) was analyzed. Grain yield of different row spacing was similar at 12.5, 20 and 30 cm row distance in all plots of organic and conventional origin in wheat and barley. Grain quality parameters of wheat storage protein fractions (albumin/globulin, gliadin, and glutenin) were analyzed by SDS-PAGE and the pattern of protein storage bands were compared qualitatively. Moreover, a quantitative analysis of all single sub fractions (bands) were performed. The concentration of storage fractions was similar at all row distances but the sub-fractions of the gliadins and glutenins varied in concentration. This indicates that both wheat flower and bread quality might differ at different row distances. This will be further analyzed in the ongoing project, in addition to other factors like with/without N-fertilization and seed coating with a plant growth promoting bacterium.

**633 - Engineering the polyphenols pathway stimulates metabolic and molecular changes during fruit ripening in “Bronze” tomato**

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Tomato (*Solanum lycopersicum*) is an important crop consumed and cultivated worldwide. Quality-related tomato compounds such as sugars, acids and flavonoids show marked differences along tomato fruit ripening. Metabolic engineering for the enhancement of nutritionally important secondary metabolites can contribute to the improvement of fruit quality and to study the metabolic and molecular changes occurring during fruit ripening. In this study, molecular and biochemical analyses were carried out on the engineered Bronze tomato line at four different ripening stages to unravel the effect of regulatory and structural transgenes on metabolic fluxes of primary and secondary metabolisms.

The present study highlights the involvement of the energy-related shikimate pathway, with a higher consumption of sugars and malate in Bronze compared to wild type Money Maker fruit, and the enhanced gene expression of transporters to compensate the engineered accumulation of anthocyanins in the vacuolar compartment. This study contributes to understanding the impact of transgenes on different metabolic pathways committed to quality and nutritional value of tomato fruit.



### **638 - Expression of genes involved in the biosynthesis of linseed oil fatty acids**

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Seeds of flax (*Linum usitatissimum* L.) are enriched with unsaturated fatty acids, including linolenic (LIN), linoleic (LIO), and oleic (OLE) ones, which role in the prevention and treatment of cardiovascular, oncological, and other diseases is extensively studied. Linseed oil is used in pharmaceuticals, nutraceuticals, functional feed, composites, and polymeric materials. The field of linseed use is determined by certain characteristics, primarily by the fatty acid composition of the oil. Flax cultivars differ significantly in oil composition (mainly in the content of LIN, LIO, and OLE). We studied the role of the expression of genes involved in fatty acid biosynthesis in the determination of the fatty acid composition of linseed oil. RNA was extracted from capsules of seven flax varieties with diverse LIN, LIO, and OLE content in the green-yellow stage of maturity with a Quick-RNA Microprep Kit (Zymo Research, USA). NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, USA) was used for cDNA library preparation. Transcriptome sequencing was performed on NextSeq 500 (Illumina, USA) and about 10 thousand 81-bp reads were obtained for each genotype. For evaluation of gene expression, reads were mapped to the *L. usitatissimum* reference genome (GenBank: GCA\_000224295.2) using STAR, quantified using BEDTools, and analyzed with edgeR. We revealed differences in the expression of genes involved in the fatty acid biosynthesis, including FAD2A, FAD2B, FAD3A, and FAD3B, for varieties with different content of LIN, LIO, and OLE in oil. In some low-LIN varieties, expression of FAD genes was downregulated, however, this trend was not observed in all studied low-LIN genotypes. The obtained results are essential for establishing the role of gene expression in the determination of the fatty acid composition of linseed oil. This work was financially supported by the Russian Science Foundation, grant 21-16-00111.

## **681 - Cowpea still has a word to say about a sustainable healthy diet**

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Cowpea is a drought and heat resistant grain legume; features highly sought in crops in the context of global warming. With high protein content it also fits an eco-friendlier diet, pursued by an exponentially growing population. Moreover, it is a promising source of anti-inflammatory compounds both in the grain and other parts of the plant. Cowpea is highly consumed in some regions of Africa where it is said to “feed people, their livestock and the next crop”. In the Mediterranean area, its consumption is not of that magnitude, but it certainly is part of the Mediterranean diet; forgotten for a period; but now with revived attention. In countries where small scale family agriculture was suddenly substituted by monoculture, many landraces empirically selected over years were set aside. In an effort to evaluate Portuguese cowpea variability, we began by collecting such landraces (21) and comparing them with a commercial variety for their physiological performance under water stress and phenolic value. Phenolic content in ultra-sound-assisted extracts varied between 50 and 250 mg EAG /100 g of grain with AO capacity ranging over 6-fold among extracts with highest values around 160  $\mu\text{mol TEAC} / \text{g}$  of grain. HPLC analysis showed peaks that indicate the presence of phenolic compounds (to be identified) that may justify the high AO values obtained for some of the landraces. Subsequently, 5 varieties pinpointed by their AO capacity, phenotypic contrast and economic/national importance were evaluated for drought resistance in pots and under rainfed Mediterranean conditions. In the field there were no differences in productivity in both irrigated and rainfed plots. In the pot trial, under severe water stress, plants produced less grain, but grain dimensions and AO activity were similar in stressed and well-watered plants. The obtained results show valuable characteristics that should be integrated in cowpea breeding programs.

**69 - Effect of salicylic acid and terpenoid containing natural products on field grown cereal plants (winter wheat and barley)**

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The role of salicylic acid (SA) in plant disease resistance is well documented for plants, where it is required for basal resistance against pathogens as well as for the inducible defense mechanism, systemic acquired resistance (SAR). The activation of SAR is associated with the heightened level of expression of the pathogenesis-related (PR) proteins, some of which possess antimicrobial activity. SA has been the focus of intensive research due to its function as an endogenous signal mediating local and systemic plant defense responses against pathogens. In future, the exogenous application of SA might act as a powerful tool in enhancing the growth, productivity and also in combating the ill effects generated by various abiotic stresses in plants. The terpenes (terpenoids), constitute the largest class of secondary metabolites. They are toxins and feeding deterrents to many herbivorous insects and mammals. Some of them are popular ingredients in commercial insecticides because of their low persistence in the environment and their negligible toxicity to mammals. Field studies were carried out to evaluate the effect of one salicylic acid and one terpenoid containing products on cereals (winter wheat and barley). Plants were treated once, twice and/or three times in small plot experiments. Physiological status of the plants was investigated according to their photosynthetic pigment content levels. Yield quantity and quality were evaluated. Based on the experimental data we can state that both products have provided positive effect on the development of winter wheat and barley. More application is beneficial compared to single one. Application of SA containing product in suboptimal conditions in autumn also caused positive effect on cereal plants. As a conclusion we can state, that one terpenoid treatment in the middle of spring, followed by one spraying of SA product could enhance the physiological properties of winter cereals which lead higher yield results.

### 637 - Chromatographic profile of antioxidants in plants of subtropical cultures

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#### Introduction

Antioxidants can neutralize free radicals in the body. Natural antioxidants are interesting, because synthetic analogues are often unsafe to use [1].

The important sources of antioxidants are tea and citrus plants which content polyphenols and amino acids (later AA). These substances are used to assess the quality of products, breed plant species [1].

But the methods used provide only for the determination of integral indicators, which is not informative enough. It is necessary to develop methods for determining individual compounds [2].

The purpose of the work: to develop an express version of the polyphenols and AAs determination in subtropical crops by HPTLC so to obtain chromatographic profiles of analytes in.

The conditions of the polyphenols, gallic acid, caffeine, AAs separation were optimized [2,3]. The factors affecting the retention parameters of analytes have been identified and a scheme of samples of tea & tangerines preparation for chromatographic analysis has been developed. Quantitative determination of analytes was carried out by video densitometry [2]. The detection limits for catechins and AAs were found.

The chromatographic profiles of polyphenols and AAs were obtained and chemometric treatment of these profiles was made. Dominant analytes which determine the differences between tea varieties were identified [4].

## **676 - Exploring variability of free asparagine content in durum wheat seeds to reduce acrylamide forming-potential**

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Acrylamide, a molecule which is known to be toxic to the nervous system and fertility and suspected to be carcinogenic, has been detected in many foods after high temperature processing. In wheat derivatives, the quantity of free asparagine (fAsn) has been identified as a key factor in acrylamide formation. Therefore, the control of fAsn content is of current interest in contemporary crop and food sciences. Exploring natural variation for fAsn content in wheat genotypes could help breeders to identify novel beneficial traits and useful genes for breeding programs.

To this purpose, 216 durum wheat (*Triticum turgidum* L. ssp. durum (Desf.)) genotypes, which originate from 35 different countries, were selected from the international Global Durum Panel (GDP), a collection which has been previously genotyped with Illumina iSelect 90K SNP array technology. The selected genotypes include 195 durum wheat landraces, which were chosen to increase genetic variability. Population structure analysis identified four groups, matching the geographic origin of the genotypes.

The 216 genotypes were grown in Fiorenzuola d'Arda (Italy) in 2019-2020. Quantification of fAsn levels in the whole-grain samples was conducted using an enzymatic/spectrophotometric method and up to now, 174 samples have been analyzed for fAsn content. The analysis indicates the presence of a good variability for this trait in the chosen population, with a fAsn content ranging from 0.12 to 0.58 mg/g dry matter. A one-year preliminary genome-wide association study (GWAS) is in progress to identify chromosomal regions and putative genes associated with grain fAsn content. In addition, NMR and HPLC-MS analysis is being exploited to monitor metabolite composition in the selected genotypes and get insight into the factors that control fAsn accumulation in durum wheat seeds.

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## TOPIC:

### From plant defence to plant immunity

#### **Oral Communications**

**64 - Orchestration of the oxidative burst in elicitor-induced immunity requires the multiple organelle-targeted Arabidopsis NPK1-related protein kinases (ANPs)**

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Recognition at the plasma membrane of danger signals (elicitors) belonging to the classes of the microbe/pathogen- and damage-associated molecular patterns is a key event in pathogen sensing by plants and consequent rapid activation of immune responses. Different subcellular compartments, including plasma membrane, chloroplasts, nucleus and mitochondria, are involved in carrying out the immune cellular program. However, how pathogen sensing is transmitted throughout the cell remains to be uncovered. Arabidopsis NPK1-related Proteins (ANPs) are mitogen-activated protein kinase kinase kinases previously shown to have a role in immunity. In this paper, we studied the in vivo intracellular dynamics of ANP1- and ANP3-GFP fusions and found that in physiological conditions these proteins are present in the cytoplasm, while ANP3 is also localized in mitochondria. After elicitor perception, both proteins localize also into plastids and nucleus, revealing a localization pattern that is so far unique. The N-terminal region was responsible for mitochondria and plastid localization of the protein kinases. Moreover, we found that ANP localization coincides with sites of elicitor-induced ROS accumulation and that plants lacking the ANP function do not produce intracellular ROS. Our results suggest that ANPs are required both for ROS generation and ROS signaling in those organelles, pointing to ANPs as central hubs in the orchestration of ROS accumulation and signaling.

## 220 - Disrupted actin cytoskeleton : a switch from immunity to senescence

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Actin cytoskeleton dynamics is crucial for plant cell, especially upon the changing environment. In stress conditions, i.e. pathogen infection, actin filaments get rapidly reorganized to enable defence responses. Chemical drugs like latrunculin B (latB), affecting actin polymerisation, also influence the infection process, and support pathogen growth while applied together with pathogen. On the contrary, being applied previously to infection, such drugs specifically trigger defence-like responses and even induce resistance to further infection. Among such responses, we detected the accumulation of salicylic acid (SA), specific activation of SA signalling pathways and SA-responsive genes, callose deposition through PMR4. Interestingly, this was specific to actin cytoskeleton, as the drugs targeting tubulin organization had no such effect.

In this study, we investigated the similarities and differences between latB-triggered reactions in *Arabidopsis thaliana* to those caused by *Pseudomonas syringae* infection or recognition of microbe-associated molecular patterns. We focused on physiological responses, callose deposition patterns, transcriptomic signatures and induced SA biosynthesis pathways. Interestingly, short-term actin disruption partly mimicked early defence events, while prolonged exposure to latB caused localized senescence, typical for the later infection stages. A sensing of disrupted actin cytoskeleton can thus be a triggering point for immune responses that escalate up to local induced senescence.

## **263 - The end of the trade wars: a new paradigm in plant defence theory**

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Why and when plants divert resources to deploying defensive chemicals instead of to energy production (photosynthesis) is still not understood. Resource allocation theories predict that diverting resources to the synthesis of defence should result in lower photosynthetic rates and a growth penalty, but this is often hard to detect. Indeed, sometimes they are positively correlated. My research uses cyanogenic glucosides (CNglc) as a model system to investigate how metabolic and energetic processes in biological systems may be so entangled that a new paradigm is required. It is important to understand these relationships in order to ensure there are no inadvertent effects arising from eliminating cyanide from staple crops such as cassava and sorghum.



## TOPIC:

### From plant defence to plant immunity

#### **Extended Elevator Pitches**

##### **146 - Host factors involved in viroid and satellite RNA infections**

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Viroids and satellites are small non-coding RNAs which may have agronomic importance. Both pathogens counteract host defense responses through replication speed but also through subcellular localization and interaction with specific host factors. Their interactions with the silencing machinery and especially DCL proteins remained elusive for many years. By focusing on DCL proteins using DCL knock-down (RNAi) plant lines we have started to unravel the complex relationship between viroids and the RNAi silencing machinery. We have shown that viroid RNA is mostly processed by DCL4 but this is not necessarily the most efficient anti-viroid silencing pathway. Here we have extended our studies by generating CRISPR DCL knock-out plants using CRISPR/Cas9 technology to study both viroid (mostly PSTVd) and satellite (SatL and B10 of tomato bushy stunt virus) interplay with the silencing machinery. Further, we have focused on subcellular localization of different RNAi proteins upon pathogen infections which is revealing aspects crucial not only for viroid biology but also for the silencing machinery per se. Finally, we have extended our search for additional host factors necessary for PSTVD. Most recent findings in these areas will be further discussed.

### 173 - Immune signaling peptides in early land plants

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Plants are constantly exposed to various pathogens and, thus, have evolved a complex innate immune system. Cell surface localized pattern recognition receptors (PRR) recognize conservative pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) released by host cells, which ultimately triggers host immune responses. Endogenous immune plant signaling peptides have recently attracted special interest. Such peptides are derived from nonfunctional or functionally active precursor proteins and trigger antiherbivore and antimicrobial defense pathways. However, evolution of peptide signals as well as components of signaling cascades are poorly studied in early land plants.

To understand whether plant peptide signaling might be conservative among angiosperms and early land plants we treated the model “basal” plant, *Physcomitrella patens*, with synthetic *Arabidopsis thaliana* peptide elicitor (AtPEP), which is known to amplify the innate immune response. We then analyzed whereas AtPEP leads to activation of defense gene expression and reactive oxygen species production. Further we performed comprehensive peptidomic analyses of the key components of immune signaling mutant lines upon chitosan treatment, which is a well-known elicitor of host defense in *P. patens*. Mass-spectrometry and bioinformatics approaches allowed us to identify thousands of extracellular peptides. We then provided a list of peptides, which are potentially involved in immune responses. Our results suggest that degradation of functionally active proteins might play an important role in defense mechanisms by generating a new source of bioactive peptides and shed more light on the plant peptide signaling in early land plants.

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#### **413 - Xylella fastidiosa in olive tree: physiological evidences correlated to the resistance**

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The identification of *Xylella fastidiosa* (Xf)-resistant olive genotypes represents the most promising strategy to control this pathogen; since the first observations in Salento (2013, Southern Italy) some cultivars showed different levels of resistance to Xf. Therefore, to identify the mechanisms explaining this resistance, we have compared susceptible cultivars to resistant ones adopting different research approaches. A first finding was the increase of quinic acid, a lignin precursor, significantly detected only in Xf-resistant trees; in addition, laser scanning microscopy observations revealed lignin deposition in xylem vessels and expression of phenylpropanoid pathway genes showed, in branches of resistant cultivars, the Cinnamoyl-CoA Reductase upregulation, described as strongly induced during “defence response associated” lignin formation. These data are in agreement with the hypothesis that increased cell wall lignification can reduce bacterial movement, thus delaying disease progression. Moreover, scanning electron microscopy-Energy Dispersive X-ray observations of olive tissues displayed starch grains accumulation (a refilling mechanism) in vessels, while anatomical measurements on healthy stems indicated that resistant trees could be constitutively less susceptible to cavitation. Gene expression patterns (upregulation of aquaporin OeTIP1.1, sucrose transporters OeSUT1 and OeMST2, amylases OeAMY and OeAMY2) suggested that the resistant cultivars are able to modulate genes involved in embolism sensing and refilling mechanisms: this ability to detect embolism and to restore hydraulic conductivity can influence symptoms severity. Furthermore, the occlusions and the *Xylella*-induced changes in wood-anatomical characteristics, such as the increase in both ring width and vessels number, were revealed by a dendrochronological approach in rings from the year 2010 to 2017 in either resistant or susceptible olive cultivars; as expected, vessel occlusions in the susceptible cultivars were significantly higher than in resistant ones. These studies indicated that the resistance to *Xylella* has a multifactorial basis, and the composition and structure of xylematic tissues play a central role in the pathogen colonization.

#### **419 - GLYI4: a potential key hub of primary metabolism and hormone signaling pathway in *Arabidopsis thaliana***

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Plant responses to biotic and abiotic stresses employ finely tuned regulatory mechanisms, largely orchestrated by phytohormones. Typically, salicylic acid (SA) plays a central role in defense responses against biotrophic and hemi-biotrophic pathogens. By contrast, jasmonic acid (JA) is usually associated with defense against necrotrophic pathogens and herbivorous insects. Moreover, plants subjected to stress can produce cytotoxic compounds, as methylglyoxal (MG), which is detrimental to the cell. MG is detoxified by the glyoxalase system composed of two enzymes, glyoxalase I (GLYI) and glyoxalase II (GLYII), which transform MG in D-lactate and GSH. Recently, by a genome-wide association study performed in *Arabidopsis*, we identified GLYI4, a member of the GLYI family, as a new actor in the crosstalk between SA and JA signaling pathways. In *glyI4* mutant plants, we observed a general stress phenotype, characterized by MG accumulation, impaired MG scavenging, accumulation of reactive oxygen species (ROS), stomatal closure and altered fitness. Interestingly, accumulation of MG in the *glyI4* mutant led to a lower efficiency of the JA pathway, causing a greater susceptibility to the pathogenic fungus *P. cucumerina*. Here, a metabolomics approach based on high resolution mass spectrometry on the *glyI4* mutant revealed a bunch of 72 differentially regulated metabolites, compared to wild-type. A bioinformatics analysis revealed accumulation of compounds related to redox reactions and in cellular energy maintenance, and impairment of those involved in plant defense and growth. Focusing on redox reactions, enzymatic assays confirmed an altered ROS scavenging. These results corroborated the role of GLYI4 as an important actor for plant metabolism and health.

**461 - The Plasma Membrane-Associated Ca<sup>2+</sup>- binding protein PCaP1 is required for oligogalacturonide and flagellin-induced priming and immunity.**

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Early signaling events in response to elicitation include reversible protein phosphorylation and re-localization of plasma membrane (PM) proteins. Oligogalacturonides (OGs) and flagellin are well-known elicitors acting as endogenous and exogenous inducers, respectively, of plant immune response. Previous data on early phosphoproteome changes in *A. thaliana* upon OG treatment uncovered the phospho-regulation of several membrane proteins including PCaP1, a PM-anchored protein with actin filament-severing activity.

PCaP1 was shown to be implicated in some development processes, however its role in immunity has not been previously investigated.

Here, we demonstrated that PCaP1 is specifically involved in the induction of elicitor-triggered late defence responses, including the response to *Botrytis cinerea* infection.

Additionally, *pcap1* null mutants were unable to mount defence responses to consecutive elicitor treatments pointing to PCaP1 as a player in the elicitor-induced priming.

The use of transgenic plants expressing a PCaP1:GFP, under the control of its native promoter, revealed that PCaP1 is localized in PM. In basal condition, PCaP1 is distributed in microdomains whose organization rapidly rearranges upon elicitor treatments. Notably, fluorescence is associated with endocytic vesicles that allow an endocytic turnover of PCaP1 for maintaining its homeostasis at the PM during the immune response.

## TOPIC:

### From plant defence to plant immunity

#### Posters

**650 - Identification of the amino acids involved in the pH-dependent activity of OGOX1 by an integrated approach of molecular dynamics and alanine scanning mutagenesis.**

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Oligogalacturonides (OGs) are released from the breakdown of the plant cell-wall by microbial polygalacturonases during infection and alert plants by acting as Damage Associated Molecular Patterns (DAMPs). After the early-phase of infection, plant cells secrete different OG-oxidases (OGOxS) paralogs, defence flavoproteins capable of oxidizing the reducing ends of OGs by concomitantly releasing H<sub>2</sub>O<sub>2</sub>; the oxidized OGs lose their elicitor nature in favor of an increased recalcitrance to enzymatic hydrolysis. Among the different plant defense responses, the increase of apoplastic pH that accompanies pathogenesis is hypothesized to be pivotal in reducing the degrading potential of microbial PGs on one side and in boosting the oxidizing activity of OGOxS on the other. In accordance with this hypothesis, all OGOxS so far characterized show an optimum activity at pH values ranging from 8 to 11. By an integrated approach of molecular dynamics and alanine-scanning mutagenesis, we identified the aminoacids D325 and D344 as responsible for the pH-dependent activity of OG-OXIDASE 1 (OGOx1) at basic pH. According to our results, D325 and D344 act in antagonistic manner in modulating the activity of OGOx1 at pH 8.5 but not at pH 5.0. Compared to the wild-type enzyme, D344A mutation increased OGOx1 activity whereas the D325A mutation reduced it; intriguingly, the activity of OGOx1 variants was altered in opposite manner by a similar extent, suggesting that pH dependent activity of OGOx1 is dependent on the antagonistic action of both aminoacids at basic pH.

**654 - Calcium phosphate nanoparticles doped with copper ions as efficient tools for downy mildew prevention**

**Dora Scarpin** <sup>(1)</sup> - **Marco Vuerich** <sup>(1)</sup> - **Enrico Braidot** <sup>(1)</sup> - **Elisa Petrusa** <sup>(1)</sup> - **Guido Fellet** <sup>(1)</sup> - **Giorgio Honsell** <sup>(1)</sup> - **Paolo Sivilotti** <sup>(1)</sup> - **Luca Marchiol** <sup>(1)</sup> - **Lorenzo Degli Esposti** <sup>(2)</sup> - **Alessio Adamiano** <sup>(2)</sup> - **Michele Iafisco** <sup>(2)</sup>

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Nowadays the management of crops requires the use of a large quantity of pesticides, herbicides and fertilizers, especially in high-income crops such as vines, which undergo several treatments per year. Their use, however, is highly inefficient, with considerable losses that have harmful effects on the environment and human health. For this reason, the interest towards methods able to make agronomic practice more sustainable is growing noticeably. Particular attention is paid to nanotechnology applied in agriculture, as this could lead to the development of nano-agrochemicals more effective than conventional ones, concerning leaching and treatment persistence on plant. In fact, nanomaterials allow to bind the molecules of interest and to transport them directly to the target site in the plant, thus reducing the overall doses used and their dispersion into the environment.

According to this perspective, in this work two types of calcium - phosphate nanoparticles were applied on Chardonnay grapevine cultivar: the aim was to evaluate their potential as vectors for low amounts of ionic elements, which may have a nourishing or biocidal function. In particular, two different methodologies have been used for functionalization with copper (Cu II), aiming to investigate its inhibitory action on *Plasmopara viticola* infection and to verify its entry into the leaves.

**674 - ARTEMISIA ESSENTIAL OIL EXTRACTS: A source of non-contaminant biopesticides protecting tomato against Fusarium solani**

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Plant essential oils (Eos) are potential biopesticide natural sources, showing high toxic activity in vitro against several high impact crop phytopathogens. The domestication of plant producing EOs is not evident, the characterization of new EOs is under progress, and the direct effect of EOs on crop protection remains poor. The EU framework aims to achieve the sustainable use of new plant protection products (PPPs). Since 2020 the use of contaminant PPPs is not allowed. Hydroponic culture emerges as an increasing farming strategy to develop sustainable crops, reducing water consumption, space, and fertilizers, under the current climatic change situation. Spain produced 5 millions of tons of tomato plants in 2019 (FAO). In this work, the effects on tomato seedlings growth and immunity response, of an EO extract from a previously domesticated Artemisia sp (AEO), was tested both in vitro and in vivo using hydroponic culture of plants, inoculated with Fusarium solani (F.s) phytopathogen. AEO showed protection capacities on tomato seedlings, against F.s, at concentrations as lower as 0.8 mg/ml. The results open new perspectives to the use of EOs as a source of non contaminant biopesticides in a new agroforestry environment.



### 683 - Combined effects of environmental factors on non-host resistance to Tobacco mosaic virus in barley

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Abiotic and biotic environmental factors like high temperature and pathogen infections can have a profound influence on disease resistance of plants. For example, it is known from the 1970s that Tobacco mosaic virus (TMV) is capable of replication and systemic spread in non-host barley plants at prolonged high temperatures (30 °C). TMV spread is further enhanced if heat-exposed barley is also infected by an adapted virus, e.g. Barley stripe mosaic virus (BSMV). We have shown previously that the non-host resistance of barley to wheat powdery mildew can be partially suppressed by a simultaneous application of heat shock and antioxidant treatments. However, the effect of a heat shock on virus infections is currently not known. In the present study we have aimed at elucidating how heat shock may influence non-host resistance of barley to a non-adapted virus, TMV.

In barley cv. Ingrid plants containing BSMV and mechanically inoculated with TMV a heat shock treatment (49 °C for 20 sec) resulted in an initial decline of TMV accumulation within the first two days after inoculation (DAI). On the other hand, at four and seven DAI TMV levels became significantly (50-100%) higher in heat shock-exposed plants. Expression of a barley pathogenesis-related gene (HvPR-1b), a NADPH oxidase gene responsible for production of the reactive oxygen species (ROS) superoxide and host disease resistance (HvRBOHF2), and HvSOD1 (superoxide dismutase) and HvBI-1 (BAX-inhibitor), genes encoding an antioxidant and a cell death regulator, respectively, correlated with TMV levels, rather than non-host resistance. Our results therefore indicate that these barley genes might function as stress/susceptibility markers, rather than defense components, at least at these relatively later time points (1 to 7 DAI). It seems that a heat shock and BSMV infection in concert can impair the non-host resistance of barley to a non-adapted virus, TMV.

This research was supported by a grant of the Hungarian National Research, Development and Innovation Office (NKFIH-OTKA, K128868)

**11 - RNA silencing functions of plant endogenous siRNAs activated by virus infection in Arabidopsis and Brassicaceae crops.**

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RNA-based silencing functions as an important mechanism of antiviral immunity in plants. Viral infections are accompanied by a massive production of small interfering siRNAs, either of viral origin (viral siRNA, v-siRNA) or of plant origin (virus-associated siRNA, va-siRNA). The latter are associated with widespread silencing of host gene expression.

Turnip and canola are respectively susceptible and tolerant species to cauliflower mosaic virus (CaMV) infections. In the present work we have used an objective and accurate phenotyping process to select the leaves of *Brassica rapa* (turnip) and *B. napus* (canola), infected by CaMV to characterize the va-siRNAs loci, i.e. transcripts producing va-siRNAs associated to symptoms of mosaic.

A group of 15 orthologues sharing turnip, canola and Arabidopsis in the production of va-siRNA in response to CaMV infection was identified. These include proteins encoding the components of the photosynthetic machinery, i.e. rubisco activase and intrinsic factors of the antenna complex, or factors sensitive to biotic and abiotic stimuli, i.e. proteins associated with senescence, heat shock protein 70 and catalase. As previously reported, the va-siRNAs loci transcripts are dramatically down-regulated. va-siRNAs of 22-nucleotides (nt) were overrepresented compared to those of 21-nt in the two pathosystems. The 22-nt va-siRNAs showed affinity with RNA-induced silencing complexes core proteins AGO1 and AGO2 and they exerted the ability to effectively drive the cleavage of the target RNAs.

Together these data suggest (i) a general mechanism of plant response shared between viruses of different taxa and including those with DNA genome and (ii) the involvement of a specific group of genes common to Brassicaceae, including core components of the photosynthesis along with specific host factors involved in stress response.

### **34 - PAMP-Responsive Long Noncoding RNA ELENA transcriptionally regulates innate immunity in Arabidopsis**

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Long noncoding RNAs (lncRNAs) have emerged as important regulatory factors of diverse biological processes. However, plant lncRNAs involved in innate immunity remain largely unknown. Plant innate immune responses are initiated upon the perception of PAMP (pathogen-associated molecular pattern) such as flagellin (flg22) and EF-Tu (elf18). In this study, we analyzed custom lncRNA array datasets generated from PAMP treatments and identified 1,370 Arabidopsis lncRNAs induced or repressed by flg22 and elf18. Real-time RT-PCR validation confirmed the differential expression of these lncRNAs in PAMP and tissue-specific manners. Out of them, we chose 13 ELENAs (elf18-induced long noncoding RNAs) for further analysis. All ELENAs are induced by PAMP treatments, and some of them are responsive to salicylic acid. ELENA3 is a natural antisense transcript of acyl-transferase induced by PAMP, and ELENA3 knockout plants show decreased callose deposition. ELENA11 is a long intergenic noncoding RNA as a negative regulator, and ELENA11 knockout plants show increased expression of nearby lipase and elevated callose deposition. Our results suggest that ELENAs play key roles in the transcriptional regulation of innate immunity.

### **35 - Gene AtGSTF11 can enhance stress tolerance in transgenic plants due to the activity of glutathione S-transferase**

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GSTs are multifunctional enzymes that play an important role in stress resistance by catalyzing the conjugation of the reduced form of glutathione to endogenous compounds and xenobiotics. They are well studied in animals, however the functions of individual GST genes in plants are not clear yet. AtGSTF11 gene of *Arabidopsis thaliana* encodes GST of the class phi and have been found in many groups of plants which means that this gene is of high evolutionary importance. In our experiments its expression was induced by salinity and drought and decreased under heat stress. This gene was cloned into binary vector pCambia 1301 with 35S promoter cassette, and resulting genetic construct was used for transformation of *Nicotiana tabacum* and spring variety of *Brassica napus*. Under normal conditions transgenic *N. tabacum* with constitutive expression of target gene had longer stems and increased raw and dry weight in comparison with control plants. The expression of AtGSTF11 gene in *B. napus* was suppressed in normal conditions, probably due to 87% identity to own *B. napus* GSTF11 gene, which resulted in shorter stems and lower weight. Experimental plants were exposed to different stress factors. Transgenic tobacco better maintained raw weight than control plants in the conditions of drought, and root growth - under salinity and cold stress. Transgenic *B. napus* better maintained raw weight under cold stress, most probably due to a 3-times increase in target gene expression. It is the most interesting, that transgenic plants also demonstrated resistance to powdery mildew. The expression level of GSTF11 gene in nontransgenic oilseed rape decreased dramatically both under cold and drought stress, so it probably has different functions than AtGSTF11, which can be used as a target to improve stress resistance in plants. Research was supported by grant of President of Russian Federation MK-1146.2020.11.

### **53 - Molecular factors underlying Arabidopsis PME activation against Botrytis**

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Cell wall (CW) is the foremost interface at which plants and fungi interactions take place. Pectin is methylesterified in the Golgi and secreted in the CW in a high methylesterified form. The activity of Pectin Methyl Esterase (PMEs) and methylesterification status of pectin are critical for the outcome of plant-fungus interaction. PME activity was associated to the production of de-methyl esterified and active Oligogalacturonides, the best characterized damage-associated molecular patterns (DAMPs) in plants. Moreover, PME is responsible for the release of methanol, which may function as DAMP alerting adjacent non infected tissues or neighbouring plants. The de-methyl esterified pectin can form crosslinks with calcium promoting wall stiffening. However, evidence indicates that pectin de-methylesterification also trigger the degradation by pectinases from pathogens. Despite this evidence, the current knowledge about the molecular mechanisms regulating pectin methylesterase activity during disease remains largely unknown. PME activity can be regulated by subtilisin-like proteases (SBTs) and by PME inhibitors (PMEIs). By using biochemical and reverse genetic approaches we demonstrate the role of specific PMEs and SBTs in plant immunity. PME17 and SBT3.3 emerged as functional pectin methyl esterases regulating PME activity and Arabidopsis resistance against Botrytis attack. The potential mechanisms of regulation and signal transduction triggered by pectin methyl esterase activity during fungal infection will be discussed.

#### 54 - A switch from peace between plants and pectobacteria to rotting: What lies beneath?

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Many agricultural plants suffer from devastating soft rot-causing bacteria, including *Pectobacterium atrosepticum* (Pba). Herewith, these bacteria often establish prolonged commensalistic interactions with plants without causing the disease. Our comprehensive study aims to understand what drives the peaceful coexistence of plants with pathogens in terms of the molecular physiology of both organisms and what disturbs the equilibrium within the pathosystem.

Different types of microscopy and NGS-transcriptomics constitute the basis of our systemic methodology providing information on 1) spatial organization of microbial population in planta and phenotypic diversity of Pba cells – both are the criteria of the infection type; and 2) plant and bacterial genes differentially expressed during latent and acute infection enabling us to assume the molecular players (including novel ones) that determine the type and outcome of plant-pathogen interaction. Then, specific test-systems (including those involving mutagenesis, cloning, chromatography, NMR-spectroscopy, phytohormone application, immunodetection, etc.) are developed to experimentally characterize these players.

The obtained results show that latent, disease-free infections are likely to be the physiological norm and reflect natural equilibrium between plants and pathogens, and the development of pathological processes is a result of the disturbance of this equilibrium due to the specific physiological reactions of both organisms. Different types of induced plant susceptible responses with different outcomes were characterized. Specific role in determination of the infection type was shown for phytohormonal status (which is actively modulated by the pathogen) and Pba-induced plant-mediated reorganization of the plant cell wall by plant cell wall proteins/enzymes and ROS. Novel determinants of plant-Pba interaction were revealed and characterized. Global comparative picture of the pathosystem at the equilibrated and disequilibrated states will be presented and maintenance of a pathosystem at the equilibrated state will be discussed as a perspective plant protection strategy. The study is supported by RSF (No 19-14-00194).

## 66 - The modulation of regulatory networks of infected plant: a view from the point of transcriptomics

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Plant responses, including those activated during biotic stress, are coordinated by the action of transcription factors (TFs) that regulate the expression of genes with a particular cis-regulatory element (CRE). Elucidation of key TFs is a significant point in the characterization of molecular mechanisms of infection. In our study, we developed a pipeline that enables the prediction of TFs taking part in disease development by the example of the tobacco soft rot caused by *Pectobacterium atrosepticum*. This pipeline includes the following steps: 1) identification of genes expressed differentially (DEGs) during the infection, including those encoding TFs; 2) extraction of DNA sequences located upstream the transcription start site of all genes in tobacco genome; 3) search for CREs in the extracted sequences; 4) clarification if a pool of genes with a particular CRE is significantly enriched with DEGs. Thus, in our analysis, we have taken into account not only the expression level of TF-encoding gene but also the expression pattern of a pool of genes that are targets of a particular TF.

The comparative transcriptome profiling of intact and infected plants revealed 8606 DEGs, including 271 up-regulated during the infection genes encoding TFs. In total, 577029 CREs of 546 annotated CRE variants were revealed in tobacco genome. In a number of cases, pools of genes with specific CREs were enriched by DEGs. Among these CREs were those for WRKY and NAC and some other TFs. Genes for some of these TFs (WRKY6, 28, 31, 40, 42, 45, 50, 51, 65, 69, 72 and NAC029) were up-regulated during the infection. Taken together, we have identified the TFs that seem to play major role in regulation of the development of a given pathological process. The study is supported by Ministry of Science and Higher Education of the Russian Federation (grant № 075-15-2019-1881).

**85 - The role of jasmonate- and salicylate-regulated plant responses in the development of infection caused by *Pectobacterium atrosepticum***

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Salicylic (SA) and jasmonic (JA) acids are key regulators of plant defense responses to pathogen invasion. However, there are examples indicating that the induction of the above systems leads to plant susceptibility to some of the pathogens. SA- and JA-induced pathways operate antagonistically. In turn, pathogens possess different degree of sensitivity to SA- and JA-induced responses and may activate the type of response preventing their development within the host to a lesser extent. For example, pseudomonads produce phytotoxin coronatin which activates JA-dependent plant responses thus blocking SA-dependent ones. Gene cluster that encodes enzymes for biosynthesis of coronafacic acid (cfa) (a structural component of coronatin) was identified in *Pectobacterium atrosepticum* (Pba). Pba is usually considered as brute force pathogen causing soft rots. However, it may also colonize the host asymptotically. We assumed that different types of interactions could be related to alterations in JA/SA balance in the infected plants. To check this, we assessed 1) the activity of JA- and SA-dependent hormonal systems by analyzing the expression levels of marker genes during latent and typical infections caused by the wild type Pba and its cfa-deficient mutant; 2) the effect of JA and SA pretreatment on plant resistance to pectobacteria; 3) the role of cfa in systemic plant colonization by pectobacteria. We have revealed that the type of the infection (typical or latent) caused by Pba is determined by the balance of JA- and SA-dependent responses. SA inhibits the propagation of pectobacteria or determines the development of asymptomatic interaction of pathogen with the host. The development of typical infection is related to the activation of JA-mediated responses induced by Pba-produced coronafacic acid. Our results show that the induction of JA-mediated responses is a criterion of induced susceptibility of plants to Pba. This study was supported by Russian Scientific Foundation #19-14-00194.



## **87 - GLYI4: a potential key hub of methylglyoxal scavenging and hormone signaling pathway in *Arabidopsis thaliana***

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Plant responses to biotic and abiotic stresses employ finely tuned regulatory mechanisms, largely orchestrated by phytohormones. Typically, salicylic acid (SA) plays a central role in defense responses against biotrophic and hemi-biotrophic pathogens. By contrast, jasmonic acid (JA) is usually associated with defense against necrotrophic pathogens and herbivorous insects. Moreover, plants subjected to stress can produce cytotoxic compounds, as methylglyoxal (MG), which is detrimental to the cell. MG is detoxified by the glyoxalase system composed of two enzymes, glyoxalase I (GLYI) and glyoxalase II (GLYII), which transform MG in D-lactate and GSH. Recently, by a genome-wide association study performed in *Arabidopsis*, we identified GLYI4, member of GLYI family, as a new actor in the crosstalk between SA and JA signaling pathways. Here, we investigated the impact of GLYI4 knock-down on MG scavenging and JA pathway. In *glyI4* mutant plants we observed a general stress phenotype, characterized by MG accumulation, impaired MG scavenging, accumulation of reactive oxygen species (ROS), stomatal closure and altered fitness. A metabolomics approach based on high resolution mass spectrometry on the *glyI4* mutant revealed an accumulation of compounds related to redox reactions and in cellular energy maintenance, and an impairment of those involved in plant defense and growth. Interestingly, accumulation of MG in the *glyI4* mutant led to a lower efficiency of the JA pathway, causing a greater susceptibility to the pathogenic fungus *P. cucumerina*. Furthermore, MeJA stimulus was able to relocate the GLYI4 protein from the plasma membrane to the cytoplasm. Based on these results, GLYI4 has emerged as an important actor for MG scavenging and plant health. Finally, it's tempting to speculate that there may be a crosstalk between the MG and JA pathway in which GLYI4 likely plays a key regulatory role.

**102 - RESISTANCE TO BOTRYTIS CINEREA IN ARABIDOPSIS PLANTS IMPAIRED IN DE-ESTERIFIED HOMOGALACTURONAN CONTENT CORRELATES TO INCREASED CUTICLE PERMEABILITY AND IS SUPPRESSED BY ABSCISSIC ACID**

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Pectin is a major cell wall component that plays several important roles in plant development and response to environmental stresses. Arabidopsis plants expressing a fungal polygalacturonase (35S:AnPGII plants) that degrades homogalacturonan (HGA), a major pectin component, show enhanced expression of defense responses and increased resistance to the fungal pathogen Botrytis cinerea. Here we show that resistance to B. cinerea of 35S:AnPGII plants, as well as of plants mutated in QUASIMODO2 (qua2-1 and tsd2-1), encoding a putative pectin methyltransferase important for HGA biosynthesis, is suppressed by exogenous abscisic acid (ABA). Notably, loss of HGA integrity results also in a dramatic increase in leaf cuticle permeability and is also suppressed by ABA. Cuticle permeability in plants with altered HGA is possibly mediated by the peroxidase-generated reactive oxygen species, as plants overexpressing the class III peroxidase AtPRX71, whose expression is enhanced in 35S:AnPGII and qua2-1 plants, show increased cuticle permeability, and the atrpx71-1 loss-of-function mutation partially suppresses cuticular permeability and resistance to fungal infection in qua2-1 plants. Mutants with altered content of cellulose, fucose or arabinose do not show increased resistance to B. cinerea or cuticle permeability though some of them have increased ROS accumulation. Hyperaccumulation of ROS mediated by class III peroxidases in plants with altered cell wall composition may therefore be necessary but not sufficient to impair cuticle deposition and increase resistance to B. cinerea, that are instead specifically affected in mutants with altered HGA.

### **130 - Trigger and suppression of antiviral defenses by Grapevine Pinot gris virus (GPGV): novel insights on virus-host interaction in Grapevine Leaf Mottling and Deformation (GLMD) disease**

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Grapevine leaf mottling and deformation (GLMD) is a novel disease that has been putatively associated with a new trichovirus, named grapevine pinot gris virus (GPGV). Yet the role of GPGV in symptom display is poorly understood since it has been detected both in symptomatic and symptomless grapevines. We exploited a recently constructed pRI-derived infectious clone (pRI::GPGV-vir) to induce an antiviral response in *Nicotiana benthamiana* plants. In silico prediction of virus-derived small interfering RNAs (vsiRNAs) and gene expression analyses revealed the involvement of DCL4, AGO5 and RDR6 genes during GPGV infection, suggesting the activation of the post-transcriptional gene-silencing (PTGS) pathway as a plant antiviral defense. Furthermore, PTGS-suppression assays were conducted in transgenic *N. benthamiana* 16c plants, revealing the ability of GPGV coat protein (CP) to overcome plant antiviral defense, exhibiting suppression activity of RNA silencing. This work provides novel insights on the interaction between Grapevine Pinot gris virus and its host, revealing the ability of the virus to trigger and suppress antiviral RNA silencing and suggesting that symptoms display in Grapevine Leaf Mottling and Deformation disease could depend on the delicate balance between antiviral RNA silencing and its virus-mediated suppression.

**159 - Antimicrobial activity of divinyl ethers - geometric isomers of etherolenic and etheroleic acids. The effect of double bond geometry on the intensity of antimicrobial effect of the divinyl ethers**

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Plant oxylipins - large group of secondary metabolites, derivatives of polyunsaturated fatty acids, which play an important role in protecting plants from phytopathogenic microorganisms. Some oxylipins are produced by plants in response to invasion of a wide range of pathogens; others exhibit a narrow specificity against certain microorganisms. They can also act as signaling molecules inducing the expression of protective plant genes.

Oxylipins are the products of lipoxygenase cascade, the key enzymes of which are lipoxygenases and cytochromes P450 of the CYP74 family: allene oxide synthases (AOSs), hydroperoxide lyases (HPLs), divinyl ether synthases (DEs), and epoxyalcohol synthases (EASs).

We showed that (11Z)-etherolenic, etherolenic and (ω5Z)-etherolenic acids are active against gram-negative bacteria: *Xanthomonas campestris* ssp. *vesicatoria*, *Pseudomonas syringae* ssp. *tomato*, *Pectobacterium atrosepticum* SCRI1043. These chemical compounds differ in geometry of the only double bond (cis and trans). There are literature data that fatty acids with cis double bonds have more pronounced antibacterial properties than fatty acids with trans double bonds. (ω5Z)-Etherolenic acid with two conjugated double bonds shows the greater biological activity than other isomers of etherolenic acid.

In order to check the double bonds geometry effect on the biological activity of divinyl ethers, in this work etheroleic acid isomers were tested. Moreover the number of phytopathogenic microorganisms was extended. The data obtained would become the basis for the development of new agricultural antimicrobial drugs that are not harmful for human health and the environment.

The reported study was funded by RFBR, project number 20-34-70126.

## **171 - Investigation of the effects of daytime on the phytohormone-mediated defence responses of tomato plants**

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The first line of defence in plants against pathogens is induced by the recognition of microbe-associated molecular patterns (MAMP). The best-characterized MAMP is the flagellin (flg22). Flg22-induced defence responses can be dependent on various external and internal factors. Here we studied the effects of the daytime-dependent flg22 treatments on salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signalling in leaves of intact tomato plants. Flg22 was applied in the afternoon (late light phase) and at night (early dark phase) and defence responses of the plants were recorded one hour later. Flg22 induced the rapid closure of stomata, but the degree of this closure was dependent on the daytime of the elicitor application. Flg22 perception in the first hours can be crucial to induce several defence responses in plants, thus signalling events after flg22 treatments were analysed following the different application times. Accumulation of reactive oxygen species (ROS) and nitric oxide (NO) was different after flg22 treatments in the late light phase and the early night. Surprisingly, ET emission and NO production were not significant in the dark phase-treated leaves. Moreover, SA and JA accumulation were also different in the two time-points. Expression of hormone response genes, Pathogenesis-related 1 (PR1), Ethylene response factor 1 (ERF1) and Defensin (Def) also showed significant differences in these daytimes upon flg22 treatments. Interestingly, we were able to detect a rapid systemic response of intact plants in the distal/upper leaves of flg22-treated ones based on the changes in stomatal closure, defence hormonal contents and hormone-mediated gene expression. These data highlight the importance of the daytime and the defence hormones in the response of intact plants to bacterial elicitor treatments. This work was supported by the National Research, Development and Innovation Office – NKFIH, (Grant no. NKFI FK 124871), and by the UNKP-19-4-SZTE-86 and 19-1-SZTE-42 Programs.

**200 - Investigating the molecular interactions of *Plasmodiophora brassicae* with *Arabidopsis thaliana* through a genome-wide association study and transcriptomics.**

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**(1)**

*Plasmodiophora brassicae* is an obligate biotrophic pathogen causing clubroot disease of oilseed rape and other brassicas. *P. brassicae* induces the formation of large galls on infected plants establishing a favourable environment for sequestration of host resources and spore multiplication. This interferes with the nutrient and water uptake ability of the host and thus leads to significant crop loss. To better understand the genetic basis underpinning resistance and susceptibility to clubroot disease we carried out a screen of *Arabidopsis* accessions, quantifying their pathogen levels when infected with an aggressive pathotype (P1b) prevalent in Poland. We inoculated 142 accessions and collected the hypocotyl and upper 1 cm of root 19 dpi. Quantitative PCR was performed on DNA extracted from these galls using primers for the pathogen gene Pb18S and the host gene AtSK11 to determine relative infection levels. 12 accessions were resistant based on pathogen quantification and absence of gall formation. Genome wide association (GWA) analysis highlighted 2 SNPs- one in a novel TIR-NBS-LRR gene and the other in AT1G32030 which is adjacent to the RPB1 resistance loci previously identified in Tsu-0. Confirmation of the NBS-LRR and RPB1 as the genes responsible for resistance in each incompatible interaction is ongoing. GWA analysis of the 130 susceptible accessions has implicated numerous genes and T-DNA knock-out lines for selected candidates are being evaluated. Gene expression responses in the compatible and incompatible interactions have also been profiled using RNA-Seq.

## **205 - Xylella fastidiosa in olive tree: physiological evidences correlated to the resistance of the Leccino cultivar**

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The identification of *Xylella fastidiosa*-resistant genotypes represents the most promising strategy to control this pathogen. Since the first observations in Southern Italy, the cultivar Leccino showed resistance to *Xylella*; in order to identify the mechanisms explaining this resistance, we compared Leccino to susceptible cultivars adopting many approaches and looking at several aspects. A first finding was the increase of quinic acid, a lignin precursor, detected only in Leccino infected leaves: increased cell wall lignification can reduce bacterial movement, thus delaying disease progression. Laser scanning microscopy observations revealed lignin deposition in xylem vessels and expression of phenylpropanoid pathway genes showed, in infected Leccino branches, the Cinnamoyl-CoA Reductase upregulation, described as strongly induced during “defence response associated” lignin formation. Scanning electron microscopy-Energy Dispersive X-ray observations of infected stem sections displayed starch grains accumulation (a refilling mechanism) in Leccino vessels, while anatomical measurements on healthy stems indicated that Leccino could be constitutively less susceptible to cavitation. Gene expression patterns (upregulation of aquaporin OeTIP1.1, sucrose transporters OeSUT1 and OeMST2, amylases OeAMY and OeAMY2) suggested that infected Leccino is able to modulate genes involved in embolism sensing and refilling mechanisms: this ability to detect embolism and to restore hydraulic conductivity can influence symptoms severity. A fluorescence in situ hybridization probe specific for *Xylella fastidiosa* (named KO210) was optimized to survey the colonization behavior in olive trees. The occlusions and the *Xylella*-induced changes in wood-anatomical characteristics, such as the increase in both vessels number and ring width, were observed in rings from the year 2010 to 2017 in both olive cultivars by a dendrochronological approach: as expected, vessel occlusions in the susceptible cultivars were significantly higher than in Leccino. These studies indicated that Leccino resistance to *Xylella* has a multifactorial basis, moreover, composition and structure of xylematic tissues play a central role in the pathogen colonization.

### **235 - Fight against biotic and abiotic stress in plants - New Ionic liquids based on the Systemic Acquired Resistance anion and polyamine cation**

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There are many compounds which can be used as enhancements of resistance to biotic and abiotic stress in plants. One of them are polyamines or compounds based on cholinum structure which increase the tolerance of plants to adverse environmental factors. However, it is possible to combine these anti-stress factors with the biologically active substance (here the plant immune inducer) into a bifunctional salt which exhibits behavior of both ions. The high biological efficiency of the elicitors combined with protective properties in one chemical compound may be an interesting possibility to show a new concept of design of plant protection agents.

We have successfully synthesized bifunctional salts with anti-stress cation and SAR activating properties anion. SAR inducing properties of synthesized compounds were examined by monitoring inhibition of the viral infection in tobacco plants as compared to control plants (not treated). Also phytotoxicity assessment was performed on tobacco N. tabacum var. Xanthi plants and Raphanus sativus seeds. Obtained results show that the presence of anti-stress cations decreased the phytotoxic effect of SAR inducers compounds. Moreover anti-stress cations have not changed the SAR-inducing properties.

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## **236 - New ionic liquids based on systemic acquired resistance inducers combined with phytotoxicity reducing cation**

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Modern agriculture at present times is facing many challenges. On the one hand, society requires crops and plant-related products free from contaminations of plant protection products (PPP) residues. On the other, crop quantity and quality should be sufficient due to continuously increasing demand for food. Unfortunately most often crop yields are reduced by pathogens, insects, other pests or weather conditions, so that tasks posed to modern agriculture focuses on finding new and more sophisticated methods of plant protection, even not chemical-based.

One of the promising method of plant protection is the activation of the systemic acquired resistance (SAR). Systemic acquired resistance has a broad spectrum action against pathogens, most often simultaneously against bacteria, viruses and fungi. This phenomenon is one of the defense mechanism against pathogens which were developed in the evolutionary process. Systemic acquired resistance is activated by pathogen attack or artificially, by compounds imitating the plant-pathogen interaction. Activation of this phenomenon is followed by transportation of the signaling substances such salicylic acid or salicylic acid methyl ester to every part of the plant. This signaling compounds activate metabolic pathways in which defense compounds and pathogenesis-related (PR) proteins are synthesized. Those proteins have antifungal, antiviral and antimicrobial activity.[1]

We have successfully synthesized new ionic liquids composed of the anion of plant resistance inducers and cholinium, betainium and chlorocholinium cations. Following the synthesis we determined phytotoxicity and SAR inducing properties.

This work was supported by the National Science Centre (Poland), project PRELUDIUM (No. 2018/29/N/NZ9/01813) - "Systemic Acquired Resistance (SAR) of plants against viruses: new elicitors and biological and molecular characterization of their mechanism of action" co-financed by European Union.

## **257 - An integrated approach to unveil Pinus spp. response to Fusarium circinatum inoculation**

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*Fusarium circinatum* Nirenberg & O'Donnell, the causal agent of pine pitch canker, represents a considerable threat to conifer forests worldwide being associated to significant economic losses. Although essential for the development of disease mitigation strategies only recently research focused on host's susceptibility/tolerance mechanisms has begun. Our current research line relies on the use of a multidisciplinary approach to study the contrasting responses of *Pinus radiata* D. Don (highly susceptible) and *Pinus pinea* L. (relatively tolerant) to *F. circinatum* artificial inoculation. In the present work, the proteomic profiles of both pine species were analysed 10 days post-inoculation with the pathogen, when all inoculated *P. radiata* seedlings presented disease symptoms (tip dye-back and needle wilting and browning). This data was integrated with physiological, hormonal and oxidative stress-related results for the same sampling point. In addition, epigenetic variation was assessed. This strategy allowed us to identify species-specific responses and provided a comprehensive overview of the mechanisms occurring upon *F. circinatum* attack in *Pinus* spp., defining new interactions, signalling pathways and related targets depending on species susceptibility/tolerance to *F. circinatum* infection. In general, this approach contributes to fulfil knowledge gaps on forest tree stress responses and is aimed at developing markers for the implementation of environmentally friendly pest management strategies to control pine pitch canker.

## 258 - Origin of dispensable chromosomes in *Fusarium verticillioides* strains

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*Fusarium verticillioides* (Fv) is considered one of the most common plant pathogenic fungi affecting Zea mays (maize) roots, stalk tissues and kernels causing diseases, such as stalk and ear rot. Moreover, Fv is capable to produce mycotoxins, which can accumulate into the kernels in storage that can be dangerous for animal's and for human's health. Although its economic importance, little attention has been given to this pathogen. Here we report a comparative genomic study between Fv Italian strains and worldwide strains. The Fv Italian strain (Fv10027) was compared to 24 Fv Italian isolates, to the Fv7600 which is the BROAD institute reference strain isolated in USA and to three strains from Australia. Pulsed-field electrophoresis analysis showed chromosomes polymorphisms between the Fv7600 and Fv10027. Therefore, the genome of Fv10027 was re-sequenced using Nanopore sequencing and used as reference for comparative genomics. The comparative analysis confirmed chromosome polymorphisms of two mini-chromosomes (Chr12 and Chr13) between Fv10027 and Fv7600 strains. Additionally, we observe chromosomes polymorphisms between Fv10027 and both Australian and Italian Fv strains. Gene prediction analysis and annotation was performed resulting in an accumulation of putative effectors and cluster for secondary metabolites on the mini-chromosomes. Phylogenetic analysis was performed to determine the origin of the mini-chromosomes. The results suggest that the mini-chromosomes follow the same evolutionary path of the core chromosomes leading us excluding a horizontal transfer from other fungi. To support this hypothesis, ~1/3 of all genes codified on the Chr12 of about 1Mbases had a homology with genes located on the dispensable Chr3 of *F. oxysporum* f. sp. *lycopersici*. Interestingly, synonymous substitution analysis showed an accelerated evolution of the genes on Chr12 and Chr13 compared to the core genome. Additional analysis are required to understand the role of these chromosomes on the virulence of Fv10027 in maize.

## 259 - Molecular insights into the direct and indirect effects of natural extracts on plant-pathogen interaction

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Chemical compounds as natural plant extracts can either directly or indirectly increase the tolerance of plant towards both environmental stresses and defence towards pathogens. The main object of this study was to test different natural plant extracts in barley plants inoculated with *Blumeria graminis*, the pathogen responsible of powdery mildew disease in barley. With this aim, either plants inoculated and non-inoculated with *Blumeria* were studied to investigate the most favourable treatment in terms of plant-pathogen interaction. The combination of untargeted metabolomics and gene expression analysis were applied not only to select the most effective treatment to enhance the plant defence, but to elucidate the plant response at molecular level.

Firstly, two different plant natural extracts (Tea Tree Oil (TTO) and a mix containing TTO, eugenol and thymol) were applied to barley plants. Afterwards, barley plants were inoculated with oidium and natural products were also tested after biotic stress application. Subsequently, a comprehensive characterization of the metabolomic profile was performed using a UHPLC chromatographic system coupled to a hybrid quadrupole-time-of-flight mass spectrometer (UHPLC/QTOF-MS). Multivariate statistics allowed to discerning the metabolic changes in plants after natural extracts application and to identify differential metabolites. Thereafter, data interpretation using the Plantyc Pathway Tool Software led to a better understanding of the physiological processes involved. The untargeted metabolomics allowed discriminating the most effective treatment against pathogen-induced stress. In order to better comprehend the complexity of the physiological response to *Blumeria*, a Chemical Similarity Enrichment Analysis for Metabolomics was also carried out, obtaining the principal classes of metabolites involved in the plant defence. Additionally, the principal genes related to plant defence were evaluated by using RNAseq.

Our findings revealed that the mix was the most efficient extract. Secondary metabolism was strongly stimulated by this treatment. Phenylpropanoids, in particular flavonoids, were up-accumulated after the mix application. Moreover, both the TTO and the mix extracts provoked a high production of phytoalexins in inoculated-plants.

To summarize, the combined use of metabolomic and transcriptomic allowed to study in depth the effect of plant extracts on plant pathogen interaction and priming induction.

**311 - Novel antimicrobial peptides from blackseed (*Nigella sativa* L.) with absolutely unique Cys-motif represent strong inhibition towards mold fungal species**

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Blackseed (*Nigella sativa* L.) is a rich source of a wide range of biologically active compounds with different activities. This plant is widely used as a medicinal plant and a spice. Several antimicrobial peptides (AMPs) belonging to some structural families were found in *N. sativa* seeds. In this study, we report the investigation of new AMPs with a previously unknown cysteine motif for all the kingdoms of living things. Six novel cysteine-rich and disulfide-linked homologous peptides were isolated from *N. sativa* seeds via acetic acid extraction followed by liquid chromatographic techniques. These peptides were found in the 0 mM NaCl fraction after cation-exchange (pseudo-affinity) medium-pressure chromatography and then further purified using reversed-phase high-performance liquid chromatography (RP-HPLC). The individual peptides were collected manually, and their primary structure was evaluated by automated Edman degradation. The unusual cysteine motif of XnC<sub>1</sub>XXXC<sub>2</sub>XXXC<sub>3</sub>XnC<sub>4</sub>XXXC<sub>5</sub>XXXC<sub>6</sub>Xn was detected. No analogous sequences were found in the databases, so we propose this motif as new AMP representatives and termed them “nigellins”.

The resulting peptides demonstrated specific antifungal activities against *Aspergillus* and *Penicillium* species over a wide range of tested micromolar concentrations but were found to be completely inactive against both Gram-negative and -positive bacteria and yeasts. These compounds were found to be similar with respect to their spatial structures, which are represented by two  $\alpha$ -helices,  $\beta$ -turn, and N- and C-terminal random coils. This folding type and secondary structure elements are typical for the previously identified plant  $\alpha$ -haipinins. Therefore, the newly described peptides are presumably members of a novel  $\alpha$ -haipinin subfamily.

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### 316 - Effect of Plant Inhibitory Proteins on Pectinases in Herbivorous Beetles

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The herbivorous mustard leaf beetle *Phaedon cochleariae* feeds on brassicaceous plants and possesses various digestive carbohydrases targeting plant cell wall polysaccharides. Amongst those, polygalacturonases (PGs) hydrolyse the polysaccharide pectin.

In beetles, PGs are mainly restricted to the Phytophaga clade (leaf beetles, longhorned beetles and weevils). Surprisingly, their PG-encoding genes originate from horizontal gene transfer from a fungus to a common Phytophaga ancestor approximately 200 million years ago. During Phytophaga radiation, the ancestral PG gene underwent extensive duplication, enabling functional divergence.

In plants, cell wall-associated PG-inhibiting proteins (PGIPs) counteract microbial PGs and thus contribute to the defence against phytopathogens. However, the impact of plant PGIPs on beetles and their PGs is unknown.

I aim to characterize the interaction of plant PGIPs with beetle PGs and the subsequent consequences on PG activity and beetle performance. Putative PGIPs were identified from Chinese cabbage, a food plant of the polyphagous *P. cochleariae*, in an unbiased interaction assay and stably overexpressed in the model plant *Arabidopsis thaliana*. Using PGIP knockout as well as overexpression lines in feeding assays revealed a negative impact of PGIPs on the beetles' performance in vivo. PG enzyme activity assays further elucidated the specificity of binding and inhibitory effect of PGIPs towards beetle PGs in vitro. These experiments provide the first insights into both the importance of PGs for Phytophaga beetles and the dual, protective role of PGIPs defending plants against microbes and insects.

**318 - Comparative characterization of qualitative and quantitative content of antimicrobial proteins and peptides from different rapeseed (*Brassica napus* L.) breeds to evaluate their resistance to diseases**

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In this study, a comparative analysis of the antimicrobial polypeptide compositions of several cultivated and related rapeseed plants (*Brassica napus*) was carried out to comprehensively analyze the polypeptides' contribution to resistance to environmental biotic stress factors, particularly fungal and bacterial plant pathogens. Overall, 10 of 12 samples showed identical protein-peptide composition profiles, and the highest degree of polypeptide representation, in all the variants, was localized to a lower-molecular-weight region (less than 10 kDa). Regarding the antimicrobial activity of the studied protein-peptide extracts of rapeseeds, at the concentration of 2 mg/ml, there was a complete absence of any inhibitory effect on the tested cultures, but increasing the load to 8 mg/ml resulted in an exclusively fungistatic effect on *C. albicans* and *F. solani*. When comparing the protein-peptide profiles of the rapeseeds to that of a closely related weed, the wild radish (*Raphanus raphanistrum*), it was found that the qualitative composition of proteins and peptides of the wild radish radically differed from that of rapeseed, mainly due to the absence of macromolecular components, presumably reserve proteins. These results make it possible to examine, in greater detail, some aspects of the molecular basis of plant immunity to diseases. This work is supported by the Russian Science Foundation (grant № 18-74-10073) and Russian Foundation for Basic Research (grant № 18-34-20058).

### **323 - Role of S-nitrosylation in plant viral immunity**

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S-nitrosylation, the addition of a NO moiety to protein cysteine thiol to form an S-nitrosothiol (SNO), has shown to be involved in plant immunity against bacterial and fungal pathogens. However, not much is known about its effect on plant's interaction with viruses. Here, we show that increased SNO levels in plants confer them with viral resistance. Interestingly, this observation opposes the trend seen with bacterial and fungal pathogen. In this study, we explore the mechanism associated with S-nitrosylation based viral resistance.



### **326 - Botrytis cinerea induces local hypoxia in Arabidopsis leaves**

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Low oxygen availability is often associated with soil waterlogging or submergence, but may occur also as hypoxic niches in otherwise aerobic tissues. Experimental evidences assign a role in Botrytis cinerea resistance to a group of oxygen-unstable Ethylene Response Factors (ERF-VII). The involvement of the oxygen-destabilized ERF-VII proteins in the response of plants to biotic stresses raises the question about the mechanism by which they get stabilized during pathogen infection. Pathogens often attack fully aerobic tissues such as leaves and, additionally, they often induce the synthesis of NO that can further destabilize the ERF-VII proteins. Given that these proteins are potentially highly unstable during pathogen infection, it is tempting to speculate that pathogen infection locally induces hypoxic conditions in an otherwise fully aerobic plant tissue such as leaves. Alternatively, a mechanism independent from oxygen might stabilize the ERF-VII proteins. We analysed the expression of hypoxia-responsive genes in infected leaves. Confocal microscopy was utilized to verify the localization of the ERF-VII protein RAP2.12. Oxygen level was measured to evaluate the availability of oxygen. We discovered that infection by Botrytis cinerea induces increased respiration, leading to a drastic drop in the oxygen level in an otherwise fully aerobic leaf. The establishment of a local hypoxic area results in stabilization and nuclear relocalization of RAP2.12. Hypoxia at the site of pathogen infection generates a nearly oxygen-free environment that allows the stabilization of ERF-VII proteins and may also affect the stability of other N-degron-regulated proteins as well as the metabolism of elicitors.

**336 - The effect of lactone-, ketone-containing brassinosteroids on physiological status Brassica napus plants in normal conditions and under salt stress**

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Salinity is one of the main stresses reducing plant growth and productivity. Impaired ionic and osmotic balance and increased levels of reactive oxygen species are the main damaging factors of salt stress. The increase of plant resistance is largely determined by hormonal factors, such as brassinosteroids (BS). The 24-epibrassinolide (EBL) exhibits high biological activity. A potentially active compound also can be its precursor – ketone-containing, 24-epicastasterone (ECS). In this work, we compared the effects of EBL and ECS on Brassica napus plants in normal conditions and under salt stress. Plants, at the age of three weeks, were pretreated with EBL or ECS at a concentration of  $10^{-8}$  M for 4 hours, after that plants were transferred to a ½ Hoagland and Snyder (HS) solution without BRs for 20 h. Then, plants were placed into HS medium in the absence (control) or presence of 150 mM NaCl (experimental variants) for 6 days. In normal conditions, ECS, in contrast to EBL, showed a growth-promoting effect on rapeseed, which consists in enhancing of the leaf surface and fresh weight of plants. In addition, exogenous ECS stimulated the accumulation of chlorophyll a and carotenoids, while EBL did not cause significant changes. To grow of rape plants on HS medium containing 150 mM NaCl resulted in a decrease of growth parameters, the content of photosynthetic pigments, and induced of osmotic and oxidative stresses. ECS pretreatment to a greater extent than EBL pretreatment reduced the negative effect of salt on leaf surface size and fresh plant weight. The specificity in the activation of antioxidant enzymes in response to short-term treatment of EBL or ECS with subsequent chloride salinity was revealed.

The reported study was funded by RFBR, project number 19-34-90093.

### **344 - CRISPR/Cas9 mediated genome editing of OsbZIP73 reduces disease susceptibility to *Xanthomonas oryzae* pv. *oryzae***

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Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of main diseases in rice field. Xoo translocates effectors including transcription activator-like (TAL) effector into the host through the Type III secretion (T3S) system. These proteins migrate to the rice nucleus and bind to the promoter of a specific gene to induce its transcription. In this study we isolated TAL effectors from a Korean Xoo strain and tried to identify host genes regulated by them. Based on search results of prediction program we carried out qRT-PCR for candidate genes. We found that OsbZIP73 was up-regulated upon the infection of a Korean Xoo strain. To investigate whether TAL effectors regulate OsbZIP73 expression, we performed a promoter transient expression assay and found that only TALE2 increased expression of OsbZIP73. In addition, chromatin immunoprecipitation qPCR assay using transgenic plants overexpressing TALE2 gene demonstrated that TALE2 directly bound to a specific nucleotide sequence of the promoter of OsbZIP73. Based on analysis of transgenic plants overexpressing OsbZIP73 we found that OsbZIP73 suppresses basal defense instead of sugar supply. Now we tried to mutagenize TALE2 target sequence of OsbZIP73 promoter through CRISPR/Cas9 system. We obtained rice lines containing various mutations of OsbZIP73 promoter such as deletion or insertion. We found that mutant rice lines showed reduced symptoms to Xoo, it is proposing that mutation in rice lines is responsible for TALE binding. This approach will provide a novel strategy to develop disease resistant rice cultivar.

### 348 - Digging into the *Arabidopsis thaliana* genome for Flavescence dorée phytoplasma resistance genes

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Phytoplasmas are phloem-restricted plant pathogenic bacteria that infect hundreds of vegetal species, causing considerable crop losses worldwide. Flavescence dorée phytoplasma (FDp) is a quarantine pest associated with a disease of grapevines causing severe damages to the viticulture in Southern Europe. FDp is transmitted by the leafhopper *Scaphoideus titanus* in a persistent-propagative manner. The management of the disease is based mainly on roguing of infected plants, planting of healthy grafted rootstocks, and compulsory insecticide treatments against the vectors. Resistant *Vitis vinifera* genotypes are not reported so far, and all the cultivars are susceptible even if in different degrees. Studying the interactions between FDp and its natural plant host is rather difficult due to the long life cycle of grapevine, the dimension of its genome, and the difficulties in obtaining genetic mutants. Therefore, the model system *Arabidopsis thaliana* was adopted to explore the plant genome and discover candidate genes for resistance to FDp. *A. thaliana*, infected by FDp in controlled conditions by the vector *Euscelidius variegatus*, show a partial tolerance/resistance to the phytoplasma, revealing a low infection rate and mild symptoms. Comparison of RNAseq data of both grapevines and *Arabidopsis* FDp-infected plants, resulted in a list of twenty genes putatively involved in plant response to phytoplasma infection, mainly belonging to the GO categories of 'plant defense', 'hormone metabolism' and 'cell development'. The relative expression of these genes was studied overtime by qRT-PCR on FDp-infected *Arabidopsis* in comparison with healthy controls. The genes involved in the metabolisms of jasmonate and callose deposition were differentially expressed, and the results were validated using *Arabidopsis* mutants in a reverse genetic approach. This study represents a first step towards the identification of new plant pathways involved in resistance to Flavescence dorée.

## **352 - Uncoupling growth and immunity by application of chemical compounds**

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Climate changes and increased temperatures support the spreading of pathogens, thus challenging future harvests. Plants have developed an effective immune response against pathogens. However, the defence often occurs at the expense of plant biomass production. We aim at the identification of chemical compounds that uncouple growth and immunity in order to obtain resistant plants with sufficient yield.

We are performing a chemical genetic screen, using the autoimmune mutant saul1-1 (senescence associated ubiquitin ligase1-1) as a marker line. In the homozygous knockout mutant saul1-1 the effector triggered immunity can be induced by low ambient temperatures below 24°C, which results in a dwarf phenotype with lesioning of all above-ground organs and an up-regulation of pathogenesis related (PR) genes. We aim at identifying chemical compounds that induce growth and prevent lesions but maintain a high level of expressed defence genes.

A previous chemical genetic screen performed in the group of Prof Xin Li at the University of British Columbia identified the chemical compound Ro8-4304 as a potential uncoupler of growth and immunity in the mutant chs3-2D (chilling sensitive3-2D. Ro8-4304 stimulated growth and kept defence gene expression slightly higher in chs3-2D than in the wild type. Here, we show that in different Ro8-4304 derivatives its efficiency is affected.

### **361 - Enhanced susceptibility to a necrotrophic fungal pathogen *Pyrenophora teres* induced by heat stress in barley**

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Previous research has shown that short term heat treatment (49 ° C for 20 to 30 seconds) increases susceptibility to a biotrophic pathogen *Blumeria graminis* f.sp. *hordei* in barley and at the same time a decrease in the level of reactive oxygen species (ROS) was detectable. In our recent project we want to study how heat shock influences the defense responses of barley to a necrotrophic pathogen *Pyrenophora teres*. We used two barley cultivars (*Hordeum vulgare* cv. Ingrid and cv. Himalaya) which are both susceptible to *P. teres* f.sp. *teres* 289 Hungarian isolate. A short term heat shock (49 ° C for 20 seconds) significantly increased both the severity of necrotic symptoms caused by *P. teres* and the accumulation of fungal biomass in both barley cultivars as detected by real-time qPCR, especially in cv. Himalaya. Among the tested defense related genes the expression of HvPR-1b increased significantly one and four days after inoculation while heat shock further induced the expression of HvPR-1b. The amount of ROS (superoxide and hydrogen peroxide) increased significantly in heat shock-exposed and infected plants, as compared to infected plants without heat treatment. Both the heat treatment and the infection induced the amount of oxidized glutathione. However, in cv. Ingrid, the amount of reduced glutathione was also increased along with the level of oxidized glutathione, whereas in cv. Himalaya, most of the glutathione was oxidized, associated with enhanced susceptibility of this cultivar to *P. teres*.

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### **374 - Effects of juvenility and cytokinins on development, resistance and gene expression of plants**

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The plant hormones cytokinins affect a various array of plant growth and development processes, in addition to the induction of juvenility/inhibition of senescence. It is generally accepted that biotrophic pathogens prefer juvenile, but necrotrophic pathogens prefer senescent plant tissues. We found that cytokinin overproducing paraquat tolerant juvenile tobaccos are more tolerant, while NahG salicylic acid deficient tobaccos are more sensitive to the reactive oxygen H<sub>2</sub>O<sub>2</sub> than their respective controls, indicating the higher and lower antioxidant capacity, respectively. The biotrophic powdery mildew (*Golovinomyces orontii*) infection slightly changed the leakage of ions from tobacco leaves. Although the necrotic leaf area was only about 35-45% larger on NahG than on Xanthi leaves by TMV infection, it led to a 6-fold and 2-fold elevation of ion leakage from HR resistant NahG and Xanthi leaves, respectively.

Furthermore, we report on the different, sometimes opposite, effect of kinetin (N<sup>6</sup>-furfuryladenine) and benzyl adenine (BA) on development, on tolerance to virus, bacteria and fungi infection of Arabidopsis and tobacco plants, in addition to the changes in gene expression profiles of Arabidopsis as reactions to treatments with the two cytokinins. Generally treatments with water solution of BA had much stronger stress protecting effect on both plants than treatments with water solution of kinetin. Similarly, in microarray tests only BA treatment upregulated more than 1000 genes, and downregulated more than 2000 genes, while only 134 genes were upregulated and 133 genes were downregulated by kinetin treatment. Even, there were 28 genes where BA and kinetin treatments caused changes of gene expressions into the opposite directions.

### **390 - Combined effects of environmental factors on non-host resistance to Tobacco mosaic virus in barley**

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Abiotic and biotic environmental factors like high temperature and pathogen infections can have a profound influence on disease resistance of plants. For example, it is known from the 1970s that Tobacco mosaic virus (TMV) is capable of replication and systemic spread in non-host barley plants at prolonged high temperatures (30 °C). TMV spread is further enhanced if heat-exposed barley is also infected by an adapted virus, e.g. Barley stripe mosaic virus (BSMV). We have shown previously that the non-host resistance of barley to wheat powdery mildew can be partially suppressed by a simultaneous application of heat shock (49 °C for 20 sec) and antioxidant treatments. However, the effect of a heat shock on virus infections is currently not known. In the present study we have aimed at elucidating how heat shock may influence non-host resistance of barley to a non-adapted virus, TMV.

In barley cv. Ingrid plants containing BSMV and mechanically inoculated with TMV a heat shock treatment (49 °C for 20 sec) resulted in the decline of TMV accumulation within the first two days after inoculation (DAI), as compared to untreated controls. On the other hand, at four and seven DAI this trend has reversed, since TMV levels became significantly (50-100%) higher in heat shock-exposed plants. Expression of a barley pathogenesis-related gene (HvPR-1b) varied in parallel with TMV levels. It seems that a heat shock and BSMV infection in concert can impair the non-host resistance of barley to a non-adapted virus, TMV.

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#### 409 - Powdery mildew and grapes: the metabolism associated with susceptibility

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Vitis vinifera berries are extremely sensitive to infection by the biotrophic pathogen Erysiphe necator causing powdery mildew disease and deleterious effects on grape and wine quality.

The combined analysis of the transcriptome and metabolome associated with this common fungal infection has not been previously carried out in any fruit. In order to identify the molecular, hormonal and metabolic mechanisms associated with infection, healthy and naturally infected Carignan berries were collected at two developmental stages: late green (EL33) and early véraison (EL35). RNA sequencing combined with GC-EI/TOF-MS, GC-EI/QUAD-MS and LC-MS/MS analyses revealed that powdery mildew-susceptible grape berries were able to activate defensive mechanisms with the involvement of salicylic acid and jasmonates and to accumulate defense-associated metabolites (e.g. phenylpropanoids, fatty acids). The defensive strategies also indicated organ-specific responses namely the activation of fatty acid biosynthesis. However, defense responses were not enough to restrict fungal growth. The fungal metabolic program during infection involves secretion of effectors related to effector-triggered susceptibility, carbohydrate-active enzymes and activation of sugar, fatty acid and nitrogen uptake and could be under epigenetic regulation. This study also identified potential metabolic biomarkers such as gallic, eicosanoic and docosanoic acids, and resveratrol, that can be used to monitor early stages of infection.

#### 459 - Impact of *Erysiphe necator* on the cell wall remodelling response in susceptible grape berries

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Grapes are of a significant worldwide economic importance, but fungal diseases pose threats to its yield and quality as well as wine production. Powdery mildew is one of the most prevalent grape diseases and is caused by the fungus *Erysiphe necator* and it can infect both leaves and grapes. Being a biotrophic fungus, *E. necator* requires a living host, having preference for green chlorophyll-containing tissues, and manipulates plant metabolic pathways for its own advantage. The plant cell wall acts as an important dynamic structure in plant-pathogen interaction and with the cuticle, functions as the first barrier against pathogens. Besides its physical barrier role, when breached, it can also trigger signaling pathways involved in defense and de novo synthesis of defensive compounds, leading to a full cellular response against the invader. The cell wall is an intricate matrix of different polysaccharides, including cellulose, pectins and hemicelluloses. However, pathogens have evolved enzymatic machinery to cleave glycosidic bonds, leading to cell wall rupture. In this detailed study, GC, FTIR and Comprehensive microarray polymer profiling (CoMPP) were used to explore how cell wall monomers and polysaccharides vary upon infection in Carignan grapes at two different time points – green (EL33) and véraison (EL35). A reduction of galactose and increase of glucose was noticed at EL33 in infected samples. To complement the metabolic data, RT-qPCR analysis was used to analyse *V. vinifera* genes involved in cell wall metabolism and pathogen defense responses. Diverse genes coding for glucosidases and galactosidases were upregulated upon infection, as well as chitinases, whereas there was a downregulation in pectinesterases' expression. These metabolic and transcriptomic results provide novel insights into the role of the cell wall in grape defense against an important biotrophic pathogen.

**478 - Virulence-related metabolism may be activated in Botrytis cinerea mostly in the interaction with tolerant green grapes that remain largely unaffected**

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Botrytis cinerea is responsible for the gray mold disease, severely affecting Vitis vinifera grapevine and hundreds of other economical important crops. However, many mechanisms of this fruit-pathogen interaction remain unknown. The combined analysis of the transcriptome and metabolome of green fruits infected with B. cinerea from susceptible and tolerant genotypes was never performed in any fleshy fruit, mostly because green fruits are widely believed to be resistant to this fungus.

In this work, peppercorn-sized fruits were infected in the field and mock-treated and infected berries were collected at green (EL32) stage from a susceptible (Trincadeira) and a tolerant (Syrah) variety. The RNAseq and GC-MS data suggested that Syrah exhibited a pre-activated/ basal defense relying on signaling pathways (enrichment in protein kinases, transcription factors, Ca<sup>2+</sup> signaling) together with jasmonates and ethylene metabolism, among several up-regulated genes involved in phenylpropanoid metabolism. In addition, metabolites such as ursolic acid, trans-4-hydroxy cinnamic acid, and epigallocatechin were more present in Syrah before infection. On the other hand, Trincadeira undergone a broad metabolism reprogramming upon B. cinerea infection, however, insufficient to contain disease progression at green berry stage.

RNA-seq analysis of the fungus in planta revealed an opposite scenario with higher gene expression activity in B. cinerea during infection of the tolerant cultivar and lesser activity in Trincadeira infected berries. The results suggested an active virulence state even without visible disease symptoms on the tolerant cultivar. Together, this study brings novel insights related to B. cinerea early infection strategies and the green berry defense regulation involved in tolerance/ susceptibility against necrotrophic fungus.

#### 480 - Can chronic ionizing radiation compromise plant immunity?

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Ionizing radiation is a ubiquitous stressor that affects growth, development, and physiology of plants. The primary exposure to this factor causes radiolysis of macromolecules. Reactive oxygen species are important byproducts of radiolysis initiating oxidative stress in plants. Besides, direct DNA damage provokes chromosomal aberrations and mutations. Notably, ionizing radiation's effect depends on its intensity and exposure period. Stressed plants synthesize specific proteins and metabolites modifying transcriptomic and proteomic profiles. Two scenarios are plausible: (i) Primed defense system may help plants to cope with pests and pathogens, or (ii) Plants can become more susceptible to invaders due to the negative effect of ionizing radiation on their morphological barriers and biochemical arsenal. Our earlier research showed increased infestation of the wild aquatic plant (common reed—*Phragmites australis*) by mites in lakes heavily contaminated with radionuclides. Such data encouraged us to design a further study for discovering biochemical mechanisms responsible for the compromised immunity in the common reed in the aftermath of ionizing radiation exposure. We collected mature leaves from contaminated (primarily with radionuclides <sup>137</sup>Cs and <sup>90</sup>Sr) and reference lakes in the Chernobyl exclusion zone. Currently, we are testing infectious bioassays to verify field effects in robust laboratory conditions. Discovery proteomics with liquid chromatography profiling and mass spectrometry quantification will detect complex changes in affected plants. Complementary analysis of carbonylated proteins, enriched with affinity chromatography, should pinpoint cellular targets suffering from oxidative stress. We aspire to solve the intriguing knowledge gap in fundamental radiobiology and produce relevant practical guidelines for the management of contaminated lakes.

**494 - Transcriptomic and biochemical analyses highlight the molecular mechanisms involved in Pinus pinaster resistance to pine wilt disease**

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Pine wilt disease (PWD) is caused by the parasitic nematode *Bursaphelenchus xylophilus*, or pinewood nematode (PWN). Since its introduction in the 90's, PWD has become a serious threat to conifer forests in Iberian Peninsula, where *Pinus pinaster* is the most affected species due to its high susceptibility. Interestingly, heritable resistance has been reported in *P. pinaster* trees, opening the possibility for selecting and breeding for this trait. Understanding the molecular basis of this resistance can be useful for future programs aiming at mitigating PWD impact in *P. pinaster* forests. In this study, we compared the transcriptional changes between resistant and susceptible plants after inoculation, highlighting the mechanisms possibly involved in *P. pinaster* resistance to PWD. Furthermore, the role of miRNAs in the regulation of *P. pinaster* response was investigated by analysing the small RNA expression in the same samples. Our analysis revealed a strong reprogramming of gene expression, with a higher number of differentially expressed genes in resistant (1916) than in susceptible plants (1226). Biochemical analyses confirmed a role of lignin synthesis and jasmonic acid defence pathway in resistance, while secondary metabolism, oxidative stress response and resistance genes also seem relevant to overcome PWD. From the over 2000 miRNAs identified, 40 were differentially expressed after inoculation, some of which with predicted targets associated to plant defence response, such secondary metabolism and resistance genes. This study provides valuable information about the molecular mechanisms involved in *P. pinaster* resistance to PWD and elucidates the role of miRNAs in the post-transcriptional regulation of *P. pinaster* defence response, which may be useful for the development of new strategies to protect *P. pinaster* forests from PWD.

**501 - Tolerance and susceptibility of grapevine leaves during interaction with *Erysiphe necator* is genotype-specific and may be associated with constitutive levels of phytohormones**

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Biotrophic fungus *Erysiphe necator* causes powdery mildew in grapevine and imposes high economical losses through reduction of grapes' yield and quality. During grapevine – *E. necator* interaction, phytohormones are major modulators of structural and biochemical defensive responses. Nevertheless, the analyses of hormonal profiling in this context are scarce. In the present work, changes in hormonal metabolism in grapevine were compared between a tolerant (*Vitis rupestris* × *riparia* cv. 101-14 Millardet et de Grasset) and a susceptible (*Vitis vinifera* cv. Aragonês) species when infected with *E. necator*.

Under greenhouse conditions, infected and mock-inoculated leaves were collected at 0, 6, 24, 96 hours post-infection for analysis of hormonal profiling and targeted qPCR analysis of genes related to hormonal biosynthesis, metabolism and signalling.

The results showed a substantial reprogramming of hormonal metabolism in response to fungal attack. Constitutive high levels of salicylic acid (SA) and auxins (IAA) together with additional induction of SA and constitutive low levels of jasmonates and ABA are likely involved in a priming stage leading to a faster response upon infection. This hormonal combination may be related to the interspecific genetic background and may be fundamental in providing tolerance or susceptibility. Finally, the present study also confirmed the well-known role of SA against biotrophic phytopathogens and a possible new role for IAA in tolerance. These insights may be used to develop strategies for conventional breeding and/or gene editing aiming at providing a durable resistance/ tolerance in grapevine against *E. necator*.

## 502 - Proteomic signatures uncover the early key players on *Vitis vinifera* cv. 'Regent'-*Plasmopara viticola* crosstalk

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The analysis of complex biological systems keeps challenging researchers. Aiming to overcome this complexity, the study of different cellular compartments, like apoplast, could allow a broader understanding of the cell dynamics. Plant apoplast, the cellular compartment external to the plasma membrane including the cell wall, is the first contact point between plant and pathogen molecules. Also, it is where the first pathogen structures develop after infection. In grapevine (*Vitis vinifera* L.), little is known about apoplast and the role of apoplastic proteins in cellular mechanisms, particularly in response to pathogens. Grapevine, which has a high economic importance due to its final products, is very susceptible to diseases, like to downy mildew, caused by the obligate biotrophic oomycete *Plasmopara viticola*. Normally, upon *P. viticola* infection the colonization of grapevine leaf tissues is rather fast. At 6 hours post inoculation (hpi), the pathogen penetrates through the stomatal opening and develops substomatal vesicles with primary hyphae. In tolerant cultivars, like 'Regent' (crossing line tolerant to *P. viticola*), the infection progress, at this early stage, is slowed down, inhibited, or completely stopped.

In this study, we have evaluated two different leaf proteomes (whole leaf and apoplast proteomes) of 'Regent' at 6hpi with *P. viticola*. Mock-inoculated leaves treated with water were used as control. Both proteomes were sequenced by nanoLC-MS/MS and the proteins identified by homology search in NCBIprot *Vitis vinifera* database. Protein differential accumulation between apoplast and total leaf proteomes, for both inoculated and mock conditions, together with functional annotation analysis, are being conducted. This analysis will allow an understanding of protein movement between the inside and outside of the cell, under non-stress conditions and after inoculation with *P. viticola*. Also, it will allow the identification of proteins directly involved in plant-oomycete communication and key players in the establishment of an incompatible interaction.

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### **503 - Tomato reprograms photosynthesis and monolignols metabolism to activate plant defense responses against *Clavibacter michiganensis* infection**

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The pathogenic bacterium *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) infects tomato plants causing yield loss and reduction of fruit quality. Cmm spreads through the xylem causing stem canker. While progress has recently been made to elucidate plant responses to pathogenic bacteria, a clear understanding how the host could block Cmm infection remains still elusive. Here, we present a deep transcriptome analysis to characterize the dynamic expression profile of tomato genes upon Cmm infection. An indigenous virulent Cmm strain was used to artificially inoculate tomato seedlings of the Ekstasis F1 hybrid variety. Symptoms of bacterial canker became evident from the third day, whereas the morphology of infected stems was completely distorted eighteen days after inoculation. Following the severity of the disease symptoms, the population of bacteria in planta increased, reaching the highest level at six days and remained constant till day twelve, before a downward slope. Given the constant pattern of symptom emergence, these two time points were selected for transcriptomics. A progressive shut down of key genes in control of photosynthesis was observed, supporting that monitoring chlorophyll levels could be useful for precision agriculture to determine the health status of tomato plants. In terms of defense mechanisms activation, PAMP-Triggered Immunity (PTI) highly responded first followed by a progressive activation of the Effector-Triggered Immunity (ETI), which reached the highest level of activity late. In support to PTI, the pathway of phenylpropanoids was also early activated by diverting the synthesis of monolignols away from S-lignin units. This twist towards G-lignin units enrichment is consistent with previous findings, highlighting a response to generate an early defensive barrier and enlightening a tight interplay between lignin recomposition and PTI. Temporal deactivation of phenylpropanoids coincided with upregulation of ETI genes supporting a functional crosstalk towards the establishment of an acute line of defense to confine pathogen invasion.



## 513 - Circadian clock and pathogen-triggered immunity in *Phaseolus vulgaris*

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The present study investigates molecular and biochemical aspects of the crosstalk between the circadian clock and the defense mechanisms in *Phaseolus vulgaris* (common bean) during the primary stages of infection by the pathogen *Pseudomonas syringae* pv. *phaseolicola* (Psp) which causes the halo blight disease. The circadian clock is the endogenous timekeeping mechanism that synchronizes the plant physiology and metabolism to the 24-hour cycles of day/night transitions. The clock has been shown to play a major role in controlling plant defense mechanisms but also to receive significant feedback from the infection process. The present work is the first study conducted with an important crop plant (common bean) that investigates the crosstalk of the circadian clock and the pathogen-triggered immunity (PTI) caused by one of the plant's major pathogens, Psp. Assays to study the in planta growth of a virulent *Pseudomonas* strain (1148A) in leaves of Red kidney beans indicated that bacterial growth was infection-time dependent. In addition, analysis of the plant oxidative burst after treatment with the bacterial elicitor peptide, flagellin 22 followed a similar pattern of variation. Therefore, we asked whether these results rely on gene expression. The study of genes involved in the oxidative stress response identified rhythmic and non-rhythmic genes that are significantly affected by the infection, an indication that they contribute to the plant's defense. As plant diseases pose a global threat to adequate food supply, the present work is essential in order to understand control mechanisms of the plant to cope with pathogenic bacteria and lays the groundwork for relevant bean research.

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project “Reinforcement of Postdoctoral Researchers - 2<sup>nd</sup> Cycle” (MIS-5033021), implemented by the State Scholarships Foundation (IKY).



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### 530 - Some Pathological Changes in Phytoplasma Infected Woody Plants

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The following research focuses on phytoplasma-induced pathological changes in *Eriobotrya japonica*, *Thuja occidentalis* L., *Abies Nordmanniana* (Steven) Spach., *Picea pungens* var. 'argentea' Rosenthal, *Picea excelsa* Link. at the dendropark of Agricultural University of Georgia.

Experiments were conducted during 2019-2020. Plants were varied to be between 40-45 years old. The plants were chosen for their visually symptoms of the disease. The phytoplasma infection in plant samples were diagnosed using Diene's cytochemical method [Srinivasan, 1982]. Rate of the disease was estimated according on how large the blue-dyed parts of the phloem. Water levels in sample plant stems were established using Weight Method. Acid-insoluble lignin content was quantified using the Klason method [Dence, 1992, Ayeni et al., 2015].

All the examined plants had the most common symptoms of the phytoplasma disease: needles hortening, yellowing, exsiccation (*A. nordmanianna*, *P. pungens*, *Th. occidentalis*), foliage deformation (*E. Japonica*). Both visually healthy and diseased plants pith, parenchyma and phloem had phytoplasmas in them. Visually infected plants usually had higher percentage of the inoculum (especially *E. Japonica*, *Th. occidentalis*, *A. nordmanniana*). In a few cases the intensity of the infection in visually healthy and diseased plants were nearly equal. In all the visually infected plant stems water volume was decreased by 5-12%, lignin quantity in *E. Japonica*, *A. nordmanianna* and *P. excelsa* was increased correspondingly by 14%, 56% and 15%. In phytoplasma-infected plant samples, we also described the following structural changes: a reduced space and diameter of the plant xylem, stem and petiole. Meanwhile, in visually infected plants, we observed an increased number of additional vascular bundles, which were arranged into one or two rows. Resin duct were also decreased in infected coniferous.

The results provide an opportunity to better understand the mechanisms of plant resistance to phytoplasmosis.

**538 - Understanding pathogen aggressiveness: Serine Protease Inhibitors as effectors in *Plasmopara viticola* – *Vitis vinifera* interaction**

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The domesticated European Grapevine *Vitis vinifera* is highly affected by pathogens, particularly *Plasmopara viticola*, the causing agent of Downy Mildew. Extensive resistance breeding activities between resistant and susceptible cultivars have succeeded in establishing tolerant to resistant varieties. However, with the emergence of *P. viticola* strains that overcome resistance it is crucial to also focus on the pathogen, as they become more aggressive and/or overcome the resistance traits. In grapevine, subtilisin-like serine proteases have been shown to play an important role in context with increased disease tolerance. Equally, recent studies show the importance of pathogen effectors as highly adaptable weapons, within those, protease inhibitors are gaining raising interest.

*P. viticola* isolates, *avrRpv3+* (unable to overcome resistance) and NW-10/16 (can overcome) were used to inoculate susceptible 'Mueller-Thurgau' and tolerant genotypes 'Calardis blanc', 'Cabernet blanc', 'Regent', and resistant 'Sauvignac'. Comparing the two isolates NW-10/16 was able to fully develop and release sporangia in all cultivars, except for 'Sauvignac', also producing more sporangia. Moreover, the growth rate of mycelia was slightly faster. Both results indicate an increase aggressiveness for NW-10/16.

Twelve putative protease inhibitors were identified in *P. viticola* genome, five as serine protease inhibitors. Gene expression for these five genes was determined for 'Mueller-Thurgau' 'Cabernet blanc' and 'Sauvignac'. Two genes presented interesting results. *Pvit\_020s* gene expression with NW-10/16 increases over time in the susceptible cultivar, however, has no relevant expression in the resistant cultivar. While *avrRpv3+* isolate presents a high expression throughout time in the resistant cultivar. *PVIT\_0007026.T1* gene expression in NW-10/16 peaks at 12 and 6 hours in the susceptible and tolerant cultivar, respectively. Still, it has no relevant expression in the resistant cultivar. This might indicate that the genes are possibly not the cause for increased aggressiveness but may be part of the pathogen weaponry to counterattack plant defenses.

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**575 - Novel antimicrobial peptides from blackseed (*Nigella sativa* L.) with absolutely unique Cys-motif represent strong inhibition towards mold fungal species**

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Blackseed (*Nigella sativa* L.) is a rich source of a wide range of biologically active compounds with different activities. This plant is widely used as a medicinal plant and spice. Several antimicrobial peptides (AMPs) belonging to some structural families were found in *N. sativa* seeds. Here, we represent the investigation of new AMPs with previously unknown cysteine motif for all the Kingdoms of Living. Six novel cysteine-rich and disulfide-linked homologous peptides were isolated from *N. sativa* seeds by acetic acid extraction followed by liquid chromatography techniques. They were discovered in 0 mM NaCl fraction after cation-exchange (pseudo-affinity) medium-pressure chromatography and further purified using reversed-phase HPLC. The individual peptides were collected manually; their primary structure was evaluated by automated Edman degradation. The unusual cysteine motif of  $XnC_1XXXC_2XXXC_3XnC_4XXXC_5XXXC_6Xn$  was firstly detected. There was no analogous sequence in databases so we suggested them as new AMP representatives, and called them "nigellins".

The peptides obtained have shown specific antifungal activity against *Aspergillus* and *Penicillium* species in a range of micromolar active concentrations tested, and was absolutely inactive against both Gram-negative and Gram-positive bacteria, and yeasts. These compounds are similar for spatial structure which is represented by two  $\alpha$ -helices,  $\beta$ -turn and N-terminal and C-terminal random coils. This folding type and secondary structure elements are typical for previously identified plant  $\alpha$ -haipinins. Therefore, the newly described peptides presumably are members of a novel  $\alpha$ -haipinin subfamily.

This work was supported by the Russian Science Foundation (grant № 18-74-10073).

**576 - Expression of micro RNAs and target genes in Scots pine (*Pinus sylvestris* L.) needles in response to methyl jasmonate treatment.**

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The aim of this study was to identify and characterize novel and conserved microRNAs (miRNAs) and to investigate the effect of methyl jasmonate (MeJA) treatment on miRNA and gene expression in Scots pine needles and to compare the differential expression of miRNAs and their target genes. MicroRNAs are small molecules, that regulate gene expression by binding and cleavage of target gene transcripts.

4975 annotated miRNA sequences were identified and assigned to 173 miRNA groups, belonging to a total of 60 conserved miRNA families. A total of 1029 potential novel miRNAs, grouped into 34 families were found, and 46 predicted precursor sequences were identified. The majority of previously reported highly conserved plant miRNAs were identified in this study, as well as some conserved miRNAs previously reported to be monocot specific. A number of potential gymnosperm or conifer specific miRNAs were found, shared among a number of conifer species. A total of 136 potential target genes targeted by 28 families were identified.

58 miRNAs with, from 28 families, were confirmed to be significantly differentially expressed (fold-change  $\geq 1.5$ ,  $p \leq 0.05$ ) between the control and MeJA treated samples. 15 miRNAs were up-regulated and 43 were down-regulated. Comparing miRNA expression data with expressed transcripts in Scots pine after treatment with MeJA, 38 differentially expressed miRNAs from 23 families targeted 44 differentially expressed gene transcripts. For 27 down-regulated miRNA sequences, their target gene transcripts were up-regulated and for 3 up-regulated miRNA sequences, their target gene transcripts were down-regulated. The same direction of transcriptional regulation was found for 9 up-regulated and 13 down-regulated miRNA sequences and their target genes.

## **623 - Salt stress response of tomato cultivars: focussing on glutathione and related processes**

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High salinity extensively affects plants life cycle, negatively influences plant development and productivity by causing ionic toxicity, osmotic stress, oxidative damage, and nutritional shortage. In the last few years large amount of information have been generated on the importance of glutathione transferases (GSTs) in response to different environmental stress conditions. Despite of this, the function of individual GSTs in abiotic stresses is still poorly understood. In our experiments we investigated the response of five-week-old tomato (*Solanum lycopersicum* L. cv. Mobil, cv. Elán F1 and cv. Moneymaker) cultivars to 100 mM NaCl applied for one week. Our aim was to reveal the differences in their salt stress response with a special attention on the glutathione and related processes. We measured the growth of plants, H<sub>2</sub>O<sub>2</sub>, malondialdehyde and non-enzymatic antioxidant (ascorbate and glutathione) levels in leaves. The activities of glutathione reductase, -transferase and -peroxidase were detected, and gene expression of SIGSTs was investigated also. After one week of salt treatment the fresh weight (FW) of Moneymaker remained on the control level, however in Elán F1 and Mobil lower FW was measured than under control conditions. Glutathione and ascorbate levels and glutathione reductase activity was also lower in Mobil compared to the other two cultivars under control condition and after salt treatment. Expression of investigated SIGST genes showed different pattern in the cultivars. Our results indicate that lower glutathione and ascorbate levels, and more positive redox potential of glutathione under control conditions influenced negatively the salt stress response of Mobil, while in Moneymaker higher glutathione reductase activity and elevated non-enzymatic antioxidant levels supported a more efficient stress response.

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## 628 - The novel model of SAUL1 reveals crucial structural and functional properties

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The membrane localized senescence associated E3 ubiquitin ligase 1 (SAUL1) is a known regulator of immune responses. However, the precise structure and function remains elusive. Lately, it was demonstrated that SAUL1 forms membrane-patches by tethering multi-vesicular bodies (MVBs) with the plasma membrane (PM). A structural analyses of SAUL1, led to the identification of an arginine-based positively charged groove that might contribute to the tethering. Here we present results confirming that the arginine-based groove is indeed involved in the patch formation. The groove is located among the ARM-like domain 7-11 of SAUL. Therefore, a truncated versions lacking parts of domain 7-11, as well as constructs with an amino acid exchange of arginine R736, R737 and R775 with alanine were generated. We can demonstrate that ARM domain 7-11 is required for the membrane association of SAUL1, since the PM localisation is lost, if just parts of the domain are present. Interestingly, the arginine exchanges of R736 is sufficient to lose patch formation, although the protein structure of the mutated version SAUL1R736A is identical to the protein structure of native SAUL1. The other mutations showed only a slight change in membrane-patch occurrence. The mechanism of the membrane association is yet unknown, but first protein-lipid assays showed an interaction between the recombinant GST-SAUL protein and lipids of the phosphatidylinositol family, making protein-lipid interactions with the plasma membrane and MVBs highly probable and revealing a potential mechanism of SAUL1 in tethering lipids.

## 652 - Calcium phosphate nanoparticles doped with copper ions as efficient tools for downy mildew prevention

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### Calcium phosphate nanoparticles doped with copper ions as efficient tools for downy mildew prevention

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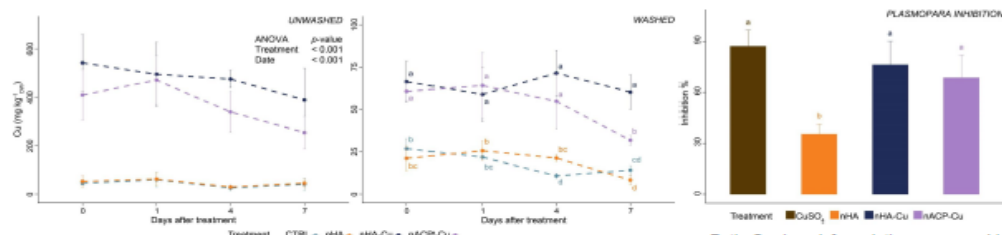
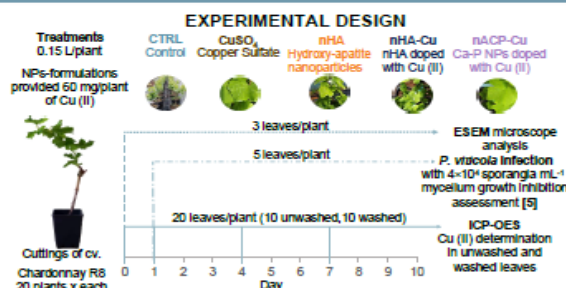


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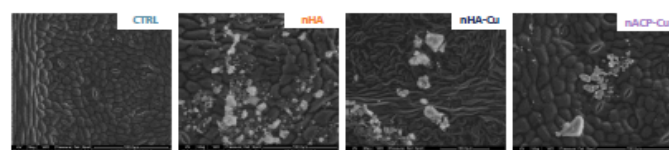
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**INTRODUCTION** Intensive application of agro-chemicals causes an increasing environmental impact in agriculture, especially in high-income crops such as grapevine. Their delivery, however, is highly inefficient, since a considerable amount is leached in the environment [1]. For this reason, nanotechnology could represent a sustainable practice able to improve the efficacy of application of agro-chemicals by increasing their permanence on the canopy [2]. In vineyard, copper compounds are intensively used as antifungal pesticides, with strict limitations in their application [3, 4]. Thus, in the present work, two types of Cu (II)-doped calcium phosphate NPs were applied at low dose on *Vitis vinifera* L. and their efficacy against downy mildew (*Plasmopara viticola*) was compared to that of conventional copper-sulfate treatment.



**RESULTS AND DISCUSSION** The profile of the total concentration of Cu (II) in unwashed leaves and that of absorbed Cu (II) (washed leaves) was followed for one week. As expected, the application of nHA-Cu and nACP-Cu caused a significant increase (~11 and 8.6 times, respectively) in the average foliar concentration of Cu (II) compared to CTRL or nHA alone. Both in unwashed and washed leaves treated by Cu-functionalized nanoparticles, there was a progressive decrease in Cu (II) concentration over time. It was more evident in nACP-Cu treatment, whose pattern was significantly lower if compared to nHA-Cu ( $p < 0.001$ ). Regarding the washed leaves, a significant decrease occurred during the terminal phases of the experiment in the nACP-Cu thesis, if compared to nHA-Cu, where Cu (II) concentration remained constant. This fact supports the hypothesis that the nHA-Cu was able to maintain a more stable and long-lasting amount of Cu (II) in comparison to the nACP-Cu formulation, where Cu (II) is more soluble.

Both Cu-doped formulations were able to prevent *P. viticola* infection by more than 60%, showing no statistically difference with CuSO<sub>4</sub> treatment, even if Cu (II) concentration after its application was 3 times higher (1381.9 ± 512.1 mg kg<sub>dw</sub><sup>-1</sup>). In case of a technical improvement in functionalization, this result opens the possibility of complying with EU regulations by administering low dosage of Cu (II) associated to a nanomaterial. The inhibition was significantly lower in the absence of Cu, as confirmed by treatment with nHA.



ESEM analysis showed the presence of Ca-P NPs in aggregates of different sizes (in the range of 0.7-30 µm) in all treatments, except in the control and in CuSO<sub>4</sub> (not shown). The resolution level obtained in low vacuum mode may not have allowed the detection of smaller particles of nanometric size.

**CONCLUSION** The application of calcium phosphate based nanomaterials doped with Cu (II) is efficient to inhibit the propagation of *P. viticola* at an extent comparable to CuSO<sub>4</sub>, even though the nanomaterial showed a yield distribution in copper of 30% if compared to the conventional treatment. The improvement of the agronomic distribution technique is clearly required. In addition, the results provide evidence that calcium phosphates functionalization allows to deliver ionic elements both on grapevine canopy and at mesophyll level. According to an improvement of the method for the application of Calcium-Phosphate nanoparticles, this technical solution will be promising as a low environmental impact treatment.

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## **671 - A PME-mediated increase of ascorbic acid level affects susceptibility of *Solanum lycopersicum* to *Botrytis cinerea***

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*Solanum lycopersicum*, the second most important vegetable crop worldwide, is an important source of antioxidants, such as ascorbic acid (AsA), exhibiting positive effects on human health. One introgression sub-line IL12-4-SL, that shows increased levels of AsA, was previously selected from a *Solanum pennellii* IL (Introgression Lines) population which consists of 76 lines with different wild chromosomal segments in the genetic background of the cultivated variety M82. The increased level of AsA in IL12-4-SL, compared with M82, was due to the activation of the alternative D-galacturonate pathway related to the up-regulation of the Solyc12g098340 and Solyc12g096730 encoding respectively a pectin methylesterase (PME) and a polygalacturonase (PG). Commercial tomato varieties are highly susceptible to microbial pathogens and in particular to fungal necrotrophs. It is well established that the orchestration of pectic enzymes activity affect plant resistance to pathogens.

The aim of this research is to study the responses of the tomato IL12-4-SL line to *Botrytis cinerea*. *B. cinerea*, the causal agent of grey mold disease, is a broad-spectrum fungal necrotroph that causes serious pre- and post-harvest rot in more than 200 species worldwide. To identify pectic genes induced in tomato by *B.cinerea* leaf infection, a bioinformatic-based data mining on the available transcriptomics data was performed. The response of M82 and IL12-4-SL to *B.cinerea* was evaluated in tomato leaves challenged with the fungal necrotroph. Our results indicate a significant reduced susceptibility of the IL lines to the pathogen. The definition of the molecular bases of the observed plant resistance will provide new insights into the regulatory control of plant resistance by antioxidant accumulation and pectin metabolism and could provide novel tools for obtaining tomato genotypes more resistant to pathogens.

Financial support: Sapienza University of Rome grant no. RM11816432F244FD, and LazioInnova-Regione Lazio

CUP: B81G18000770002.

### **673 - Unveiling the genetic components of resistance to powdery mildew in grass pea through a genome-wide association approach**

**Davide Martins**<sup>(1)</sup> - **Pedro Crespo**<sup>(1)</sup> - **Ana Margarida Sampaio**<sup>(1)</sup> - **Priscila Pereira**<sup>(1)</sup> - **Carmen Santos**<sup>(1)</sup> - **Diego Rubiales**<sup>(2)</sup> - **Maria Carlota Vaz Patto**<sup>(1)</sup>

**ITQB NOVA, Universidade Nova de Lisboa, Oeiras, Portugal**<sup>(1)</sup> - **CSIC, Instituto de Agricultura Sostenible, Cordoba, Spain**<sup>(2)</sup>

Lathyrus sativus (grass pea) plants are impacted by biotic constraints, compromising yield stability. Powdery mildew (PM) disease in grass pea is mainly attributed to Erysiphe pisi (Ep), causal agent for pea PM. Nevertheless, Erysiphe trifolii (Et), known to overcome Ep-resistance genes in pea, could also impact grass pea productivity, given its broad host-range. Breeding grass pea for PM resistance would be more effective if the genetic basis of resistance against these two potential pathogens would be clarified.

To tackle this we explored the natural variation of a worldwide grass pea accessions collection through a Genome-Wide Association Study (GWAS) to identify resistance associated genomic regions. Accessions were repetitively and independently inoculated with both pathogens under controlled conditions, being this the first report of grass pea response to Et. Phenotypic response was scored as the percentage of leaf covered by mycelia. The same collection was genotyped with high-throughput single nucleotide polymorphism (SNP) approaches and SNP-resistance associations tested using mixed models accounting for population structure. For GWAS validation, candidate genes expression was analyzed by quantitative PCR in leaf samples collected at different time-points of inoculation from phenotypically contrasting accessions.

A wide-range of responses to PM was observed, with high levels of resistance more frequent in response to Ep than to Et. Distinct SNP-trait associations were detected in response to each pathogen, suggesting that resistance to each pathogen may be under different oligogenic control. Nevertheless, one SNP marker was associated with the response to both pathogens. Candidate genes underlying the detected SNP-trait associations were predicted to be involved in gene expression regulation, ATP-metabolism, retrotransposon, and protein-protein interaction.

By exploring grass pea natural variation, this study clarified common and unique genetic components of oligogenic grass pea resistance to PM, identifying new resistance sources and genomic targets for the development of molecular tools for precision breeding.



# ARTEMISIA ESSENTIAL OIL EXTRACTS: A source of non-contaminant biopesticides protecting tomato against *Fusarium solani*

Serine Soudani<sup>1</sup>, Maria Fe Andres<sup>2</sup>, Azucena Gonzalez-Coloma<sup>2</sup> and Marta Berrocal-Lobo<sup>\*1</sup>

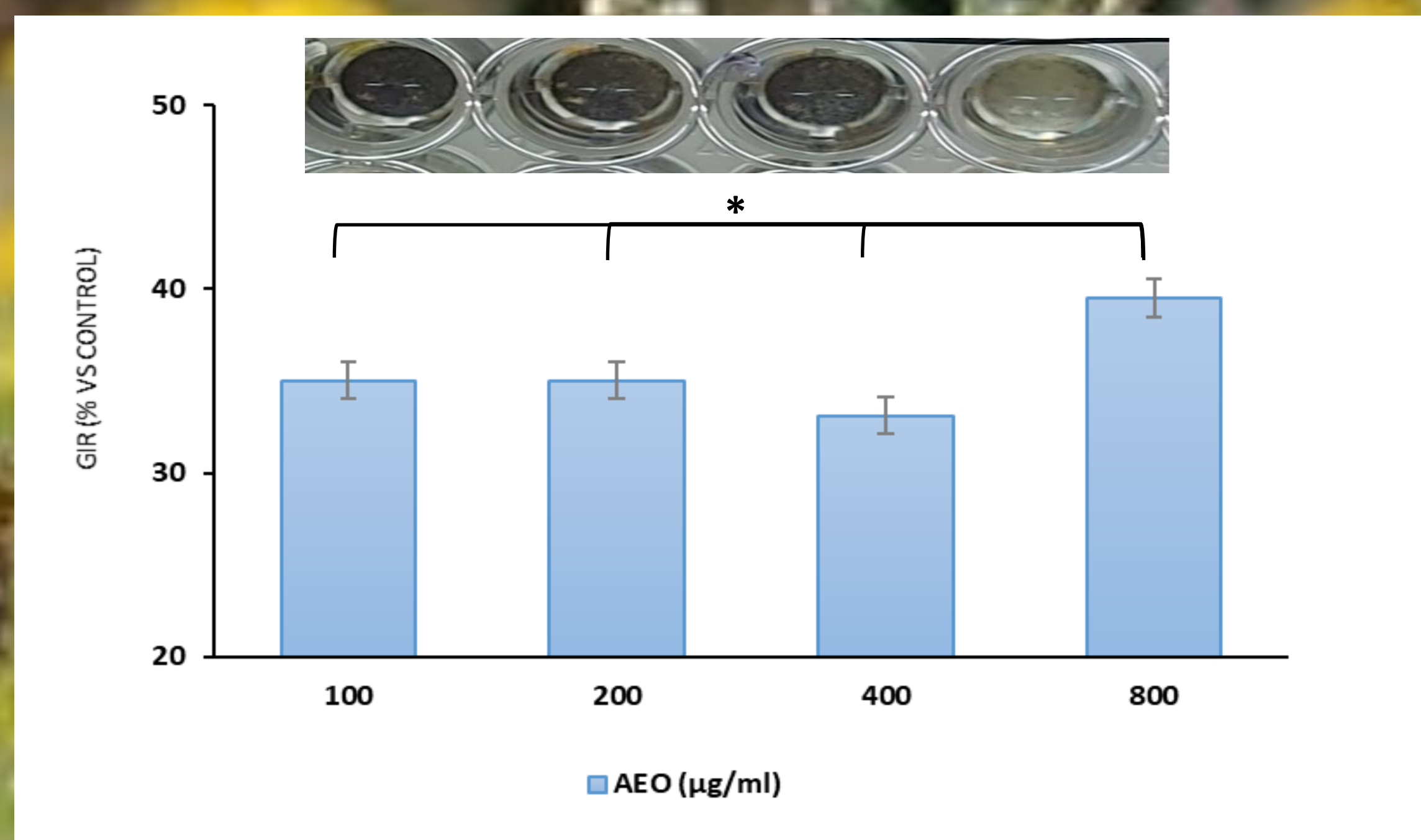
<sup>1</sup> School of Forest Engineering and Natural Resources (ETSIMFMN, UPM), Madrid, Spain

<sup>2</sup> Spanish National Research Council (CSIC), Agrobiology and Natural Products Institute, Madrid, Spain

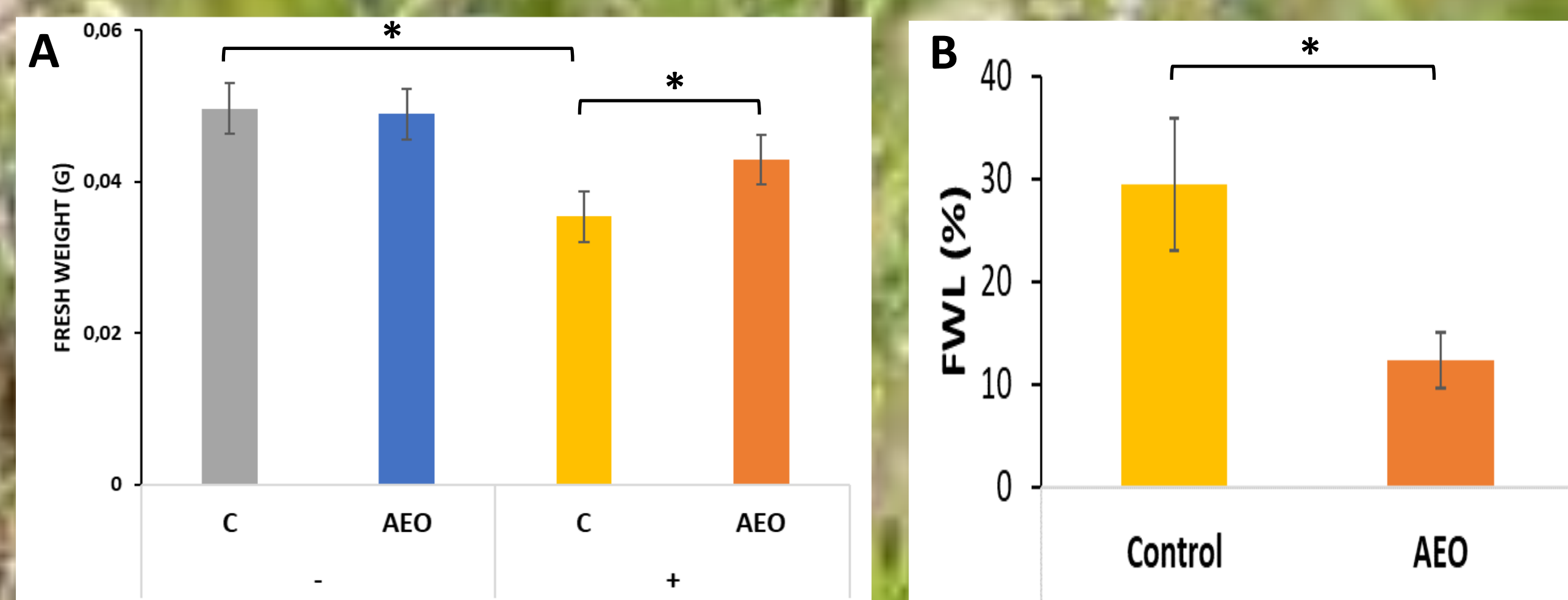
Contact: m.berrocal@upm.es

## Abstract:

Plant essential oils (EOs) are potential biopesticide natural sources, showing high toxic activity *in vitro* against several high-impact crop phytopathogens. The domestication of plant producing EOs is not evident, the characterization of new EOs is under progress, and the direct effect of EOs on crop protection remains poor. The EU framework aims to achieve the sustainable use of new plant protection products (PPPs). Since 2020, the use of contaminant PPPs is not allowed. Hydroponic culture emerges as an increasing farming strategy to develop sustainable crops, reducing water consumption, space, and fertilizers, under the current climatic change situation. Spain produced 5 millions of tons of tomato plants in 2019 (FAO). In this work, the effects on tomato seedlings growth and immunity response, of an EO extract from a previously domesticated *Artemisia sp.* (AEO), was tested both *in vitro* and *in vivo*, using hydroponic culture of plants, inoculated with *Fusarium solani* (F.s) phytopathogen. AEO showed protection capacities on tomato seedlings, against F.s, at concentrations as lower as 0,8mg/ml. The results open new perspectives to the use of EOs as a source of non-contaminant biopesticides in a new agroforestry environment.



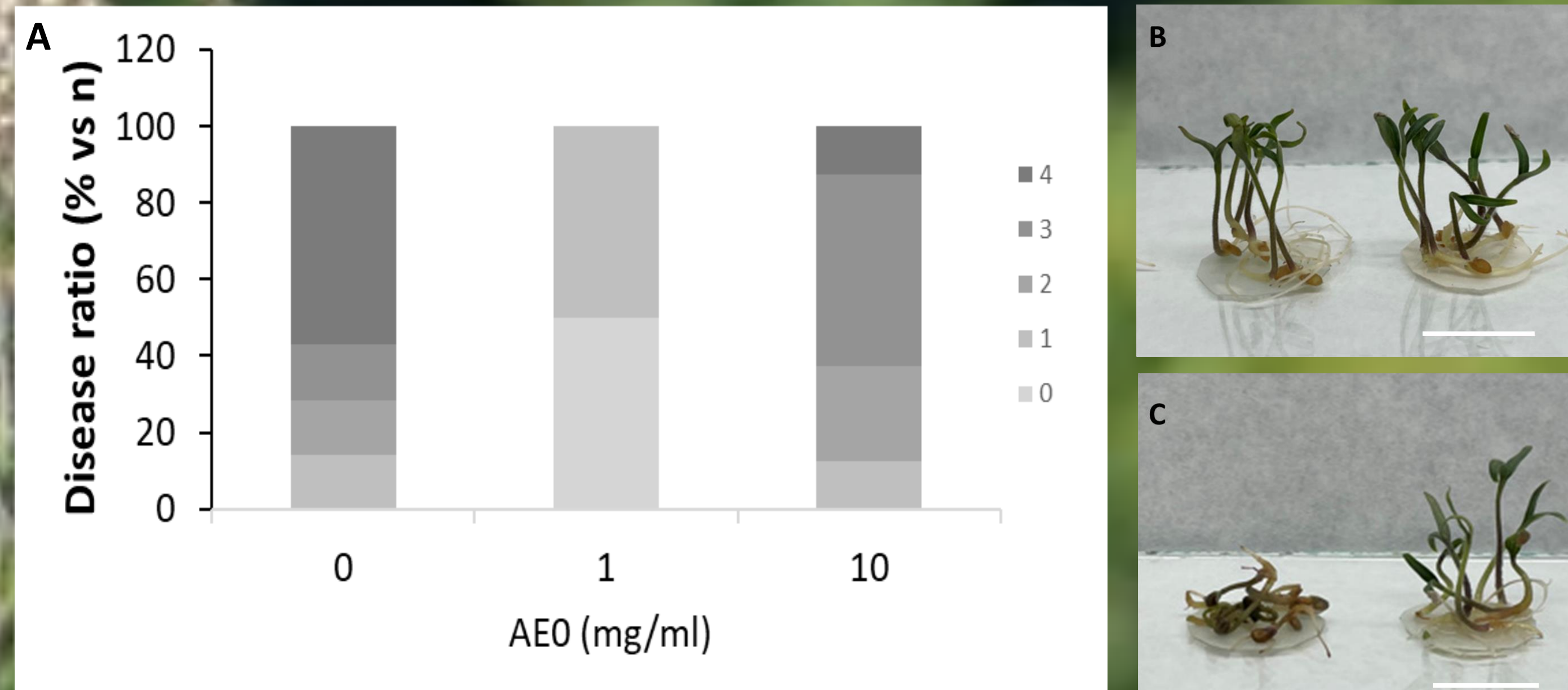
**FIGURE 1.- *In vitro* analysis of toxic activity of AEO on *F. solani* spores.** Histogram showing different active concentrations of AEO (µg/ml), tested on *Fusarium solani* spores 10<sup>5</sup> spores/ml by MTT/ DMSO assay (Abs 630 nm).



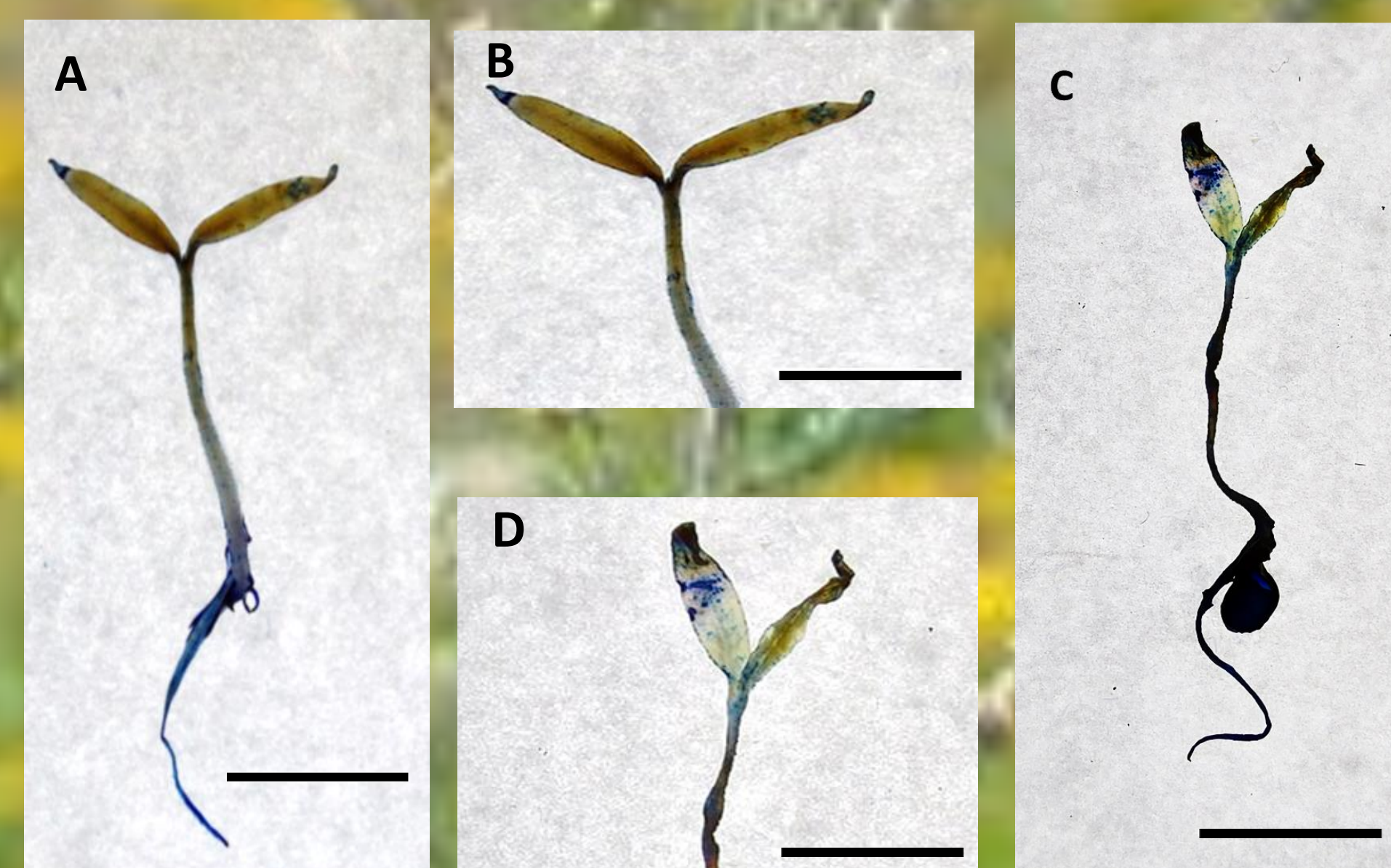
**FIGURE 2.- AEO direct effect on tomato seedlings. (A).** Plant fresh weight of 12 days old control plants (C) non inoculated (-), or inoculated (+), with *F. solani* (10<sup>6</sup> sp/ml), with or without AEO (1mg/ml) pre-treatment. **(B).** Plant fresh weight lost (FWL), related to non-inoculated plants, 12 dpi with *F. solani*, non-treated (control) or treated with AEO at 1mg/ml.

## CONCLUSIONS

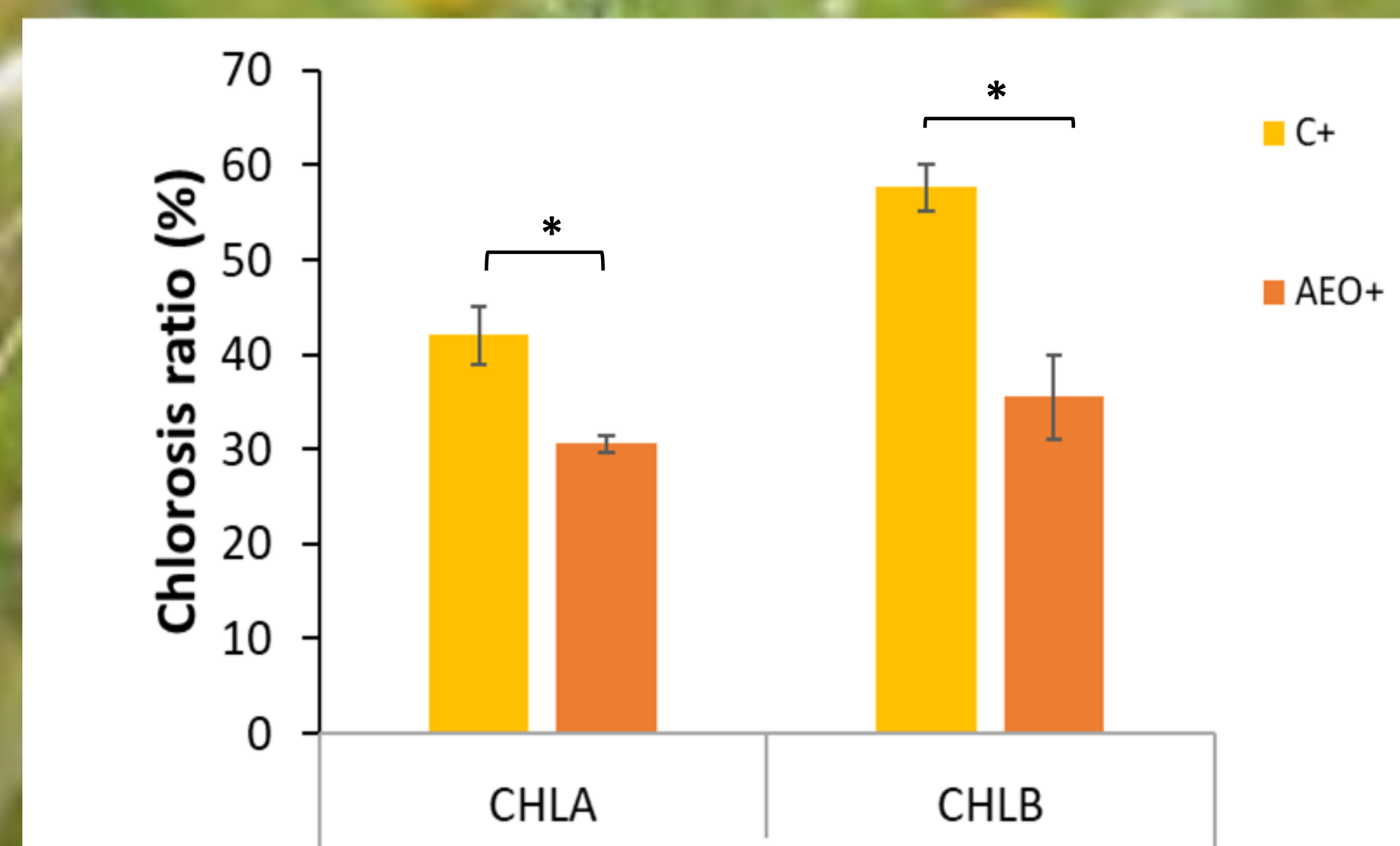
AEO inhibits spore germination of *F. solani* at 0.8mg/ml *in vitro*.  
AEO pre- treatment protects fungal penetration on tomato seeds  
AEO improves tomato tolerance to *Fusarium solani* infection increasing photosynthetic activity, related to non-treated seedlings.  
AEO might be used as non-contaminant biopesticide alternative.



**FIGURE 3.- Effect of AEO protecting tomato against *F.s olani*. (A).** Disease ratio (% related to total number of plants), of tomato seedlings, 12 days after inoculation with *F. solani* (Fs, 10<sup>6</sup>esp/ml) where 0: no symptoms, 1: light inhibition of growth, chlorosis and necrosis, 2: medium symptoms, 3: heavy symptoms, 4: decayed necrotic plants. **(B).** Non-inoculated seedlings, control (left), or treated (right) with AEO (1mg/ml). **(C).** Inoculated seedlings with *F. solani*; in absence (left) or presence of AEO (1mg/ml) 12dpi. Size bars: 20mm.



**FIGURE 4.- Disease symptoms on tomato seedlings. (A-B).** Inoculated tomato seedlings with *F. solani* (10<sup>6</sup> sp/ml), 12dpi, pre-treated with AEO (1mg/ml). **(C-D).** Seedlings without AEO treatment. Size bars: 10mm.



**FIGURE 5.- Chlorosis ratio.** Chlorophyll A (CHLA) and Chlorophyll B (CHLB) were measured by absorbance (430 to 665 nm) on leave tissues, 12 days after inoculation with *F. solani* (+), 10<sup>6</sup> sp/ml. Seedlings were pre-treated with AEO at 1mg/ml (AEO+) or not (C+). Statistical significance (p.value 0.05).

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**Funding:** MICINN, Spain, 2020-2024. SS has a PhD fellow of the Algerian Government-UPM collaboration.



**680 - New insight into the biochemical features of AtPME17, a functional Arabidopsis PME affecting plant resistance to pathogens, regulated by its pro-region**

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**Sapienza University of Rome, Department of Biochemical Sciences “A. Rossi Fanelli”, Pasteur Institute-Fondazione Cenci Bolognetti, Rome, Italy <sup>(1)</sup> - Sapienza University of Rome, Department of Biochemical Sciences “A. Rossi Fanelli”, Pasteur Institute-Fondazione Cenci Bolognetti, Rome, Italy <sup>(2)</sup> - Sapienza University of Rome, Department of Biology and Biotechnologies “Charles Darwin”, Rome, Italy <sup>(3)</sup> - National Research Council, Researcher Institute of Molecular Biology and Pathology,, Rome, Italy <sup>(4)</sup> - Aix-Marseille Univ, CNRS, Aix-Marseille Univ, CNRS, Centrale Marseille , iSM2, Marseille, France <sup>(5)</sup>**

PMEs are widespread in plants and belong to a large multigene family whose members display different expression profiles, mediate different physiological responses and play critical role in the outcome of plant-pathogen interaction. Differently from fungi and bacteria, higher plants PMEs are classified in two groups on the basis of their structures: group1 contains only the catalytic PME domain; group 2 defined as ProPME, possesses, in addition to the catalytic domain, a N-terminal Pro-region. The Pro-region shows sequence similarities with characterized PME inhibitors proteins. Between Pro-region and PME domain there is a conserved Serine-like proteases (SBTs) processing site cleaved for the secretion and activation of the PME domain in the apoplast. Much remains to be discovered about the peculiar mechanism of intramolecular regulation of group 2 PMEs.

The heterologous overexpression of functional ProPMEs is a major bottleneck of structural biology studies for understanding their SBT-mediated post transcriptional regulation. We have previously demonstrated that AtPME17, a ProPME isoform, from Arabidopsis highly induced in response to several pathogens strongly contributes to resistance against *B. cinerea*.

We have expressed and purified the AtPME17 catalytic region using *P. pastoris* and the characterization of the biochemical features of the enzyme are in progress. In *E. coli*, we demonstrated that the PRO region acts as an intramolecular inhibitor of AtPME17 activity. The optimization of the bacterial expression system is in progress to obtain Pro-AtPME17 amounts useful to solve the 3D structure. These studies will give more insight into the peculiar on-off mechanism of post transcriptional regulation of this class of enzymes.

Financial support: Sapienza University of Rome grant no. RM11816432F244FD, and LazioInnova-Regione Lazio CUP: B81G18000770002

## TOPIC:

### Genomics and genome editing for crop design

#### **Keynote Lecture**

##### **Transforming the food system with the Inari SEEDesign™ platform**

**Catherine Feuillet** <sup>(1)</sup>

**Inari Agriculture, industry, Cambridge, United States** <sup>(1)</sup>

Inari is designing seeds to help address one of the greatest challenges of our times - growing enough nutritious calories for a growing population while reducing the footprint of agricultural production on the environment. Embracing the complexity and diversity of nature, we use our SEEDesign™ platform to overcome these challenges. Our platform integrates Predictive Design and advanced Multiplex Gene Editing tools to develop resilient seeds that require fewer natural resources and inputs, in a drastically shorter time and lower costs than current approaches. In Predictive Design, we harness the power of Artificial Intelligence and cell-based assays to gain a deep understanding of the sequence polymorphisms that underpin crop performance. Once the target sequences have been identified, we generate new allelic diversity using our comprehensive Multiplex Editing toolbox to deliver the changes into elite parental lines. We then provide our improved seeds to our customers through a simple and collaborative go-to-market strategy. Results and illustrations of our technical approaches and product concepts will be presented.

## TOPIC:

### Genomics and genome editing for crop design

#### Oral Communications

##### **72 - CRISPR/Cas9-Based Mutagenesis of PPO genes in eggplant for the improvement of the berry quality**

**Andrea Moglia**<sup>(1)</sup> - **Silvia Gianoglio**<sup>(2)</sup> - **Alex Maioli**<sup>(1)</sup> - **Alberto Acquadro**<sup>(1)</sup> - **Danila Valentino**<sup>(1)</sup> - **Anna Maria Milani**<sup>(1)</sup> - **Jaime Prohens**<sup>(3)</sup> - **Diego Orzaez**<sup>(2)</sup> - **Antonio Granell**<sup>(2)</sup> - **Sergio Lanteri**<sup>(1)</sup> - **Cinzia Comino**<sup>(1)</sup>

**DISAFA, University of Torino, Grugliasco, Italy**<sup>(1)</sup> - **IBMCP (CSIC-UPV), IBMCP, Valencia, Spain**<sup>(2)</sup> - **Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain**<sup>(3)</sup>

The polyphenol oxidase enzymes (PPOs) are implicated in undesirable enzymatic browning of eggplant fruit due to the oxidization of polyphenols after cutting.

Following a survey of the eggplant genome, ten ppo genes (named ppo1-10) were isolated and four of them (ppo1-3-4 and 5) highlighted a strong increase in transcript levels in flesh after cutting.

A CRISPR/Cas9 system, integrated into the GoldenBraid (GB) cloning standard tool, was applied to knock-out the ppo genes. An eggplant breeding line of the variety 'Black Beauty' and a double haploid of the variety 'Ecavi' were selected for Agrobacterium-mediated transformation. Seed-derived cotyledons were transformed with a CRISPR/Cas9 construct targeting a conserved region of ppo4-5 as well as ppo6 (due to the high homology between these gene family members).

Genotyping of these plants through Illumina deep sequencing of amplicons revealed that the plants were successfully edited in PPO4,5 and 6 loci, with the insertion of a single nucleotide as the most frequent mutation. We investigated potential off-target effects through deep sequencing of amplicons of the putative off-target sites identified in silico and the lack of off-target mutations confirmed the high specificity of the system. The mutations were stably inherited in the T<sub>1</sub> and T<sub>2</sub> progeny in which some individuals, due to segregation, no longer carried out the transgene construct. A reduction of browning potential as well of PPO activity were highlighted in our knock out T<sub>1</sub> and T<sub>2</sub> edited lines.

Altogether, this study provides for the first time a useful methodology involving the use of the CRISPR/Cas9 system for mutagenesis studies in eggplant.

## **115 - Rice growing in high density: Elucidating the genetic networks of weed-competitive rice architectures**

**Martina Huber** <sup>(1)</sup> - Virender Kumar <sup>(2)</sup> - Rashmi Sasidharan <sup>(1)</sup> - Ronald Pierik <sup>(1)</sup>

**Plant Ecophysiology, Utrecht University/Department of Biology, Utrecht, Netherlands** <sup>(1)</sup> - **Crop and Environmental Sciences Division, International Rice Research Institute, Los Banos, Philippines** <sup>(2)</sup>

How does a rice plant in the field fight against the weeds growing next to it?

Weeds are the major constraint in modern rice farming. Since rice feeds more than half of the world's population as a staple food, tackling this issue is of great relevance. Driven by climate change, the traditional rice farming of transplanting rice into flooded paddy fields, needs to adapt. Currently, a transition from transplanting to direct-seeded rice is occurring. Besides the advantages of water saving and less labour, the major constraint of this system is the weeds. This raises an urgent need for a sustainable weed control.

The main goals of this project are:

- First, to explore the plethora of different rice varieties searching for relevant traits that make rice more competitive against weeds. For this, we phenotyped a rice diversity panel of 344 varieties for traits related to shading and early plant vigour.
- Second, to connect the phenotype with its underlying genes. With the known genomes of the screened rice population, we performed a genome-wide association study. We identified genetic loci associated with shoot architectural traits and hubs involved in several aspects of plant architecture.

To get a holistic picture, a functional validation of the selected varieties carrying the candidate genes is tested in the field.

Ultimately, the identification of target genes regulating the shade-casting traits of competitive phenotypes can be integrated in future breeding programmes. This will help to reduce the amount of herbicide usage and enable us to make rice-farming more sustainable.

## **247 - Overcoming genetic redundancy and lethality: Novel CRISPR tools for plant loss-of-function studies**

**Marie Pfeiffer** <sup>(1)</sup> - **Roman Hudeček** <sup>(1)</sup> - **Rafael Andrade Buono** <sup>(1)</sup> - **Ward Decaestecker** <sup>(1)</sup> - **Kevin Debray**<sup>1,2</sup> - **Nick Vangheluwe** <sup>(1)</sup> - **Joris Jourquin** <sup>(1)</sup> - **Mansour Karimi** <sup>(1)</sup> - **Gert Van Isterdael** <sup>(2)</sup> - **Tom Beeckman** <sup>(1)</sup> - **Tom Ruttink** <sup>(3)</sup> - **Moritz Nowack** <sup>(1)</sup> - **Thomas Jacobs** <sup>(1)</sup>

**VIB-UGent, Center for Plant Systems Biology, Ghent, Belgium** <sup>(1)</sup> - **VIB-UGent, Center for Inflammation Research, Ghent, Belgium** <sup>(2)</sup> - **Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Melle, Belgium** <sup>(3)</sup>

The generation of stable, inheritable loss-of-function mutant alleles has been indispensable for functional genomic studies in plants. In recent years, the high efficiency and simplicity of designing CRISPR-mediated mutagenesis in plants has revolutionized the generation of loss-of-function mutants. However, standard mutagenesis approaches are limited by two main factors: severe pleiotropic phenotypes or even lethality upon knockout of essential genes and genetic redundancy among gene family members masking phenotypic consequences in single knockout mutants. To overcome these limitations, we devised novel strategies using the CRISPR-Cas9 technology. We devised CRISPR-TSKO that enables the generation of somatic mutations in particular plant cell types, tissues, and organs. This system can circumvent pleiotropic or lethal phenotypes when generating knockouts. We created *Arabidopsis thaliana* CRISPR-TSKO mutants in essential genes, which caused well-defined, localized phenotypes in the root cap, stomatal lineage, or entire lateral roots. To address genetic redundancy, we are using high throughput multiplex CRISPR screens. While already an established routine in animal systems, CRISPR screens are still not widely employed in plant research. The resulting combinatorial mutant collections can be screened for phenotypes, and a high-throughput HiPlex amplicon sequencing approach will facilitate mutant genotype identification.



## 279 - Developing a viral based genome editing tool for plant editing

Enikő Lőrincz-Besenyi<sup>(1)</sup> - Rabih Mehdi<sup>(2)</sup> - Thorben Sprink<sup>(3)</sup> - Uwe Sonnewald<sup>(2)</sup> - Björn Krenz<sup>(1)</sup>

Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, VirusInteract, Braunschweig, Germany<sup>(1)</sup> - Friedrich-Alexander University Erlangen-Nürnberg, Department of Biology, Erlangen, Germany<sup>(2)</sup> - Julius-Kühn Institute,, Institute for Biosafety in Plant Biotechnology, Quedlinburg, Germany<sup>(3)</sup>

Genome editing with site directed nucleases such as Cas9 is a powerful tool in plant breeding. Moreover, it has proven its efficiency, versatility and simplicity being already applied to numerous applications.

A key requirement for better public acceptance of plant gene editing by the CRISPR/Cas9 system is the avoidance of any foreign DNA sequence during the process of plant manipulation. Besides other RNPs delivery methods we established an RNA based plant virus delivery method. We developed a tomato bushy stunt virus (TBSV)-based genome editing CRISPR/Cas9 system. TBSV is a small icosahedral virus whose genome is a single copy of a positive sense single-stranded RNA. It encodes five open reading frames (ORFs) which were modified to express Cas9 and the sgRNA. The synthetic viral based RNA strand it is able to replicate, but is attenuated, thus it's not able to systemically infect plant tissue. As proof of the concept, we have successfully induced mutations in the GFP gene in *N. benthamiana* 16 c line protoplasts. Using deep amplicon sequencing we were able to see small deletions three nucleotide upstream the PAM (protospacer adjacent motif). In a next step, we will apply the system to improve important agronomic traits in potato.

This project is founded by the German Federal Ministry of Education and Research, (DeviCCpo project).

## **427 - Single-nuclei sequencing reveals drought's impact on hormone activity and leaf development**

**Joseph Swift** <sup>(1)</sup> - **Stephen Gonzalez** <sup>(1)</sup> - **Joseph Nery** <sup>(1)</sup> - **Joseph Ecker** <sup>(1)</sup>

**The Salk Institute for Biological Studies, Plant Biology Laboratory, La Jolla, United States** <sup>(1)</sup>

Drought stress can cause plants to slow their leaf growth rate and limit their leaf size. While this is a well-known physiological response, at the molecular level it remains unclear how drought signaling pathways influence leaf development to arrest growth. This is in part because the onset of drought as well as leaf development are both dynamic processes, making it difficult to measure how one influences the other.

To address this challenge, we used single nuclei transcriptome sequencing to determine how different Arabidopsis leaf cell-types respond to drought over time; sequencing nuclei using both fluidic and plate-based methods. To ensure we captured how a change in water availability impacts leaf development, we sequenced the nuclei of over 100 individual leaves, each of which was at a different developmental stage and experiencing a different level of drought severity. Additionally, since hormone signaling is known to play a role in arresting plant growth during drought, we tested how each of the 8 major hormones informed leaf cell-type specific transcriptome responses.

Thus, by untangling the effects of drought severity, developmental stage, and hormone signaling within each leaf cell-type, we gained insight into the transcriptional signaling events that work to arrest leaf growth in response to drought with unprecedented cell-type resolution. By these means, our work helps identify new loci that can assist boosting leaf growth and crop productivity in the face of drought.

#### 437 - The pattern of genome-wide polymorphisms in natural and crop polyploid species

**Kentaro K. Shimizu** <sup>(1)</sup> - Gwyneth Halstead-Nussloch <sup>(1)</sup> - Tim Paape <sup>(1)</sup>

**Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland**  
**(1)**

Polyploid species are common in plants, including crop species. Although advantages and disadvantages of polyploidization have long been discussed, genome-wide analysis of allopolyploid species has been difficult due to high sequence similarity of duplicated genes, or homeologs. We have developed bioinformatic workflows HomeoRoq and EAGLE-RC for RNA-seq and resequencing of polyploid species. To examine the pattern of selection at an early stage of allopolyploid speciation, we resequenced 25, distribution-wide individuals of the model allotetraploid *Arabidopsis kamchatica*. Negative selection was only slightly weaker than in diploid parental species, showing that homeologs are not totally redundant. We found a significantly positive value of alpha, the proportion of adaptive substitutions, in contrast to most diploid plant species. Interestingly, the pairs of homeologs showed only weak correlation in diverse indices measuring diversity and selection, including the HEAVY METAL ATPASE4 (HMA4) homeologs responsible for zinc hyperaccumulation. Our data of zinc concentration in soils and leaf tissues supported that *A. kamchatica* inherited the ability to survive in contaminated soils and elemental defense against herbivores from one of the parental species *A. halleri*. This shows that pairs of homeologs evolve independently in the allopolyploid species. To examine whether similar patterns can be found in crop polyploid species, we analyzed the 10 genome sequences of hexaploid bread wheat. Consistent with the results above, the trios of homeologs showed weak correlation in Tajima's D. These data suggested that polyploidy increased the number of targets for natural or artificial selection, and enabled the polyploids to combine environmental adaptations of different parental species.

## TOPIC:

### Genomics and genome editing for crop design

#### **Extended Elevator Pitches**

##### **264 - Genomic prediction to deliver heat tolerant wheat to the Senegal River basin**

**Filippo Bassi <sup>(1)</sup> - Hafssa Kabbaj <sup>(1)</sup> - Amadou Tidiane Sall <sup>(2)</sup> - Rodomiro Ortiz <sup>(3)</sup>**

**ICARDA, Biodiversity and Crop Improvement Research Program, Rabat, Morocco <sup>(1)</sup> - ISRA/ICARDA/CRA, Biodiversity and Crop Improvement Research Program, Saint Louis, Senegal <sup>(2)</sup> - Swedish University of Agricultural Sciences, Plant Breeding, Alnarp, Sweden <sup>(3)</sup>**

This research provides insights on durum wheat tolerance to warming climates with the aim of pyramiding multiple sources of heat tolerance through genomic prediction of breeding values (GEBV) for selection. Days to heading, days to maturity, plant height, tillering capacity, grain yield (GY) and 1,000-kernel weight were the traits evaluated in the Senegal River Basin, where temperatures vary across the growing cycle with warmer temperatures during the grain filling period. The analysis of variance across target population of environments revealed significant difference among durum wheat landraces, cultivars and breeding lines; environments across the Senegal River; and their interaction effect. Association genetics (GWAS) led to identifying most significant quantitative trait loci (QTL) across sites for various agronomic traits. The haplotype of the 64 markers underlining 32 QTL was used to describe allelic variations occurring for the 9-top yielding durum wheat genotypes, which allows targeting crossing between the top yielding lines to maximize the number of positive alleles. Genomic prediction for GY works using simple models. Likewise, a small number of DNA markers (< 300) and small training populations (TP) may be enough for getting suitable GEBV, particularly when the TP relates highly to the breeding population (e.g. full-sibs). Such an approach may decrease the accuracy of genomic selection but brings savings on expenditures. Furthermore, QTL analysis or using DNA markers linked to target genes increase drastically accuracy. These results suggest therefore, the value of combining genomic selection with marker-aided breeding (e.g. based on GWAS outputs) to improve GY under stress.

## TOPIC:

### Genomics and genome editing for crop design

#### Posters

#### **618 - IMPROVING ABIOTIC STRESS TOLERANCE IN TOMATO BY CRISPR/CAS9 EDITING OF KEY STRESS RESPONSE REGULATORS**

**Paola Punzo** <sup>(1)</sup> - **Alessandra Ruggiero** <sup>(2)</sup> - **Nunzio D'Agostino** <sup>(3)</sup> - **Stefania Grillo** <sup>(2)</sup> - **Teodoro Cardi** <sup>(1)</sup> - **Alessandro Nicolai** <sup>(1)</sup> - **Giorgia Batelli** <sup>(2)</sup>

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In a world of constant population increase, the impact of climate change on environmental resources requires breeding efforts to develop stress-tolerant crops. Here, we used the CRISPR/Cas9 technology to edit two genes involved in proline metabolism and the Salt Overly Sensitive (SOS) pathway to obtain tolerant tomato plants. Proline is a multifunctional amino acid acting as a compatible osmolyte under osmotic stress to maintain cell turgor pressure. To generate plants with increased proline levels, we selected the gene encoding PYRROLINE-5-CARBOXYLATE DEHYDROGENASE1 (P5CDH1), involved in proline catabolism, as target to be edited for generating loss-of-function mutants. The Na<sup>+</sup>/H<sup>+</sup> antiporter SOS1 controls ion homeostasis by reducing the concentration of toxic Na<sup>+</sup> ions in plant cells during salt stress. To obtain mutants producing constitutively active SOS1, we targeted the protein autoinhibitory domain that keeps the protein inactive in unstressed conditions. Constructs containing two single guide RNAs (sgRNAs) for each gene were tested using the hairy root system. Genotyping through high resolution DNA fragment analysis showed high mutation efficiency in the target sites. To obtain stable edited plants, *Solanum lycopersicum* cotyledons were transformed using *Agrobacterium tumefaciens* carrying the validated vectors. Through DNA sequencing we selected mutations resulting in premature stop codons in p5cdh1 and large deletions in the C-terminal domain of sos1. These results demonstrate that the CRISPR/Cas9 system and the tested sgRNAs can be used to successfully edit the two tomato genes previously mentioned. Progenies of independent transformants harbouring different p5cdh1 alleles were used to measure proline content. Compared with wild-type, p5cdh1 mutants showed higher proline levels in leaves. Phenotyping of these lines and of sos1 mutants in stress conditions is in progress to verify the tolerance to suboptimal environmental conditions.

## **645 - The Role of StBEL11 Transcription Factor in Potato (*Solanum tuberosum*) Tuber Formation**

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*Solanum tuberosum* is the fourth most important crop in the world, so understanding the mechanism of tuber formation regulation has a great potential for practical use, like design new approaches to achieve increased tuber yield and quality. Regulation of the onset of tuberization via environmental conditions have been known for a long time, but the molecular basis of this process has only recently started to be uncovered. The timing of tuberization is, besides external factors, affected by overall plant condition reflected by energy status, particularly carbohydrate metabolism, and the equilibrium of mobile signals transported from leaves to target tissues (stolons) by phloem. BELL (BEL1-like) transcription factors are among the important components of the regulatory signalling network. A role in the regulation of tuberization has been demonstrated in three BELL transcription factors: StBEL5, which has a positive effect on tuber formation, and StBEL11 and StBEL29, which in turn act as repressors. This work is focused on characterization the role of transcription factor StBEL11, whose transcript acts as a mobile signal repressing the onset of tuberization. We prepared StBEL11RNAi transgenic potato plants (*Solanum tuberosum* cv. Kamýk) to test effect of reduced StBEL11 expression on parameters related to initiation of tuberization and characterize the role of selected mobile signals regulating this process and carbohydrate status.

The work was supported by the Grant Agency of the Charles University: Project number 1308119.

### 3 - Karyotypic studies of four *Physalis* species from Nigeria

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The chromosome numbers in *Physalis peruviana*, *P. pubescens* and *P. micrantha* have not been ascertained in Nigeria. In addition, there is lack of consensus on the chromosome number of *P. angulata*. In view of the above, this study was carried out to investigate the mitotic chromosomes of these four *Physalis* species from Nigeria in order to elucidate the karyotypic variation that exists among them with respect to their karyotype. The mitotic chromosomes of four Nigerian *Physalis* species were studied from root tips pretreated in 0.002 M 8-hydroquinoline using squash method according to standard procedure and stained in FLP orcein. *Physalis angulata*, a tetraploid was found to have chromosome number of  $2n = 48$  with average centrometric index of 34.04%. The proportion of chromosomes in *P. angulata* with arm ratio greater than 2 was 0.38 placing it in 2A stebbins category having karyotypic formula of  $2M + 5m + 16sm + 1st$ . *Physalis micrantha*, *P. peruviana* and *P. pubescens* that are diploids showed chromosome number of  $2n = 24$ ; belong to Stebbins categories 2A, 2B and 3A with centromeric indexes of 35.21%, 42.77% and 43.18% respectively. Their karyotypic formulae were shown to be  $1M + 1m + 9sm + 1st$ ,  $4M + 6m + 1sm + 1st$  and  $1M + 1m + 2sm + 8st$  respectively. It can be concluded from this study that *P. angulata* is more advanced when compared to the other three diploids studied. Polyploidy is an evidence of advancement. Among the diploids, *P. pubescens* is the most advanced.

## 67 - Comparative genomics of ACGT Cis regulatory elements in Plant Gene expression

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Gene expression is an extensively controlled process that occurs at various levels, transcription being the central, It depends on a variety of interactions mediated by the core promoter region, sequence specific DNA binding proteins and their cognate promoter elements. Cis regulatory elements are short nucleotide sequences that provide binding sites for transcription factors. According to PLACE database, there are 469 cis regulatory elements have been reported in plants. The ACGT core sequence has been established as a functionally important cis element in several promoters that respond to different stimuli like light, jasmonic acid ,salicylic acid, abscisic acid. Promoter activity is largely affected by the copy number, inter motif distance, position of cis elements.

We did comparative genomic analysis of four different plant genomes In this study, we used two monocotyledonous – *Oryza sativa* and *Sorghum bicolor*, and two dicotyledonous species – *Arabidopsis thaliana* and *Glycine max* to analyze the conservation of co-occurring ACGT core elements in plant promoters with respect to spacer distance between them. Using data generated from *Arabidopsis thaliana* and *Oryza sativa*, we also identified conserved regions across all spacers and possible conditions regulating gene promoters with multiple ACGT cis-elements. Our data indicated specific predominant spacer lengths between co-occurring ACGT elements, but these lengths were not universally conserved across four species under analysis. However, the frequency distribution indicated local regions of high correlation among monocots and dicots. Sequence specificity data clearly revealed a preference for G at the first and C at the terminal position of a spacer sequence. Using gene expression databases, we also observed trends suggesting that co-occurring ACGT elements are responsible for gene regulation in response to exogenous stress. Data obtained in this study will be presented in the conference.



## **70 - The Effect on gene flow from GM to non-GM rice by heading date difference**

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Genetically modified (GM) crops have been increased continuously over the world and concerns about the potential risks of GM crops have also been increasing. Even though GM crops have not been cultivated commercially in Korea, it is necessary to develop technology for safety assessment of GM crops. In this study, we investigated the influence of heading date difference on gene flow from GM to non-GM rice. In the experimental design, The PAC gene GM rice was placed in the center as a pollen donor and non-GM rice were placed in eight directions as pollen receivers. Five pollen receiver rice cultivars were Unkawng, Daebo, Saegyejinmi, Nakdong, and Ilmi which had different flowering times. A total of 266,436, 300,237, 305,223, 273,373, and 290,759 seeds were collected from Unkawng, Daebo, Saegyejinmi, Nakdong, and Ilmi, respectively, which were planted around PAC GM rice. The GM×non-GM hybrids were detected by repeated spraying of herbicide and PAT immunostrip assay. Finally, the hybrids were confirmed by PCR analysis using PAC gene specific primer. The hybrids were found in Nakdong which had the same heading date with PAC GM rice. The hybridization rate was 0.0007% at Nakdong. All of GM×non-GM hybrids were located within 2 m distance from the PAC rice zone. The physiological elements including rice heading date were found to be important factors to determine GM rice out crossing rate. Consideration should be taken for many factors like the physiological elements of field heading date of rice cultivars to set up the safety management guideline for prevention of GM rice gene flow.

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## 71 - 'B.Y.O.R.' – Bring Your Own RuBisCO

### A *Nicotiana sylvestris* RuBisCO System Recipient line

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The Calvin-Benson-Bassham (CBB) cycle is the metabolic pathway by which plants assimilate CO<sub>2</sub> into biomass. One limiting factor in photosynthetic CO<sub>2</sub> fixation, and, ultimately, in biomass accumulation, is the activity of the CO<sub>2</sub>-fixing enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) [1]. Although all land plant RuBisCOs amino acid sequences are highly conserved, RuBisCOs fall in a broad spectrum of enzyme speed and affinity to CO<sub>2</sub>. This led researchers to propose that other variants of RuBisCO might be introduced into crops to improve yield.

However, there are several technical and biological obstacles to apply this idea. RuBisCOs from any photosynthetic eukaryote cannot be properly folded and assembled in *E. coli*, which has impeded structure-function studies of higher plant RuBisCOs [2]. Genes that code each of the subunits are in separate cellular compartments. The LSU is coded by the plastidial gene *rbcl*, and the SSu is coded by a nuclear gene family (*RbcS*). RuBisCO activation regulation is complex and involves another enzyme, RuBisCO activase (RA) coded by a nuclear gene family (*Rca*) that co-evolved with the other RuBisCO genes. Finally, precise genetic manipulation of the chloroplast genome is only achieved in a limited number of species.

Here we present the pipeline applied for the development and future use of a *Nicotiana sylvestris* RuBisCO genes recipient line that we hope will help the study of mutated and/or non-native sequences of the LSU and SSu, and RA. We choose *N. sylvestris* as the model organism to create this line because both transformation of the plastome and the genome is feasible and efficient [3]. Using several rounds of transformation and regeneration we: (i) knocked-out the nuclear genes using CRISPR/Cas9; (ii) removed *rbcl* from the plastome; (iii) reinstated the photosynthetic capacity of the line using a synthetic nuclear cassette.

## 241 - Genome Editing to Help Us Eat More Fresh Produce

**Shai Lawit** <sup>(1)</sup>

**Pairwise, Genome Editing Technologies, Durham, United States** <sup>(1)</sup>

Nutritional deficit is the leading health risk factor globally. Consumption of fruits and vegetables – the most effective solution to malnutrition – can be limited by low production or high cost in some countries and by poor quality characteristics or inconvenient preparation in others. Americans, for example, consume only half the recommended daily intake of fruits and vegetables despite widespread availability. Pairwise seeks to address these challenges regionally and globally by making healthy food more convenient, affordable, and sustainable. To fulfill this mission, we have assembled a world class portfolio of CRISPR technologies and a state-of-the-art R&D capability focused on delivering differentiated products to the fresh produce aisle. We have established base editing and cutting tools with industry-leading performance profiles in plants, including editing efficiencies as high as 85% and extensive target sequence accessibility through gene and protein engineering. We are applying these tools for novel consumer traits in cane berries. To enable this, we recently developed transformation capabilities in these species. This presentation will describe Pairwise progress in developing genome editing tools and transformation systems in crops, as well as our vision for opportunities in fresh produce.

## **288 - Development of genomic tools for cultivation of local avocado varieties in Northwestern México.**

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Avocado (*Persea americana* Mill.) is a climacteric fruit well recognized by its benefits on human health, mainly because of the high vitamin contents, fatty acids and other lipids that accumulate over its ripening. Most avocado orchards in Mexico are located in temperate areas and dedicated to the production of the Hass variety. However, Hass is not able to grow well in hot weather and low elevation or sea level soils. There is a large and unexplored stock of adapted avocado genotypes at northwestern Mexico. This represents an outstanding opportunity to explore new materials of local genotypes for breeding programs. This research aims to characterize the genetic diversity and gene content of avocado genotypes in northwestern Mexico by using two genomic tools: Large-scale SNP detection by genotyping by sequencing (GBS) and RNA-sequencing for obtaining transcriptomes of contrasting genotypes. GBS allowed to identify 13426 SNP using 60 avocado genotypes from 4 different regions. Values of  $H_e = 0.284$  and  $PIC = 0.35$  were obtained. Our findings suggest a high genetic diversity for local avocado genotypes in northwestern Mexico. Despite the high diversity, low levels of heterozygosity were observed in the populations, which suggest the presence of inbreeding. RNA sequencing generated a total of 8383 and 7360 annotated transcripts from two local genotypes, and 29159 annotated genes from Hass. A functional analysis focused on fatty acid, lipids and secondary metabolism was detailed. The generated knowledge would help in the understanding of fruit metabolism and its genetic control in lowland avocado resources.

### **353 - Microinjection of microspores and its application for the introduction of mutations in wheat cells**

**Bartosz Szabała**<sup>(1)</sup> - **Magdalena Świącicka**<sup>(1)</sup> - **Monika Rakoczy-Trojanowska**<sup>(1)</sup> - **Leszek Łyżnik**<sup>(1)</sup>

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Genome editing and single-cell DNA/RNA sequencing are two major developments that advance the interest in micromanipulations of plant cells. While microinjections have become standard procedures for studying cellular activities and for the production of genetically modified metazoan organisms, plant cells do not readily yield to micromanipulations. We found that microspores reprogrammed for embryogenic development are suitable for microinjection-facilitated delivery of DNA and protein molecules to metabolically active wheat cells. The system is compatible with the CRISPR technology, delivering mutations at the sites of double-strand breaks formed by the Cas9 protein. This is a natural, streamlined procedure for single-cell cellular/genetic research as well as for genetic micromanipulations towards crop improvement.

### **359 - Evaluation and optimization of a direct DNA extraction procedures for the analysis of genome-edited tomato plants**

**Ju Hui Do** <sup>(1)</sup> - **Se Hee Park** <sup>(1)</sup> - **Hyun Min Kim** <sup>(1)</sup> - **Seo Young Park** <sup>(1)</sup> - **Thanh Dat Mai** <sup>(1)</sup> - **Won Choi** <sup>(1)</sup> - **Hye Min Jang** <sup>(1)</sup> - **In Yeong Na** <sup>(1)</sup> - **Jae Sung Shim** <sup>(1)</sup> - **Young Hee Joung** <sup>(1)</sup>

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Genomic DNA isolation is a crucial technique for the PCR-based characterization of genome-edited plants, but at the same time, it is also time-consuming step for large-scale analysis. Here, we developed direct DNA extraction procedures and evaluated their efficiency for PCR-based characterization of genome-edited tomato plants. To achieve rapid extraction of genomic DNA without homogenization of tomato tissue, we used intact tomato tissues and evaluate efficiency of genomic DNA extraction by three different factors (heat, proteinase K, and  $\beta$ -mercaptoethanol). PCR analysis showed that the genomic DNA extracted by heat treatments was sufficient for robust amplification of target fragments by the PCR reaction. Further sequencing analysis using the amplified fragment showed clear separation of peaks, which is sufficient for identification of mutation patterns of the target region. We then tested the procedures with various tomato tissues harvested from different stage of growth, and found that our methods can be applied for analysis of tomato leaves and fruits (from green to breaker stages). In addition, we confirmed that the genomic DNA extraction procedures can be used for analysis of genome-edited rice plants. Our results suggest that the developed heat-based genomic DNA extraction procedures can be used for rapid screening of genome-edited plants.

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### **364 - Direct delivery of CRISPR/Cas9 machinery in grapevine protoplasts and plant regeneration**

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Plant genetic engineering has seen great improvements thanks to the availability of new biotechnological tools, including nucleases like TALEN and ZINC-FINGERS, meganucleases and, over the last ten years, the CRISPR/Cas9 system. The latter has become the predominant choice as it is cost-effective and easier to design, it allows for precise modification of target gene (or genes), it has been delivered to plant and animal cells to perform functional studies and to genetically improve desired traits. The plasmid-mediated CRISPR/Cas9 genome editing by means of *Agrobacterium tumefaciens* infection consistently provides efficient transfection of the desired expression cassette; however, it implies that foreign DNA can persist in the host organism and can potentially have undesired off-target effects. Instead, direct delivery of CRISPR/Cas9 machinery into protoplasts shows interesting advantages: it offers lower likelihood of off-target effects and ensures a transient presence of the foreign elements into the plant cell. The ribonucleoprotein complex (RNP complex) of Cas9 and gRNA is degraded shortly after its transfection, thus allowing the regeneration of tDNA-free gene-edited plants. In grapevine (*Vitis vinifera*), gene editing via CRISPR/Cas9 shows up in very few studies, mainly because of the general recalcitrance of this species to transformation and the highly cultivar-dependent responses to in vitro culture. Herein we present the results concerning the direct delivery of CRISPR/Cas9 RNPs targeting the *pds* gene in grapevine protoplasts obtained from embryogenic calli. Furthermore, we illustrate our progress in the optimization of a protocol for grapevine plant regeneration from edited protoplasts.

#### 446 - Targeting plant architectural traits for yield enhancement in chickpea

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Chickpea (*Cicer arietinum* L.), an important legume crop cultivated and consumed worldwide, requires a breakthrough in its yield and productivity. Since land resources are limited, the focus needs to shift towards altering the plant types to enhance the net seed yield output. The development of high-yielding cultivars with restructured plant architecture is required to bridge the gap between demand and production of this crop. Being indeterminate in nature, chickpea exhibits enormous vegetative growth when subjected to proper irrigation, which demands more space and creates a competition between vegetative and reproductive tissue for photosynthates. The semi-determinate plant types with restricted vegetative growth, early flowering, maturation, and synchronized growth pattern would be more remunerative to farmers in this regard. The inflorescence architecture, comprising its development timing and features, holds the key to yield stability with optimal resource use. The developmental events also directly influence the flowering time, a crucial parameter for stress avoidance in crops like chickpea. Thus, the plant architectural traits have immense potential for yield enhancement and stability. Considering the relevance of plant architectural traits, a comprehensive understanding of its genetic regulation and molecular signaling is necessary for future crop improvement programs in chickpea through translational genomics approaches. Our study integrates various classical and molecular genetics, and advanced next generation sequencing driven genomics strategies to delineate major QTLs/genes and natural alleles regulating desired architectural traits. Functional validation of these molecular signatures in near-isogenic lines (NILs) and transgenics and their subsequent introgression in chickpea cultivars will help us to develop a high-yielding restructured new plant type in chickpea.



#### **467 - PhyloGenes: an online resource for plant gene function prediction using phylogeny and prior knowledge**

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One of the great challenges facing plant biology is accurately and efficiently transferring knowledge gained from model organisms to other plants. Individual gene duplications and whole genome duplications are common in plant lineages, which result in complex evolutionary relationships between related genes. Such genes can have similar sequences but highly divergent functions. Therefore, function inference often requires integration and analysis of multiple types of information beyond sequence similarity, such as phylogenetic relationships. We have developed a new resource PhyloGenes ([phylogen.es.org](http://phylogen.es.org)) that presents precomputed phylogenetic trees of gene families along with known function information for individual members. By displaying experimentally validated gene functions associated to individual genes within a tree, PhyloGenes enables functional inference for genes of uncharacterized function, based on their evolutionary relationships to experimentally studied genes, in a visually traceable manner. For the many families containing genes that have evolved to perform different functions, PhyloGenes also facilitates the observation and study of function evolution. PhyloGenes includes 40 plant genomes and 10 non-plant model organisms represented in over 8,000 gene families. Trees can be customized to display only genes of the desired species. A tool allows a user to add a protein sequence from other species to a matching family tree. Over two-thirds of the families have at least one member with a validated known function as GO terms. The gene function information displayed adjacent to a tree can be swapped with a multiple sequence alignment with conserved residues highlighted. Future work will incorporate additional functional datasets such as gene expression and enable automated genome-scale function prediction. This presentation includes an introduction to PhyloGenes and a case study of using PhyloGenes for inferring gene function in a crop species.

#### **474 - Genetic Dissection of Complex Plant Architecture and Yield Component Traits in Chickpea**

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<sup>(1)</sup>

Chickpea (*Cicer arietinum*) is an important legume food crop of profound economic and nutritional value. This is commonly represented by two types of cultivars, desi and kabuli whose genomes have been sequenced recently. Enhancing chickpea productivity by developing high pod/seed-yielding-cultivars of restructured new plant types with desirable architecture (erect/semi-erect and semi-dwarf) is very essential in order to sustain global food security amidst climate change scenario. For instance, in case of the “Green Revolution”, significant increase in grain yield/productivity has been achieved by growing lodging-resistant semi-dwarf (ideal plant architecture) varieties of wheat and rice. In this context, genetic dissection of complex plant architectural quantitative traits is vital to enhance yield, productivity and yield stability in chickpea. Plant height (PH) and plant width (PW), two of the major plant architectural traits determining the yield and productivity of a crop, are defined by diverse morphometric characteristics of the shoot apical meristem (SAM). The identification of potential molecular tags/QTLs and genes that simultaneously modulates these plant/SAM architectural traits is prerequisite to achieve enhanced yield and productivity in crop plants including chickpea. The present study integrated a genome-wide association study (GWAS), regional gene-by-gene association analysis with QTL/fine-mapping and map-based cloning, molecular haplotyping and transcript profiling, for the dissection of plant/SAM architectural traits in chickpea. These exertions delineated CabHLH121 transcription factor (TF) gene and its derived natural alleles and superior haplotypes of a major QTL governing said architectural traits in chickpea. These molecular signatures have potential to regulate both PW and PH traits by modulating proliferation, differentiation and maintenance of the meristematic stem cell population in the SAM. The salient outcomes thus be very useful in developing high-yielding chickpea cultivars restructured with ideal plant architecture and enhanced productivity.

#### **497 - Role of SIGRAS9 and SIGRAS10 in tomato fruit ripening**

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Fleshy fruits, like *Solanum lycopersicum* (tomato), comprise an indispensable commercial and nutritional food commodity, being an important crop in agriculture worldwide. Tomatoes are climacteric fruits, and these fruits continue their fast process of ripening after harvest making it much more difficult to store and distribute, compared to other fruits. These processes of ripening and post-harvest decay are very complex. The trigger to the reprogramming of fruit development and ripening comprises several transcription factors with GRAS as putative regulators. These transcription factors are plant-specific and involved in plant growth and development. However, their role in fruit development is not fully understood yet. Recent genome-wide analysis of the GRAS gene family in tomato showed that the duplicated genes SIGRAS10 and SIGRAS9 might be involved in tomato fruit ripening.

In this study, we have first developed SIGRAS10 mutant plants using CRISPR/Cas9 which generated mutations in two different regions of the SIGRAS10 gene. Phenotypically, no major differences were found in mutated fruits compared with wild type fruits. However, molecular analyses using RT-qPCR genes showed that genes involved in the ripening process were differentially expressed in the mutated fruits compared to the wild type, in particular, RIN, ACS2, ACO1, PYL9 and PSY. Currently, we are quantifying carotenoid accumulation in order to integrate with this information that suggests a role of the SIGRAS10 in tomato ripening. Additionally, we have developed double mutants for SIGRAS10 and SIGRAS9 that may exhibit functional redundancy and/or neofunctionalization.

## 507 - How to Study Plant Transcriptome Data? Using a Shiny-Based Application Called “NORMALIX”!

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Microarray and RNA-Seq technologies allow the investigation of gene expression data for several organisms under different conditions. Both are widely used to study transcript profiles in plants; however, researchers without programming skills cannot easily approach and use the massive plant transcriptome data available online. NORMALIX is an application/open-source web tool implemented in R using the Shiny framework for the analysis of plant microarray and RNA-Seq data. It is organized in 10 tabs allowing users to perform different operations, such as the normalization of Affymetrix microarray data for 11 plant species, the generation of several plots (heatmaps, PCA, dendrograms, scatterplots), Pearson correlation (between hybridizations and genes) and differential gene expression analyses. Several datasets of normalized microarray will be freely downloadable and questionable. NORMALIX source code will be available at GitHub, therefore, users will be able to launch NORMALIX in any operating system with the R programming environment and RStudio IDE installed. Furthermore, NORMALIX will be also provided online as a shiny application in web browsers for users who are not familiar with R. NORMALIX is the first tool specifically designed for plant transcriptomics analysis, developed with a user-friendly web interface for plant researchers to help them analyzing plant gene expression data. We aim to develop an application that makes plant transcriptomics approachable to everyone and we hope to upgrade NORMALIX with other features. Therefore, we encourage users to contribute to future developments of NORMALIX through comments and suggestions.

## **665 - The CHIC project: New Plant Breeding Techniques; Chicory as a multipurpose crop for dietary fibre and medicinal terpenes**

**Katarina Cankar**<sup>(1)</sup> - Dirk Bosch<sup>(1)</sup>

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The CHIC project (Chicory Innovation Consortium), which is funded by the EU Horizon 2020 programme, consists of 17 partners from 12 different countries. The overall objective is to implement New Plant Breeding Techniques (NPBTs) in root chicory in order to establish it as a multipurpose crop and as a sustainable approach to molecular farming, i.e. the production of health-related products with clear benefits for consumers. CHIC will develop root chicory varieties that on the one hand produce more and healthier inulin food fiber and on the other hand produce sufficient amounts of medicinal terpenes. CHIC is highly interdisciplinary and focussed on interaction with stakeholders. The Consortium will evaluate the technical performance of different NPBTs, as well as the safety, environmental, regulatory, socio-economic and broader societal issues associated with them. At the same time CHIC gives great emphasis to communication about the project and about gene editing in general, also implementing innovative communication methods. For example, artists will make themselves familiar with genome editing techniques and express their feelings and views in artworks to inspire a broader public debate. By involving stakeholders and by raising public awareness at all phases of the project, CHIC strives to ensure responsible and desired innovation.

## TOPIC:

### Long-distance messages in plants

#### **Keynote Lecture**

##### **Developmental boundaries: choosing between division and differentiation**

##### **SABRINA SABATINI**

Organogenesis is a complex process in which it is necessary to coordinate tissues and cells at different stages of development to guarantee the final functional shape of an organ. One strategy to organize the orchestration of organ development is the separation of cells into distinct functional units. The interpretation of morphogenetic gradient and cell intrinsic cues sort cells in different fields, generating different domains of activities. Developmental boundaries are established that separate these domains and preserve over time their identity and activity. Understanding how these developmental boundaries are first established and subsequently kept in position during organ growth is a fundamental question in the biology of all multicellular organisms. In the root of the model plant *Arabidopsis thaliana*, the Transition Zone (TZ) is a crucial developmental boundary that separates dividing cells from differentiating ones in the meristem. Combining molecular genetics with computational modelling we identified several important molecular mechanisms involved in establishing and maintaining the TZ during root organ growth. Here some of these new identified molecular networks will be discussed.

## TOPIC:

### Long-distance messages in plants

#### Oral Communications

**52 - Dissecting the cytokinin genetic pathway and the main gene regulatory networks in Cichorium endivia leaves: fundamental biology in leafy crops**

**Giulio Testone <sup>(1)</sup> - Elena Baldoni <sup>(2)</sup> - Maria Adelaide Iannelli <sup>(1)</sup> - Chiara Nicolodi <sup>(1)</sup> - Elisabetta Di Giacomo <sup>(1)</sup> - Fabrizio Pietrini <sup>(3)</sup> - Giovanni Mele <sup>(1)</sup> - Donato Giannino <sup>(1)</sup> - Giovanna Frugis <sup>(1)</sup>**

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Cytokinins (CKs) regulate important aspects of plant development including leaf shape. Being potent antagonists of senescence, CKs might be exploited to extend the shelf life properties of leafy vegetables. Endives (*Cichorium endivia* var. *crispum*) and escaroles (*C. endivia* var. *latifolium*) are leafy crops belonging to the Cichorieae tribe which leaves are widely consumed as fresh, minimally processed and cooked food. Similar to lettuce, their leaves display a wide range of morphology diversity, ranging from smooth (escaroles) to extremely curly leaves (endives). CK homeostasis and responses may also contribute to leaf morphology diversity among *C. endivia* cultivars.

We assembled de novo the transcriptome of *C. endivia* and reconstructed the genetic pathway of cytokinins: genes of CK metabolism, transport, signaling and response were annotated, and their expression analyzed in edible leaves of two endive and two escarole cultivars by RNAseq. Metabolite analysis identified cis-zeatin as the main cytokinin type in mature leaves, which accumulated as reversibly inactivated zeatin O-glycosylated storage forms. Metabolite-transcript correlation identified candidate genes for cis- and trans-zeatin reversible O-glycosylation, and for the two-step interconversion of CK bases, nucleosides and nucleotides, which have remained elusive for a long time. Gene Co-expression Network (GCN) analyses identified an AHK4/CRE1 receptor-based module as a major CK regulatory network in edible leaves. K-means clustering and GCN analyses of transcription factors (TFs) and target pathways (CK, photosynthesis, senescence, leaf development) identified eight major modules of co-regulated genes, amongst which the two most abundant ones were anti-correlated and comprised genes promoting photosynthesis or genes involved in oxidative stress/senescence responses. Most CK-related genes placed within these two clusters. Developmental genes at the boundary between photosynthesis and leaf development were identified by a “guilt by association” approach. Finally, differential expression analysis between broad and curly leaves identified novel candidate TFs putatively involved in leaf shape diversity.

## TOPIC:

### Long-distance messages in plants

#### **Extended Elevator Pitches**

**463 - At the edge of the gate: discerning the GLUTAMATE RECEPTOR-LIKE ligand-binding role in plant systemic responses**

**Matteo Grenzi** <sup>(1)</sup> - **Andrea Alfieri** <sup>(2)</sup> - **Ambra Selene Parmagnani** <sup>(1)</sup> - **Francesca Resentini** <sup>(1)</sup> - **Laura Luoni** <sup>(1)</sup>  
- **Stefano Buratti** <sup>(1)</sup> - **Maria Cristina Bonza** <sup>(1)</sup> - **Alex Costa** <sup>(1)</sup>

**University of Milan, Department of Biosciences, Milan, Italy** <sup>(1)</sup> - **University of Pavia, Centro Grandi Strumenti, Pavia, Italy** <sup>(2)</sup>

Throughout their life plants being sessile organisms are continuously exposed to environmental challenges that need to be properly perceived and that require appropriate local and systemic responses. Systemic responses are mediated by long-distance signaling that require the activity of Glutamate Receptor-Like channels (GLRs). GLRs are homologs of animal Ionotropic Glutamate Receptors (iGluRs) which are ligand-gated cation channels in the central nervous system. Despite the fact that iGluRs are gated through the binding with glutamate, the mechanism throughout GLRs are activated is poorly understood. As an example, we still do not know if the GLRs binding of amino acids is necessary for their activity. Here, I report the setup of a reliable protocol to visualize long-distance calcium waves in flowers that are almost abolished in the *glr3.3* KO mutant. In order to define the role played by the GLR3.3 amino acid-binding in the long-distance signaling, I took the advantage of the recently obtained crystal structure of the GLR3.3 Ligand-Binding Domain (LBD) with the identification of the residues involved in the amino acid-binding. I, therefore, introduced single point mutations in the genome sequence of the GLR3.3 gene to prevent or abolish its amino acid-binding, and with the obtained constructs I complemented the *glr3.3* KO. By performing calcium imaging analyses using the established protocol I will define, in vivo, the role of the GLR3.3 amino acid-binding in the long-distance signaling.



## TOPIC:

### Long-distance messages in plants

#### Posters

##### **151 - Sugar hormone message in aspect of apple tree biennial bearing**

**Giedrė Samuolienė<sup>(1)</sup> - Darius Kviklys<sup>(1)</sup> - Kristina Laužikė<sup>(1)</sup>**

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Aiming to explore the reasons of biennial bearing, the effect of apple rootstock, scion cultivar and crop load, metabolic changes of endogenous phytohormones (zeatin, jasmonic acid, indole-3 acetic acid, abscisic acid and gibberellins, 1,3 and 7) and soluble sugars (glucose, fructose and sorbitol) and their connections with return bloom and yield in apple tree buds was analysed. Cvs. 'Ligol' and 'Auksis' on five rootstocks contrasting in induced vigour were tested: semi-dwarf M.26; dwarf M.9, B.396 and P 67; and super-dwarf P 22. Crop load levels were adjusted before flowering leaving 75, 113 and 150 inflorescences tree<sup>-1</sup>. The significant increase of inhibitor to promoter hormones ratio was in both cultivars grafted on B.396 rootstock, while the significant increase of hexoses and sorbitol was observed in cv. 'Auksis', grafted on B.396 rootstock. The significant increase of glucose and sorbitol was also found in both cultivars grafted on M. 26 rootstock. Moderate crop load resulted an increase, while The highest crop load lead to decrease of promoter and inhibitor phytohormones in 'Ligol', while no interaction between crop load was found in 'Auksis'. Besides, negative correlation between promoter and inhibitor phytohormones was found in both varieties. Such data suggest, that B.396 rootstock ensures the most stable return bloom irrespectively on previous year crop load. The most significant decrease of return bloom was resulted by the highest crop load in cv. 'Auksis' grafted on M.9 and P 22 rootstocks. Average difference in number of flowers between 75 and 150 fruits/tree treatments of cv. 'Ligol' was 68%, while for P 22 this difference reached ~90; for M.9 and M.26 rootstocks ~75%. Besides, return bloom depended both on crop load in previous year, cultivar and rootstock. Such data suggest that flowering inhibition depended on the phytohormones that were exported to buds and on sugar hormone signalling cross-talk.

**Key words:** biennial bearing, *Malus × domestica* Borkh., phytohormones, rootstock, sugars

## 661 - Involvement of ACA8/ACA10 in wounding-induced stomatal closure in Arabidopsis

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The *Arabidopsis thaliana* ACA8 and ACA10 encode two distinct plasma membrane-localized auto-inhibited  $\text{Ca}^{2+}$ -ATPases which are expressed in different tissues including stomatal guard cells. Years of intense studies have shown that ABA, plasma membrane hyperpolarization, ROS, external  $\text{Ca}^{2+}$ , among other stimuli, activate plasma membrane  $\text{Ca}^{2+}$  permeable channels leading to cytosolic  $[\text{Ca}^{2+}]$  increases. Interestingly, in guard cells cytosolic  $[\text{Ca}^{2+}]$  increases often occur in the form of repetitive  $[\text{Ca}^{2+}]$  oscillations whose frequency, duration, amplitude and transient number, which are linked to fluctuations in the membrane voltage and in ions' fluxes, regulate the stomatal aperture. In this study, the role of ACA8 and ACA10 in stomatal closure induced by root or leaf wounding was investigated by genetic approaches with loss and gain of function approaches. In wild-type seedlings, leaf/root wounding induced both local and systemic signaling leading to stomatal closure after 5' (local response) as well as after 5' or 60' (systemic responses to root or distal leaf wounding, respectively). In contrast, *aca8/aca10* insertional double mutants were unresponsive. Interestingly, also 35S::ACA8 over-expressing lines were found to be unresponsive to stomatal closure induced by leaf or root wounding. No differences in stomatal closure between wild-type and null mutants or 35S::ACA8 over-expressing lines were observed in control conditions. RT-qPCR analysis showed an induction of both ACA8 and ACA10 expression upon leaf wounding after 24h, that was stronger for ACA10 (10 fold) in respect to ACA8 (3 fold). As a whole, these results suggest the involvement of ACA8/ACA10 in stomatal closure induced by leaf or root wounding and led us to hypothesize that these  $\text{Ca}^{2+}$  pump isoforms may be necessary for the correct modulation of the cytosolic  $\text{Ca}^{2+}$  levels in the guard cells (locally) as well as in the other tissues involved in the long-distance wound response.

## TOPIC:

### Molecular and cellular organization of the photosynthetic system

#### **Keynote Lecture**

**Mitigating the impact of a warming climate on photosynthetic carbon gain with an alternative photorespiratory pathway.**

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Agricultural production faces numerous global change-related abiotic stresses, including rising temperatures, which pose a threat to global food production and sustainability. Global temperatures are rising, and higher rates of temperature increase are projected over land areas that encompass the globe's major agricultural regions. In addition to increased growing season temperatures, heat waves are predicted to become more common and severe. Among the physiological processes that underlie crop yield that are susceptible to high temperatures, photosynthetic carbon gain is among the most important where the effects high temperature can interact with other climate change factors. Strategies to adapt photosynthesis to the warming climate and episodes of extreme high temperature will be critical to sustaining productivity much less to increase productivity to meet anticipated increasing agricultural demand. There are numerous points of high temperature sensitivity in photosynthesis that could be targeted including photorespiration. Photorespiration is a very large energetic cost to C3 plants and its rate increases with temperature, increasing the overall energetic cost and lowering net photosynthesis. We have investigated installing more energetically efficient non-native pathways that substitute for the native pathway to mediate the impact of increasing temperature on net carbon gain with promising preliminary results.

## TOPIC:

### Molecular and cellular organization of the photosynthetic system

#### Oral Communications

##### 17 - Molecular determinants of grana stacking in plant thylakoid membranes

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The chloroplast thylakoid membrane is the site of the light reactions of photosynthesis. The plant thylakoids are spatially divided into the stacked grana regions and the interconnecting unstacked stromal lamellae regions. The photosynthetic complexes that perform the light reactions are heterogeneously distributed among these two regions, with Photosystem II (PSII) and its associated light-harvesting complex (LHCII), together forming the PSII-LHCII supercomplex, predominantly located in the grana stacks. Grana stacking in plant chloroplast thylakoid membranes dynamically responds to the light environment.

We performed a structural and proteomic investigation on the dynamic thylakoid stacking in pea (*Pisum sativum*) plants grown under different light intensities. As starting material, we used either thylakoid membranes isolated in close-to native state or purified PSII-LHCII supercomplexes. For our investigations, we adopted an integrated approach based on: 1) cryo-electron tomography and cryo-transmission electron microscopy to get insights on the structural determinants of the stacking of grana regions and 2) top-down and cross-linking mass spectrometry to detect at molecular detail the protein-protein interactions responsible for the thylakoid stacking.

Through this integrative structural biology approach: 1) we detected PSII-LHCII supercomplexes clearly facing in adjacent thylakoid membranes structurally interacting through stromal connecting densities; 2) we uncovered at molecular detail the spatial organization of PSII-LHCII supercomplexes arranged in a paired conformation across the stromal gap within the grana thylakoid membranes. These findings highlight a basic molecular mechanism whereby plants maintain grana stacking at changing light conditions. This mechanism relies on interactions between stroma-exposed N-terminal loops of LHCII trimers and Lhcb4 subunits of PSII-LHCII supercomplexes facing each other from adjacent membranes. The combination of light-dependent LHCII N-terminal trimming and extensive N-terminal  $\alpha$ -acetylation likely affects interactions between pairs of PSII-LHCII supercomplexes across the stromal gap, ultimately mediating membrane folding in grana stacks.

### **111 - The influence of LHCII complex composition on the grana structural regularity in Arabidopsis thaliana plants**

**Radosław Mazur**<sup>(1)</sup> - Łucja Kowalewska<sup>(2)</sup> - Sylwia Chromiec<sup>(1)</sup> - Rafał Luchowski<sup>(3)</sup> - Wiesław I. Gruszecki<sup>(3)</sup> - Maciej Garstka<sup>(1)</sup>

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The chloroplast thylakoid network forms an intricate spatial structure composed of stacked grana membranes and unstacked stroma thylakoids. The ideal granum structure is built with multiple thylakoid membranes with the same diameter forming a perfect cylindrical shape. However, in most plant species, grana structures are highly irregular – with a variable diameter of particular thylakoid layers and their shift in the lateral plane. Such irregular granum arrangement results in a significant increase in the area of granum end membranes and, as a consequence, change of the biochemical composition of the granum. Although there is a large amount of data concerning thylakoid network structural reorganization in different conditions, the influence of grana structural irregularity on these processes is completely unknown.

The aim of this study was to characterize the grana structural regularity in Arabidopsis wt plants and aslhcb2-12 mutant. The studied mutant, characterized by lowered photosynthetic capacity, is depleted of Lhcb1 and Lhcb2 proteins playing the main role in grana stacking. Microscopic analysis showed that the grana structures in the aslhcb2-12 mutant were smaller compared to wt plants. However, there was a higher number of grana stacks per chloroplast cross-section in aslhcb2-12, and these grana were extremely regular compared to less abundant irregular grana stacks of wt plants. Biochemical data confirmed mutant phenotype i.e. strong depletion of Lhcb1 and Lhcb2, and the increase of Lhcb5 protein contents. Different organization of the thylakoid membrane complexes was also reflected by increased chlorophyll fluorescence lifetime in aslhcb2-12 compared to wt plants.

In conclusion, the lack of two main LHCII proteins did not retard the grana formation process. However, it resulted in the presence of unusual for Arabidopsis plants highly regular grana stacks that maintain their regularity during illumination. This result suggests the potential role of grana irregularity in photosynthetic efficiency tuning in changing light conditions.

## 181 - Novel function for an iron regulator: OsbHLH60 activates a C<sub>4</sub> PEPC1 promoter

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Rice is the staple food for over half of the world population and its yield must increase in the next decades. Engineering C<sub>4</sub> photosynthesis into rice (C<sub>3</sub> plant) may help meeting this goal, since C<sub>4</sub> plants outperform C<sub>3</sub> in hot and dry climates. C<sub>4</sub> evolution was preceded by major regulatory changes in genes encoding key enzymes. A good example is phosphoenolpyruvate carboxylase (PEPC) whose promoter evolved to drive high expression in mesophyll cells of C<sub>4</sub> plants. Interestingly, this C<sub>4</sub> promoter also drives mesophyll-specific gene expression in C<sub>3</sub> species, such as rice. It is therefore fundamental to identify rice regulators of C<sub>4</sub> PEPC1 promoters. Using a Yeast One-hybrid system, we identified the transcription factor OsbHLH60 as binding to the C<sub>4</sub> *Setaria viridis* PEPC1 promoter (promSvPEPC1). To investigate the function of OsbHLH60 regulating promSvPEPC1, we constructed a rice transgenic line harbouring  $\beta$ -glucuronidase (GUS) driven by promSvPEPC1, which drives mesophyll-specific gene expression in rice, and knocked out OsbHLH60 in this reporter line using CRISPR-Cas9. These transgenic lines showed a clear reduction in GUS expression, indicating that OsbHLH60 acts as an activator of PEPC1 expression. Since OsbHLH60 was known to be stabilized under iron deficiency and to understand whether promSvPEPC1 regulation by OsbHLH60 was somehow modulated by iron, the promSvPEPC1::GUS lines, with wild-type OsbHLH60, were subjected to iron deficiency. Although OsbHLH60 should be stabilized under iron deficiency and thus activate GUS expression, we observed reduced GUS expression, suggesting that activation of the promSvPEPC1 by bHLH60 is iron independent. This work suggests that a C<sub>3</sub> pre-existing gene regulatory network was co-opted by C<sub>4</sub> plants to regulate PEPC1 expression. This regulation might have been involved in the high PEPC1 expression that allowed the appearance of C<sub>4</sub> photosynthesis. In the near future, we aim to characterize the biological function of bHLH60 in C<sub>4</sub> plants.

## TOPIC:

### Molecular and cellular organization of the photosynthetic system

#### **Extended Elevator Pitches**

**476 - Toward an effective use of microalgae by disentangle LHCSR role on non photochemical quenching (NPQ) in *Chlamydomonas reinhardtii***

**Federico Perozeni** <sup>(1)</sup> - **Stefano Cazzaniga** <sup>(1)</sup> - **Julianne M Troiano** <sup>(2)</sup> - **Raymundo Moya** <sup>(2)</sup> - **Luca Zuliani** <sup>(1)</sup> - **Giorgia Beghini** <sup>(1)</sup> - **Kwangyrul Baek** <sup>(3)</sup> - **EonSeon Jin** <sup>(3)</sup> - **Gabriela S Schlau-Cohen** <sup>(2)</sup> - **Matteo Ballottari** <sup>(1)</sup>

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Photosynthetic organisms evolved a photoprotective mechanism inducing thermal dissipation of excessive light energy, called Non-photochemical quenching (NPQ). The proper balance between photoprotection and light conversion for carbon fixation has been proposed as domestication target both in plants and in algae. In the model organism for green algae, *Chlamydomonas reinhardtii*, pigment binding proteins LHCSR(s), were reported as the main actors in NPQ. In this work the mechanism of action of LHCSR was studied using a combination of in vivo and in vitro approach. Despite high sequence identity of the two isoforms of LHCSR present in *C. reinhardtii*, LHCSR3 showed a stronger quencher activity compared to the LHCSR1 due to a different occupancy of L2 carotenoid binding site. Two distinct quenching processes, individually controlled by pH and zeaxanthin, were identified within LHCSR3. Either pH- or zeaxanthin-dependent quenching are able to protect against reactive oxygen species, and thus the two quenching processes may together provide different induction and recovery kinetics for photoprotection in a changing environment. By site-specific mutagenesis on chlorophyll binding sites the molecular details of NPQ induction was further investigated at intramolecular level in LHCSR3. Multiple quenching sites, cooperatively dissipating the excitation energy, were revealed with a peculiar role of Chl 613, a chromophore located a close distance to carotenoid binding site L1. These results allow to better understand the NPQ mechanism in *Chlamydomonas reinhardtii* opening the possibility to the generation of mutants with modified NPQ induction fully exploiting the microalgae potential for a growing world.

## TOPIC:

### Molecular and cellular organization of the photosynthetic system

#### Posters

#### **79 - LHCII proteins phosphorylation changes involved in the dark-chilling response in plant species with different chilling tolerance**

**Małgorzata Krysiak** <sup>(1)</sup> - Anna Węgrzyn <sup>(1)</sup> - Maciej Garstka <sup>(1)</sup> - Radosław Mazur <sup>(1)</sup>

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Under constantly fluctuating environmental conditions, the thylakoid membrane protein network evolved the ability to dynamically respond to changing biotic and abiotic factors. One of the most important protective mechanism is rearrangement of the chlorophyll-protein (CP) complexes, induced by protein phosphorylation. In a temperate climate, low temperature is one of the abiotic stresses that heavily affect plant growth and productivity.

The aim of this study was to determine the role of LHCII antenna complex phosphorylation in the dark-chilling response. The study included an experimental model based on dark-chilling at 4 °C of detached chilling sensitive (CS) runner bean (*Phaseolus coccineus* L.) and chilling tolerant (CT) garden pea (*Pisum sativum* L.) leaves. This model is well described in the literature as used for the analysis of chilling impact without any additional effects caused by light. We examined changes in thylakoid membrane protein phosphorylation, interactions between phosphorylated LHCII (P-LHCII) and CP complexes, and their impact on the dynamics of photosystem II (PSII) under dark-chilling conditions. Our results showed that the dark-chilling treatment of CS bean leaves induced a substantial increase of phosphorylation of LHCII proteins, as well as changes in CP complexes composition and their interaction with P-LHCII. The PSII photochemical efficiency measurements showed that in bean, PSII is overloaded with light energy, which is not compensated by CP complexes rearrangements. On the contrary, no significant changes in PSII photochemical efficiency, phosphorylation pattern and CP complexes interactions were observed in CT pea. In conclusion, our results indicate that different responses of the LHCII phosphorylation to chilling stress take place in CT and CS plants, and that kinetics of LHCII phosphorylation and interactions of P-LHCII with photosynthetic complexes may be crucial to chilling stress response.



#### **104 - Single-walled carbon nanotubes affect the thylakoid membrane structure and photosynthetic light utilization in pea plants**

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Increased environmental pollution with carbon-based nanomaterials raises the question of their modes of interaction with plants and therefore with the whole terrestrial ecosystem. On the other hand, carbonaceous nanoparticles such as single-walled carbon nanotubes (SWCNTs) have demonstrated promising applicability as novel carrier molecules, optical probes, etc. The reports on the physiological effects of SWCNTs on higher plants are highly contradictory and fragmentary, i.e. detailed data on their impact on plant photosynthetic apparatus is still missing.

We have studied the photosynthetic activity, architecture and macroorganization of thylakoid membranes of pea plants sprayed with various concentrations (10-300 mg L<sup>-1</sup>) of SWCNTs functionalized with Pluronic P85 copolymer.

PAM imaging of intact pea plants treated with SWCNTs revealed no significant alterations in the maximum photochemical efficiency of photosystem II. Nonetheless, a concentration-dependent effect of SWCNTs on the kinetics of the non-photochemical quenching of chlorophyll a fluorescence was established. In addition, SWCNTs were found to affect the xanthophyll cycle pigments content.

Transmission electron microscopy images of pea chloroplasts showed that spraying with increasing concentrations of SWCNTs leads to progressive thylakoid swelling and, at 300 mg L<sup>-1</sup> - to significant alteration of the thylakoid architecture. Circular dichroism spectra of isolated thylakoids indicated rearrangement of the pigment-protein complexes in the photosynthetic membranes of SWCNTs-treated plants with respect to their untreated counterparts.

Our data reveal that the foliar application of SWCNTs affects both the functional and structural properties of the photosynthetic apparatus of pea plants.

### **137 - Blue light signaling components interacts with the microRNA biogenesis and processing machinery in *Arabidopsis thaliana* pif mutant plants**

**Pavel Pashkovskiy** <sup>(1)</sup> - **Alexander Kartashov** <sup>(1)</sup> - **Ilya Zlobin** <sup>(1)</sup> - **Alexandra Khudyakova** <sup>(2)</sup> - **Valeria Strokina** <sup>(2)</sup> - **Vladimir Kreslavski** <sup>(2)</sup> - **Vladimir Kuznetsov** <sup>(1)</sup>

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Light can affect MIR gene transcription, miRNA biogenesis, and RNA-induced silencing complex (RISC) activity, thus controlling not only miRNA accumulation but also their biological function. It has been found that miRNAs are regulated by transcription factors (TFs) involved in plant responses to light of varying spectral composition. It is known that the interaction of PIF4 (phytochrome interacting factor 4) with the photoactivated form of phytochrome B (PHYB) regulates a subgroup of downstream TFs by binding to the promoters of these genes. At the same time, PIF4 regulates microRNA biogenesis by binding directly to the promoters of their Dicer-like1 (DCL1) and dsRNA binding protein (HYL1) genes, contributing to the destabilization of these important processor proteins capable of regulating mature microRNA under red light. PIF4 can regulate the expression of light-dependent miRNAs when exposed to red light, however, to what extent PIF4 regulates miRNA expression in blue light remains unstudied.

We showed that under the conditions of blue light, the expression of miR160a, miR167a, miR165a and miR833a-5p miRNAs in the *A. thaliana* pif4 mutant was significantly changed. We found that PIF4 plays a negative role in the PHYB signaling pathway and found that PIF4 integrates blue light signal transmission and miRNA biogenesis by regulating transcription and miRNA processing by acting on DCL1. In addition, we found that under blue light, the CRY1 photoreceptor is likely to interact with PIF4 and suppress its interaction with PHYB, which confirms the presence of coordination between the blue and red light signals transduction. At the same time, the light phytochrome signaling components COP1 and PIF4 regulate the stability of the HYL1. Therefore, light can affect miRNA levels by regulating light signaling components that modulate the amount of miRNA biogenesis and processing factors.

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**265 - Impact of photosynthesis and respiration on ATP levels in different subcellular compartments of *Physcomitrella patens*.**

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Life depends on the ability of photosynthetic organisms to exploit sunlight for carbon dioxide fixation into organic carbon molecules. In the chloroplast, ATP and NADPH are the final products of light-driven photosynthetic linear electron flow. At the same time though, alternative electron flow (AEF) pathways are suggested to modulate ATP:NADPH ratio to satisfy the demand of the Calvin–Benson cycle. In particular, cyclic electron flow (CEF) transfers electrons from photosystem I to the plastoquinone pool according to two mechanisms: one depends on proton gradient regulators (PGR5/PGRL1), while the other on type I NADH dehydrogenase (NDH) complex. Another pathway, called pseudocyclic electron flow (PCEF), uses electrons from photosystem I to reduce oxygen; in several groups of photosynthetic organisms excluding angiosperms, PCEF is sustained by flavodiiron proteins (FLVs). Interestingly, these three pathways are all active in the moss *Physcomitrella patens* and we have demonstrated that the mutant plants depleted in the corresponding proteins show damage to photosystem I and severe growth defects, meaning that they are functionally redundant and essential. The picture we obtain is that AEF pathways are not “regulatory” mechanism that helps responding to environmental dynamic changes but rather an essential component, indispensable for photosynthesis.

Now we are investigating their role in energetic metabolism by analyzing the ATP dynamics in *Physcomitrella patens* plants. The fluorescent protein biosensor ATeam1.03-nD/nA expressed in the cytoplasm, chloroplasts and mitochondria detected differences in ATP compartmentation in the protonema and the gametophore of *Physcomitrella patens*. The multiple subcellular localization is of interest, given that chloroplasts and mitochondria are two main organelles that participate in the supplying of ATP pools to support the metabolism of plant cells. In fact, employing a combination of photosynthetic mutants and respiration inhibitors with compartment specific ATeam biosensor, highlighted bioenergetic communication in *Physcomitrella patens* cells.

### 309 - Real-time monitoring of the dynamics of NADPH and NADH/NAD<sup>+</sup> ratio in *Arabidopsis thaliana* during photosynthesis

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The malate-OAA shuttles mediated by malate-OAA transporters and NAD(P)-dependent malate dehydrogenases (MDHs) in plastids, cytosol, peroxisomes and mitochondria enable indirect exchanges of reducing equivalents between these subcellular compartments. The MDHs often play a vital role during photosynthesis and photorespiration. It is well known that mitochondrion is the major organelle that produces adenosine triphosphate (ATP) through the mitochondrial electron transport chain (mETC). However, the sources of reducing equivalents for mETC during illumination remains unclear. In this study, we employed two circularly permuted fluorescence proteins, namely iNAP and SoNar, to monitor the dynamic changes of NADPH and NAD(H) statuses in planta. We found that illumination increased the NADPH level and NADH/NAD<sup>+</sup> ratio in the chloroplast stroma. A gradual increment of cytosolic NADH/NAD<sup>+</sup> ratio can also be detected upon 120 sec of illumination. To study the possible source of NADH in mitochondria, we investigated the effects of photosynthesis, photorespiration and mETC inhibitors on the flow of reducing equivalents. Surprisingly, when the photorespiration was inhibited by aminoacetronitrile, a photorespiratory inhibitor, the increases in plastid stroma NADPH level and NADH/NAD<sup>+</sup> ratio disappeared upon illumination, suggesting a significant amount of reducing equivalents was exported from the chloroplast through the malate-OAA shuttles when the production of NADH by glycine decarboxylase (GDC) in mitochondria was inhibited. These data imply that, under normal photorespiratory condition, the production of NADH by GDC in mitochondria may exceed the NADH-dissipating capacity in the mETC. As a consequence, the surplus NADH has to be dissipated by exporting malate from the mitochondria to the cytosol through the mitochondrial malate valve. Taken together, our findings suggest that photorespiration is the major source of reducing equivalents in mitochondria during photosynthesis.

### 346 - Mechanisms of photoprotection in photosynthesis of winter wheat

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Field-grown plants are exposed to highly variable sunlight intensity as a result of shifting cloud cover, seasonal changes, canopy shading, and other environmental factors. A number of different photoprotective approaches are employed by plants in environments where low temperatures occur on a seasonal basis. The goal of this study was to investigate photoprotective mechanisms in winter wheat by monitoring the changes in photophysical properties, chlorophyll content and pigment composition during the whole vegetative growth period in the field. We observed that winter wheat maintains its maximum photosystem II efficiency (Fv/Fm) during the winter period. Two very distinctive responses were observed in NPQ and electron transport between winter and spring seasons. Non-photochemical quenching (NPQ) capacity was higher in winter than in the spring and was accompanied by lower electron transport rate (ETR). Winter NPQ development exhibited much faster evolution even at low irradiance levels in comparison with spring NPQ. Xanthophyll cycle pigment pool size was increased in the winter period. The Chl a/b ratio increased in winter and declined in spring. A specific response accompanied long term frost conditions. We observed an accumulation of zeaxanthin and significant temporary lowering of Fv/Fm parameter in prolonged periods with temperatures below 0°C. We show that the xanthophyll cycle is operating in full extent even in temperatures approaching freezing. The results show that winter wheat maintains the function of photosynthetic apparatus throughout winter season without entering a phase of permanent quenching, which differs from other overwintering evergreen plants.

### 394 - OsPAP3 is a key regulator of chloroplast development in rice

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Transcription of chloroplast genes is a key step determining chloroplast development, and plastid-encoded RNA polymerase (PEP) is a major RNA polymerase governing the transcription of chloroplast genes. The activity of PEP largely relies on at least 12 PEP-associated proteins (PAPs) encoded in the nuclear genome of plant cells. A recent model proposed that these PAPs regulate the establishment of the PEP complex through broad PAP-PEP or PAP-PAP interactions. Previously we identified AtPAP3, which plays a pivotal role of chloroplast development. Expression of AtPAP3 is tightly regulated by light conditions, and knock-out mutants of AtPAP3 have severe defects in chloroplast development. Rice is an important staple crop supporting approximately two-third of the world's population, and rice productivity largely depends on photosynthetic activity and chloroplast development. In this study, we identified rice PAP3 (OsPAP3), which plays a crucial role in chloroplast development in rice. Expression analysis showed that transcript level of OsPAP3 is much higher in leaves with chloroplasts than in roots, and the expression is regulated by light conditions, similar to AtPAP3. Furthermore, CRISPR/Cas9-mediated mutation of OsPAP3 induced whitening phenotype, which is caused by defects of chloroplast development. Together with the result that OsPAP3 proteins specifically localize in chloroplasts, these findings suggest that OsPAP3 is a key regulator controlling chloroplast development in rice.

## **482 - How light influence the rearrangements of super- and megacomplexes in the non-appressed thylakoid membranes of maize mesophyll chloroplasts?**

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“State transitions” is a short-term mechanism of balancing energy distribution between photosystem I (PSI) and photosystem II (PSII). When PSII is preferentially excited (state 2), a phosphorylated pool of mobile LHCII antenna migrate from PSII to PSI. Plants are in state 1 when LHCII are dephosphorylated and migrate back to PSII. Analysis of the non-appressed thylakoid membranes of maize mesophyll chloroplasts after exposure of plants to changing light conditions revealed that light leads to a remodeling of the protein complexes composition in stroma thylakoids. We revealed for chloroplasts isolated from low and high light grown plants that PSI-LHCI-LHCII complex contain trimers with Lhcb1 and Lhcb2 proteins and also Lhcb4 protein but does not contain Lhcb3 protein. We identified three megacomplexes of PSI-LHCI-LHCII-PSII with different amount of PSII polypeptides (D1, Lhcb1, 2, 3, 4 and 5), indicating that megacomplexes had a different molecular mass according to the level of PSII and PSI proteins. It is of great interest that megacomplexes contained all antenna proteins of PSII in different composition. LHCII trimers are composed with Lhcb1, 2 and 3 proteins and also are the trimers containing additionally Lhcb4 protein. We found that far red light differently influenced remodeling of complexes for low and high light grown plants. Up to date the movement of LHCII has been understood as a mechanism for balancing light absorption between the photosystems. The behavior of the complexes that we have described does not seem to us to be well explained by this model. Rearrangement of super- and megacomplexes also seem to play a role for PSI in excitation quenching and in PSII turnover.

**573 - Impact of long-term high-intensity light on photosynthetic processes in *Solanum lycopersicum* hp photomorphogenetic mutants**

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In nature, the intensity of sunlight can exceed 2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . In this regard, a large number of mechanisms have been formed in plants that protect leaves from photoinhibition. One of the molecular mechanisms that protect plants from high-intensity light (HIL) is the DNA BINDING PROTEIN DAMAGE (DDB) reparasases, which prevent and repair DNA damage in the cell nucleus. In addition, DDB is involved in the transmission of light signals through the negative regulation of DET1 and COP1 proteins involved in the proteosomal degradation of transcription factors HY5, HYH, LAF1, HFR1, which provide a positive regulation of photomorphogenesis and are associated with the accumulation of various pigments such as carotenoids and flavonoids. Using photomorphogenic mutants hp of *S. lycopersicum* plants, we were able to show the role of deficiencies in DDB1 and DET1 proteins in the accumulation of leaf pigments and in the adaptation of the photosynthetic apparatus (PA) to long-term HIL. The increased ability of the hp-1,2 mutant to adapt to HIL is most likely associated with both a complex of morphological features (increased leaf thickness, high transpiration, and reduced plant height) and the ability to accumulate a large number pigments that serve as an optical filter and neutralize reactive oxygen species. An increased expression of genes of transcription factors HY5, HYH, LAF1 and HFR1 and enzymes of pigment biosynthesis (PAL1, CHS, ANS) was also found in the mutants. We assume that, along with an increased content of protective pigments, one of the explanations for the observed phenomena may be an increased expression of a number of light-depended genes, the regulatory properties of which may be broader than previously thought.

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**578 - Cryptochrome 1 is important for sustainability of photosynthetic apparatus of *A. thaliana* plants under high irradiance conditions**

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Photoreceptor cryptochrome 1 (CRY1) that mediates primarily blue light-induced inhibition of hypocotyl elongation and photoperiodic control of floral initiation, and regulates other light-regulated responses, including circadian rhythms, stomatal opening etc. Using the *A. thaliana* hy4 mutants with cry1 deficiency, grown under blue and red light we studied the relationship between stress-resistance of the plant photosynthetic apparatus to high intensity white light (HIL). HIL inhibited PSII photochemical activity and photosynthesis rate ( $P_n$ ) in plants grown both at low ( $30 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) and higher ( $130 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) intensity of blue and red light. However, decreasing the activity of photosystem 2 (PS2) and  $P_n$  in hy4 mutant grown at higher blue light intensity was higher than in WT. In red and low blue light plants no difference between hy4 and WT was found. In plants grown in blue light of higher intensity, the expression levels of genes of key transcription factors (HYH, HY5, FHY1, LAF1, FHY3, HFR1), enzymes of carotenoid and flavonoid biosynthesis (CHS, FLS1, PAL1, LDOX, PSY) and antioxidant enzymes (GR, GP, APX1) were enhanced after HIL irradiation than in plants of low blue light. We hypothesize that this difference in adaptation of plants to HIL depends on blue light intensity and it is likely linked to the carotenoids and UV-absorbing pigments content, which was significantly higher in the WT than in the hy4 mutant. A hypothesis has been put forward about the presence of a new property of cryptochrome 1 - its importance for maintaining PA stress resistance when growing plants in white light containing a sufficiently high intensity of blue light in its spectrum.

This work was supported by the RFBR grant No. 20-04-00512.

## 627 - State transitions - a side effect of redox signaling cascade caused by dark-chilling?

**Malgorzata Krysiak** <sup>(1)</sup> - **Anna Wegrzyn** <sup>(2)</sup> - **Jan Mazur** <sup>(1)</sup> - **Maciej Garstka** <sup>(1)</sup> - **Radoslaw Mazur** <sup>(1)</sup>

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Under constantly fluctuating environmental conditions, the thylakoid membrane protein network evolved the ability to dynamically respond to changing biotic and abiotic factors. In a temperate climate, low temperature is one of the abiotic stresses that heavily affect plant growth and productivity. One of the most important protective mechanisms is the rearrangement of the chlorophyll-protein (CP) complexes, induced by protein phosphorylation. The mechanism of this process under dark-chilling conditions is still unknown. That is why this study aimed to determine the role of light-harvesting complex II (LHCII) phosphorylation in the dark-chilling response. The study included an experimental model based on dark-chilling at 4 °C of detached chilling sensitive (CS) runner bean (*Phaseolus coccineus* L.) and chilling tolerant (CT) garden pea (*Pisum sativum* L.) leaves. This model is well described in the literature as used for the analysis of chilling impact without any additional effects caused by light. We examined changes in thylakoid membrane protein phosphorylation, interactions between phosphorylated LHCII and CP complexes, and their impact on the dynamics of photosystem II (PSII) under dark-chilling conditions. Our results showed that the dark-chilling treatment of CS bean leaves induced a substantial increase of phosphorylation of LHCII proteins, as well as changes in CP complexes composition and their interaction with phosphorylated LHCII. On the contrary, no significant changes in phosphorylation pattern, CP complexes interactions, and PSII photochemical efficiency were observed in CT pea. We propose that role of dark-chilling induced LHCII phosphorylation is not a protection of photosynthetic machinery from damage, but rather a side effect of the redox signaling cascade triggered by chilling stress, and this unnecessary activation of metabolic processes might be the foundation of chilling sensitivity.

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## 640 - The crucial roles of mitochondria in supporting C<sub>4</sub> photosynthesis

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C<sub>4</sub> photosynthesis involves a series of biochemical and anatomical traits that significantly improve plant productivity under conditions that reduce the efficiency of C<sub>3</sub> photosynthesis. With efforts being made to engineer the C<sub>4</sub> photosynthetic machinery into C<sub>3</sub> crops, there is an urgent need to understand the direct and indirect contributions mitochondria make to the functioning of C<sub>4</sub> photosynthesis. Such knowledge is also needed if we are to improve representation of leaf respiration of plants using the C<sub>4</sub> pathway in global models that predict rates of CO<sub>2</sub> exchange between vegetation and the atmosphere. In this poster, we explore how evolution of the three biochemical types of C<sub>4</sub> photosynthesis (NADP-ME, NAD-ME and PCK types) has affected the functions and properties of mitochondria. We then examine the extent to which these changes in properties have contributed to variation in rates of C<sub>4</sub> leaf respiration. Mitochondria in C<sub>4</sub> NAD-ME and PCK types play a direct role in decarboxylation of C<sub>4</sub> metabolites and/or production of ATP for C<sub>4</sub> photosynthesis. Such involvement has increased mitochondrial abundance/size and associated enzymatic capacity, and has altered mitochondrial location, ultrastructure and role in cellular carbon metabolism in the NAD-ME and PCK types. By contrast, these changes in mitochondrial properties are absent in the C<sub>4</sub> NADP-ME-type and C<sub>3</sub> leaves, where mitochondria play no direct role in driving photosynthesis. From an eco-physiological perspective, the available data – limited as it is – suggests that rates of leaf respiration in darkness vary considerably among species but does not differ systematically among the three C<sub>4</sub> types at a common measuring temperature. This poster outlines further mitochondrial research in key areas central to the engineering of C<sub>4</sub> pathways into C<sub>3</sub> plants and highlights the need for further work characterising phylogenetic variation, environmental impacts, and biogeographical patterns in rates of leaf respiration of C<sub>4</sub> plants.

## **677 - Influence of prolonged darkness and dark-chilling conditions on the phosphorylation status of LHCII proteins in *Arabidopsis thaliana***

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Reversible phosphorylation of photosystem II light-harvesting complexes (LHCII) is well established protective mechanism enabling efficient response to changing light conditions due to its regulation of LHCII affinity to photosystems. However, changes in LHCII phosphorylation were observed also in response to abiotic stress regardless of photoperiod. Regulation of this process under dark-chilling conditions remains unexplained. Consequently, this study aimed to investigate the response of *Arabidopsis thaliana* LHCII phosphorylation mutants, *stn7* and *tap38*, to dark-chilling. 6-week old *A. thaliana* plants were treated with dark-chilling or dark conditions in reference to regular photoperiod for 3-days and examined in selected time points. We identified the pattern of LHCII phosphorylation by application of electrophoresis followed by immunoblotting. Analysis of low temperature (77K) chlorophyll a fluorescence provided information about the organization of photosynthetic complexes. We also monitored the efficiency of photosynthetic apparatus *in vivo* by measuring Pulse Amplitude Modulated chlorophyll a fluorescence. Our results showed that changes in the maximal quantum yield of PSII (Fv/Fm) during dark-chilling were similar in all examined lines, however, altered kinetics of nonphotochemical quenching parameter was observed in the *stn7* line. LHCII phosphorylation level was constantly increasing from 24 h of dark treatment in all lines, excluding *stn7*, which correlated with a decrease of Fv/Fm. Moreover, *stn7* mutant with inactive LHCII phosphorylation process showed significantly lower Fv/Fm values measured after a long dark period compared with other lines. Collected data created a view of the relation between LHCII phosphorylation status and dark-chilling response, enabled identification of key time points of *A. thaliana* response to dark-chilling, and shed new light on the influence of prolonged darkness on LHCII phosphorylation status.

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## TOPIC:

### Phenotyping plant performance under abiotic stress

#### **Keynote Lecture**

**Plant performance under abiotic stress: combining multi scale phenotyping, modelling and genomic prediction**

**Francois Tardieu**

The plant science community takes for granted that the triplet mechanism (QTL)-phenomics-genomics is the best, perhaps the only way towards efficient breeding in a changing climate. It is healthy to recognise that, for good reasons, most breeding companies have essentially abandoned this view in favour of genomic prediction approaches. Why invest in costly phenotyping platforms that do not represent the field, are more heterogeneous than any field and do not improve heritability? Field phenotyping is now fashionable but why invest in vectors and sensors whose outputs are well correlated to yield, whereas it is simpler and more accurate to measure yield itself and to predict it for hundreds of genotypes via big data approaches? I firmly believe that phenomics keeps its interest in this context, but with renewed objectives and techniques that makes it complementary to big data. I shall present elements to back this statement, and discuss consequences in terms of modelling the genotype x environment interaction ('where and when every combination of alleles?', 'what happens if?').

## TOPIC:

### Phenotyping plant performance under abiotic stress

#### Oral Communications

##### **27 - Redundancy in tomato gibberellin receptors contributes to phenotypic stability under changing environments**

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Gibberellin (GA) promotes growth and reduces tolerance to drought. Inhibition of GA activity reduces transpiration and increases tolerance to water-deficit stress. Although the effects of GA on transpiration is not linked to growth, reduced GA affects both, limiting the use of GA deficiency to develop drought-tolerant crops. Tomato has three GID1 GA-receptors; GID1a, GID1b1, and GID1b2. To test if mutation in a single receptor reduces transpiration without a significant effect on growth, we generated CRISPR-Cas9 derived *gid1* single, double and triple mutants and studied their contributions to GA-regulated growth and transpiration. The *gid1* triple mutant was extremely dwarf and fully insensitive to GA. Under optimal growth conditions, the three receptors function redundantly in the regulation of germination, growth and gene expression. Among the three receptors, GID1a had the strongest effects on these processes. Yeast two-hybrid assays suggested that GID1a has the highest affinity to the tomato DELLA protein PROCERA. Under controlled growth-conditions the *gid1* mutants showed reduced transpiration and increased tolerance to drought stress, without a significant effect on growth. However, when these mutants were grown in the field under ambient changing environments, they showed phenotypic instability, the high redundancy was lost and *gid1a* and its double mutants exhibited dwarfism. These results suggest that redundancy in GA sensing contributes to phenotypic stability under environmental extremes.

**100 - Arabidopsis ARGONAUTE 1, ARGONAUTE 3, and ARGONAUTE 4 regulate gene expression and hypoxia tolerance.**

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Hypoxia exerts major effects on gene expression, which is reconfigured to meet the needs of the stressed plant. However, still little is known about the possible role of RNA-signaling in the regulation of gene expression under hypoxia. Here, we utilized an ARGONAUTE1 (AGO1) mutant (ago1-27) defective in post-transcriptional gene silencing to evaluate the contribution of AGO1 to plant tolerance to submergence and its effects on gene expression. We found that ago1-27 plants are less tolerant to submergence than the wild-type and transcriptome analysis allowed us to identify genes whose regulation requires a functional AGO1 protein. We found that miR2936 and miR398c were regulated by submergence, although without effects on their relative target mRNA levels. Further analysis of mutants affected in various branches of RNA signaling highlighted the convergence of AGO1 signaling with the AGO4-dependent RNA-directed DNA methylation (RdDM) pathway. AGO4-dependent RdDM represses the expression of HOMOLOG OF RPW8 4 (HR4) and alters its response to submergence. Remarkably, methylation of the second exon of HR4 is not only reduced in ago4-1 but also in plants overexpressing a constitutively stable version of the oxygen sensor RELATED TO AP2 12 (RAP2.12), indicating convergence of oxygen signaling with epigenetic regulation of gene expression. Interestingly, we observed that among the AGO genes only AGO3 was upregulated by submergence. The expression of AGO3 was already high in 35S::RAP2.12 while its induction by submergence was abolished in the erfvi mutant, indicating that AGO3 response to submergence is likely regulated by RAP2.12 stabilization under hypoxia. Moreover, the ago3-3 mutant was found to be less tolerant to submergence than wild-type plants suggesting that the AGO3 protein can play a role in the response of plants to hypoxia. Therefore, our results suggest a role for AGO1, AGO3 and AGO4 RNA-silencing pathways in low-oxygen signaling in Arabidopsis.

## **189 - A specific intra-species modulation of redox balancing systems is crucial for the tolerance of Baldo rice cultivar against salt stress**

**Sara Cimini**<sup>(1)</sup> - **Valentina Giacinti**<sup>(1)</sup> - **Vittoria Locato**<sup>(1)</sup> - **Laura De Gara**<sup>(1)</sup>

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Reactive Oxygen Species (ROS) over-production and redox-homeostasis unbalance can arise as a consequence of plant exposure to adverse environmental conditions. Despite their harmful nature, ROS also acts as signaling molecules involved in response to stress stimuli. A complex and differentiated redox network modulated by several adverse stimuli is responsible for the generation of ROS signature that is pivotal for the activation of homeostatic responses and for guaranteeing plant fitness in resistant species or varieties.

Nowadays, adverse climatic factors, such as drought, waves of temperatures far from the optimal ones and soil salinization drastically affects crop growth and productivity worldwide. Rice is one of the most sensitive cereals to abiotic stresses, salinity first. Considering the central role of rice in human nutrition, the comprehension of its resistance mechanisms represents a crucial area of plant science.

In order to increase the knowledge regarding the signaling pathways triggering defence responses against salt stress, two rice varieties showing contrasting salt sensitivity, Baldo the tolerant variety and Vialone Nano the sensitive one, have been investigated. Analysis of key metabolites and related genes/enzymes have been performed on the two varieties subjected to salt stresses of different intensity. An in-depth study centered on ascorbate and glutathione metabolism, cellular redox state and markers of cell viability and death has been carried out over treatment time.

These results wish to describe a specific ROS signatures and modulation of antioxidative pathways as a part of a complex redox network differentially activated in the tolerant rice cultivar after salt stress exposure. This knowledge advance will contribute to draw effective strategies aimed at improving rice resilience toward salt stress.



#### **443 - The characterisation of ion transport in stalk cells reveals their role in salt sequestration in quinoa epidermal bladder cells**

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Halophytes are an exciting group of plants that shows an elevated tolerance, thriving with salt concentrations in the root-zone damaging for most other angiosperms. A large fraction of halophytes form characteristic epidermal bladder cells that play a key role in their salt tolerance by acting as an external salt dump. A crucial element in the transport of salt between the epidermal and bladder cell is the stalk cells, which act as a selectivity filter and flux controller at the same time. Here, by voltage recording and ion selective electrodes, we characterised ion fluxes ( $\text{Cl}^-$ ,  $\text{K}^+$  and  $\text{Na}^+$ ) and membrane potentials in isolated stalk cells from leaves grown under saline or non-saline conditions. The results from non-saline plants reveal that stalk cells constitutively transport large amounts of  $\text{Cl}^-$  and  $\text{K}^+$ , and to a smaller extent  $\text{Na}^+$ . Nevertheless, the addition of 200 mM NaCl in the root-zone led to a plasma membrane depolarization by 40 mV which was concomitant to an increase in  $\text{Na}^+$ ,  $\text{Cl}^-$  and even  $\text{K}^+$  efflux. Subsequent comparative transcriptomics revealed the key molecular players involved in  $\text{K}^+$  and  $\text{Cl}^-$  transport from the epidermal and the bladder cells, via stalk cells.

#### **481 - Functional characterization of drought-responsive RING E3-Ubiquitin ligases in rice**

**Fredilson Melo** <sup>(1)</sup> - **Margarida Oliveira** <sup>(1)</sup> - **Nelson Saibo** <sup>(1)</sup> - **Tiago Lourenço** <sup>(1)</sup>

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**(1)**

Rice (*Oryza sativa*) is the staple food for more than half of the world population and its production is highly affected by various abiotic stresses, such as drought and high salinity. To cope with adverse environmental conditions, plants have evolved several molecular and physiological mechanisms. Among those, the post-translational ubiquitination/proteasome system (UPS) has emerged as an important mechanism underpinning stress response. Within the UPS, the E3-ubiquitin ligases are the most abundant and studied component of the system. In order to functionally characterize E3-ubiquitin ligases in response to abiotic stress in rice, we conducted an in silico study and selected 16 Really Interesting New Gene (RING) E3-ubiquitin ligases that showed gene expression changes in response to several abiotic stresses. We validated these results using in-house stress assays and selected two genes, OsRING-1 and OsDIRP1 (*Oryza sativa* Drought-Induced RING Protein 1), which showed to be highly induced by drought. To functionally characterize their role, we have developed loss-of-function knockout CRISPR mutants and overexpression transgenic rice lines that are currently being evaluated for stress tolerance. Furthermore, we performed sub-cellular localization using transient expression assays in *Nicotiana benthamiana* leaves. Our results showed that OsDIRP1 localizes to the nucleus and OsRING-1 localizes to a cell membrane. Using Yeast Two-hybrid screening and bimolecular fluorescence complementation assays we were able to show that OsDIRP1 interacts with a heat-shock protein in the nucleus, while OsRING-1 interacts with an uncharacterized protein in both the nucleus and elsewhere in the cell. A further understanding of the molecular and physiological mechanisms underlying the function of OsRING-1 and OsDIRP1 in the response to abiotic stress will provide us with important insights into the roles of E3 ligases in plants stress response network and the prospect of development of abiotic stress-tolerant crops.

## **542 - Redundancy in Tomato Gibberellin Receptors Contributes to Phenotypic Stability Under Changing Environments**

**Natanelia Illouz-Eliaz**

Gibberellin (GA) promotes growth and reduces tolerance to drought. Inhibition of GA activity reduces transpiration and increases tolerance to water-deficit stress. Tomato has three GID1 GA-receptors; GID1a, GID1b1, and GID1b2. To test if mutation in a single receptor reduces transpiration without a significant effect on growth, we generated CRISPR-Cas9 derived *gid1* single, double and triple mutants and studied their contributions to GA-regulated growth and transpiration. The *gid1* triple mutant was extremely dwarf and fully insensitive to GA. Under optimal growth conditions, the three receptors function redundantly in the regulation of germination, growth and gene expression. Among the three receptors, GID1a had the most prominent role in the regulation of these processes. Yeast two-hybrid assays suggested that GID1a has the highest affinity to the tomato DELLA protein PROCERA. Under controlled growth-conditions *gid1a* mutants showed reduced transpiration and increased tolerance to drought stress, without a significant effect on growth. We further examined how the *gid1* mutants perform in the field. Under these conditions, the high redundancy of GID1s was lost and *gid1a* plants were semi-dwarf and showed a high degree of phenotypic instability. When exposed to drought conditions in the field, *gid1a* performed similar to the wild type M82. These results suggest that redundancy in GA sensing contributes to phenotypic robustness under environmental extremes and demonstrate the importance of field experiments to stress-tolerance research.

## TOPIC:

### Phenotyping plant performance under abiotic stress

#### **Extended Elevator Pitches**

##### **168 - RootForce – Automated segmentation algorithm for root growth analysis**

**Stefan Gerth** <sup>(1)</sup> - Joelle Claußen <sup>(1)</sup> - Norbert Woerlein <sup>(1)</sup> - Michael Waininger <sup>(1)</sup> - Norman Uhlmann <sup>(1)</sup>

**Fraunhofer IIS, Development Center X-ray Technology, Fuerth, Germany** <sup>(1)</sup>

X-ray computed tomography (CT) is increasingly applied for the non-destructive visualization of belowground structures in pots. With X-ray CT the 3D volume information of objects can be visualized using X-ray projections of the object from different points of view. Due to the non-destructive nature of CT it is possible to track the growth of below ground structures. However, to do so robust segmentation algorithms for virtual root excavation is needed. This demand is increasing because current generations of conveyor integrated CT systems are able to generate 3D volume data with a throughput below 5 Minutes per pot. However, the data analysis pipeline remains complex and in many times no automation is done in the post-processing of the data.

Within the presentation, I will show results of the RootForce algorithm applied to different plants (e.g. Corn, Wheat, Potato, Cassava). This is especially of interest, to demonstrate the versatility of the algorithm in terms of multiscale extraction (from thin Wheat roots over Maize roots to Tubers and Storage roots). Additionally, a time resolved experiment is shown. In this experiment the parameter of the RootForce algorithm are kept constant to simulate an autonomous high throughput data analysis pipeline. However, each of the scans inhibits changes in soil moisture or compactness resulting in a complex task for the segmentation algorithm. As ground truth the result of the algorithm is compared to WinRhizo data, and manual segmented data. After this, I will show how to integrate the RootForce algorithm in an automated data analysis pipeline for a complete autonomous trait extraction of root traits.

## **284 - Screening for drought stress tolerance of maize genotypes using seedling traits, chlorophyll fluorescence and photosynthesis parameters**

**Abdelfattah badr** <sup>(1)</sup>

**Helwan University, Faculty of Science, Botany and Microbiology, Cairo, Egypt** <sup>(1)</sup>

Maize is known to be susceptible to drought stress which negatively affects growth and biomass production as well as the formation of reproductive organs and yield parameters. Drought stress tolerance of 40 maize genotypes was screened using 27 germination and seedling traits under simulated drought of three PEG concentrations. The heritability ( $H^2$ ) and correlations of the traits were estimated and drought tolerance indices (DTIs) were calculated for traits and accessions. The used approach is a cost-effective and time-consuming method for identifying tolerant and sensitive maize genotypes. However, resemblance in DTIs value for accessions does not clearly reflect their origin or taxonomic assignments to subspecies and varieties. Comparative analysis of drought stress response of ten maize genotypes was evaluated using chlorophyll fluorescence measurements and leaf relative water content. The initial photochemical quantum efficiency of photosystem II ( $F_v/F_m$ ) and performance index (PI), describing the ability of the photosynthetic apparatus to collect light energy, have been used to screen tolerance to drought stress by ten maize accessions, monitored by leaf relative water content (RWC) and soil water content (SWC). The analysis of chlorophyll a fluorescence induction rise from the basic dark-adapted fluorescence yield to the maximum (OJIP transient) distinguished the most tolerant and the least tolerant genotypes. Twenty genotypes were exposed to drought for 17 days pre-anthesis and three parameters of photosynthesis as well as chlorophyll content were measured. The measured traits are  $\Phi_2$  (the fraction of light energy captured by the photosystem I1 to make ATP and NADPH),  $\Phi_{INPQ}$  (the fraction of light energy captured by the photosystem I1 for non-photochemical quenching and is dissipated as heat inside the leaf) and the  $\Phi_{INO}$  (the fraction of light energy captured by the photosystem I1 to unregulated processes). The value of  $\Phi_2$  decreased and the value of  $\Phi_{INPQ}$  and  $\Phi_{INO}$  generally increased as drought increased in correlation with wilting symptoms and growth retardation and yield reduction.

#### **406 - Impact of experimental soil moisture manipulation on tropical tree seedling demographic fates and functional traits**

**Roel lammerant** <sup>(1)</sup> - **David Matlaga** <sup>(2)</sup> - **J.Aaron Hogan** <sup>(3)</sup> - **Jess Zimmerman** <sup>(4)</sup> - **Robert Muscarella** <sup>(5)</sup>

**Uppsala University, Department of Ecology and Genetics, Uppsala, Sweden** <sup>(1)</sup> - **Susquehanna University, Department of Biology, Sellingsgrove, United States** <sup>(2)</sup> - **Florida International University, Department of Biological Sciences, Miami, United States** <sup>(3)</sup> - **Universidad de Puerto Rico-Río Piedras, Department of Environmental Science, San Juan, Puerto Rico** <sup>(4)</sup> - **Uppsala University, Department of Ecology & Genetics, Uppsala, Sweden** <sup>(5)</sup>

Climate change is predicted to increase the frequency and intensity of drought in tropical regions. The extent to which these changes will result in compositional shifts of tropical tree communities is unknown due to a limited understanding about the responses of tree species to soil moisture deficit. The degree to which drought impacts demographic rates may be mediated by species functional strategies. We conducted a seedling drought experiment in the Luquillo Experimental Forest, Puerto Rico, where we exposed seedlings of eight tree species, representing different successional stages, to a gradient of soil moisture. We evaluated (1) relationships between species-mean functional trait values and growth and survival responses to drought, (2) relationships between intraspecific functional trait variation and soil moisture, and (3) the extent to which demographic responses to short-term experimental drought mirrored demographic responses to natural variation in soil moisture using long-term seedling plot data from a nearby forest dynamics plot. Species with more 'conservative' resource strategies (e.g., low specific leaf area and high wood density) had higher survival and tended to be more drought tolerant with respect to growth and survival compared to species with more 'acquisitive' strategies. Within species, traits varied with respect to soil moisture, suggesting a role for phenotypic plasticity in response to drought. Species demographic responses to soil moisture in experimental and long-term studies were weakly positively correlated but more variables are at play under natural conditions, which partly decouples these responses. Overall, our results suggest that tree species with more 'conservative' functional strategies may become selected under increasing drought frequency and intensity in the rain forests of Puerto Rico. However, understanding the broader implications of our finding will require considering the effects of other disturbances, including hurricanes, which may have contrasting effects.

#### **424 - ARSENITE PROVIDES A SELECTIVE SIGNAL THAT COORDINATES ARSENATE UPTAKE AND DETOXIFICATION IN ARABIDOPSIS**

**Cristian Mateo** <sup>(1)</sup> - **Cristina Navarro** <sup>(1)</sup> - **Mohan Thotegowdanapalya** <sup>(2)</sup> - **José M. García-Mina** <sup>(3)</sup> - **Javier Paz-Ares** <sup>(1)</sup> - **Gabriel Castrillo** <sup>(4)</sup> - **Antonio Leyva** <sup>(1)</sup>

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In nature, plants need to acquire nutrients from soils to sustain growth, and at the same time they need to avoid uptake and/or be endowed with tolerance systems to cope with toxic compounds. This is particularly challenging when the toxic compound and the nutrient are chemically similar, as it is the case of phosphate and arsenate. Here we demonstrate that regulatory elements of the phosphate starvation response (PSR) coordinate the arsenate detoxification machinery in the cell. We show that arsenate repression of the phosphate transporter PHT1;1 is linked with the degradation of the PSR master regulator PHR1. Once arsenic is sequestered into the vacuole, PHR1 stability is restored, and PHT1;1 expression is recovered. We also identified an arsenite responsive SKP1-like protein and a PHR1 interactor F-box (PHIF1) as constituents of the SCF complex responsible for PHR1 degradation. Furthermore, we found that arsenite, the form to which arsenate is reduced towards compartmentalization in vacuoles, represses PHT1;1 expression, providing a highly selective signal versus phosphate to control PHT1;1 expression in response to arsenate. Our results provide the molecular insights into a sensing mechanism that regulates arsenate/phosphate uptake depending on the plants' detoxification capacity.

#### 439 - Identification of downstream targets of MAPKKK17/18 cascade in Arabidopsis

sivakumar Krishnamoorthy <sup>(1)</sup> - Michał Szczęśniak <sup>(2)</sup> - Agata Cieśla <sup>(1)</sup> - Agnieszka Ludwików <sup>(1)</sup>

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In Arabidopsis, the current knowledge about the molecular mechanisms underlying Absciscic acid pathway (ABA) perception and signal transduction is limited. In ABA Core Signaling Pathway, Group A protein phosphatase type 2C (PP2C) ABI1 acts as a negative regulators of the Map kinases MAPKKK17/18 through MKK3, which in turn activates MPK 1/2/7/14. Furthermore, the transcription factors involving downstream cascade are unknown. This study focuses on contribution of dynamics events in response to ABA treatment. In total, we isolated 24 mRNA samples from four genotypes WT, abi1td, mkkk17-1, mkkk18-1 and performed paired end sequencing by using illumina platform to produce 100 M reads per sample. On an average, 95% of the reads were uniquely mapped against ab initio transcriptome with the phred score above 33. The reads were preprocessed using various bioinformatics algorithms, 23000 genes containing 52544 transcripts were obtained after adapter trimming. In addition, gene quantification, differential expression analysis (DE) were performed using Salmon and DESeq2 respectively. The total number of genes that undergoes DE when compared to WT Col-0 is 20,015 in abi1td, 19,147 in mkkk17-1, and 20,403 in mkkk18-1 (8,475). Among those, number of significant genes based on FDR value ( $>0.05$ ) are 2,350 in abi1td, 743 in mkkk17-1 and 936 in mkkk18-1. Additionally, we bifurcated the putative genes that are involved in either induction or repression in response to exogenous ABA treatment based on fold change (1.5). Among 119 identified Transcription factors (TF) expressed in mkkk17 and mkkk18 mutant lines we selected several targets that may work downstream MAP cascade pathway. Some of the common TF identified in both in both mkkk mutants belongs to basic helix-loop-helix and MYB family of TFs. Here we presents current results on regulatory networks involved ABA signalling via MAPKKK17/18 pathway.



#### **447 - REGULATORY INTERPLAY BETWEEN ROOT DEVELOPMENTAL PROGRAMS AND THE ARSENIC RESPONSE IN ARABIDOPSIS**

**Micaela Andrea Navarro Correa** <sup>(1)</sup> - **Jose L. Jose L. Pruneda-Paz** <sup>(2)</sup> - **Yogev Burko** <sup>(3)</sup> - **Jose Manuel Franco** <sup>(1)</sup> - **Oscar Urrutia** <sup>(4)</sup> - **Javier Paz-Ares** <sup>(1)</sup> - **Jose M. Garcia Mina** <sup>(4)</sup> - **Joanne Chory** <sup>(3)</sup> - **Antonio Leyva** <sup>(1)</sup>

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Arsenic is a metalloid classified as one of the main carcinogenic compounds present in the biosphere. It is widely distributed in nature, particularly in soils and waters facilitating the entry of the metalloid into the food chain, which threatens the health of millions of people. As sessile organisms, plants have developed efficient strategies to survive in the presence of arsenic, mainly, the accumulation into the vacuole or its extrusion to the soil. In fact, some plants have an extraordinary ability to extract and accumulate high amounts of arsenic. In order to clean up arsenic from the environment, we aim to characterize the signal transduction pathway involved in plant arsenic perception and accumulation. For this aim, we have performed a high-throughput yeast one-hybrid screening to identify transcription factors binding two arsenic inducible promoters. In addition, we have conducted an in-silico enrichment analysis of transcription factor binding sites in clusters of genes differentially expressed under arsenic stress. Our studies have allowed us to identify several mutants of transcriptional regulators that exhibit arsenic-sensitive/tolerant phenotypes compared to that observed in the wild-type strain. Here, we have found that GLABRA2 and PLETHORA5, the classical regulators involved in root hair and lateral root development, respectively, are also responsible for the regulation of the arsenic response. The most recent advances in our understanding of the interplay of root developmental programs with arsenic detoxification mechanisms will be presented.

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#### **470 - A bHLH transcription factor gene modulating Absciscic Acid signaling enhances drought tolerance, photosynthetic efficiency and crop yields**

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The major staple food cereal and legume crops are severely affected by drought stress causing substantial loss in yield and productivity across the globe. Identifying potential molecular signatures conferring drought tolerance is essential for achieving higher yield and productivity of crops under water scarce conditions. Several efforts have led to identification of multiple quantitative trait loci (QTLs)/genes governing drought tolerance traits. However none of these identified major effect QTLs and potential genes governing the said target traits have been fine-mapped or utilized in marker-aided genetic crop improvement for development of high-yielding drought tolerant crops. To accomplish this, a near about homozygous introgression lines (ILs)-derived diversity association panel and as well as recombinant inbred lines (RILs)/NILs-based mapping population of chickpea were developed utilizing multiple elite breeding cultivars and wild chickpea accession as parents. Large-scale replicated multi-environment field phenotyping for drought tolerance and yield related traits under irrigated (unstressed) vis-à-vis un-irrigated (drought stress) conditions across three individual years and overall years exhibited wide phenotypic diversity and high heritability. Through an integrated genomics-assisted breeding (GAB) and functional genomics strategy involving association mapping, QTL/fine-mapping, map-based cloning, molecular haplotyping and transcript profiling we delineated natural alleles and superior haplotypes of a CabHLH10 transcription factor gene regulating drought tolerance component traits contributing for higher yield in chickpea. We showed that CabHLH10 modulates expression of a known drought-responsive gene harbouring a trans-eQTL (expression QTL) and two strong yield-enhancement photosynthetic efficiency (PE)-associated genes potentially as a transcriptional activator. A superior haplotype of this gene introgressed in NILs through marker (haplotype)-assisted selection not only confer drought tolerance but improved root and shoot biomass including PE and thereby enhanced yield and productivity during drought without compromising agronomic performance. The essential information generated can drive translational genomics for crop improvement and development of genetically-tailored, climate-resilient high-yielding cultivars of chickpea.

## 514 - Influence of salinity and different light regimes on growth and metabolite profiles of halophytes

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Halophytes occur in many different plant families and can survive and reproduce in saline environments. To cope with salt stress, there are three different salt tolerance mechanisms in halophytes, the salt excluding, the salt excreting and the salt accumulating mechanism, which are reflected in different salt tolerance levels from low to high. In this study, five different halophytes (*Cochlearia officinalis*, *Salicornia europaea*, *Chenopodium quinoa*, *Atriplex hortensis*, *Brassica oleracea* var. *palmifolia*), with different salt tolerance mechanisms were investigated. The plants were grown in a phytochamber (Ph) and a greenhouse (Gr) under different light conditions and, three different salt concentrations (NaCl) from 50 mM to 600 mM. The two light regimes differed in their light intensities (Ph 350  $\mu\text{mol s}^{-1} \text{m}^{-2}$ ; Gr 90  $\mu\text{mol s}^{-1} \text{m}^{-2}$ ), light qualities (artificial/natural) and the photoperiod (14 h light/10 h dark). The results showed reduced growth and biomass especially for quinoa (*Chenopodium quinoa*) and the garden orache (*Atriplex hortensis*) for greenhouse grown plants. Changes in carotenoid and chlorophyll contents were found to be dependent on the salt concentration and varied among light conditions and plant species. However, an increasing trend in carotenoid accumulation with increasing salt concentration was observed in the greenhouse, while a decrease was observed in the phytochamber. The content of lutein in quinoa leaves was increased by 121% (200 mM NaCl) in the greenhouse compared to the phytochamber. The stress level was determined by the analysis of changes in plant hormone concentrations and salt accumulation in the leaves by ion chromatography. The results indicate that the different light regimes have an effect on the response of plants to salinity. Plant species with the same salt tolerance mechanism show the similar response. However, it is not clear whether the light quality or quantity or a combination of these causes these effects.

## 517 - Molecular analysis of abiotic stress response in different Capsicum genotypes

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Abiotic stress can negatively influence plant growth, development and yield. Also, abiotic stresses instigate plants to synthesize various protective compounds as a defense mechanism. Some of these protective compounds, like flavonoids, have properties which are health supporting and relevant for industrial use as e.g. pharmaceuticals or as biocides. From an agricultural and ecological point of view, a deeper understanding of the plant stress response on the molecular level (transcriptomics and metabolomics) is needed to optimize the utilization of crops.

Pepper (*Capsicum annum* L.) is cultivated all over the world. To investigate the effect of different abiotic stresses on a cultivated bell pepper (Mazurka) and a chili (CAP1035), young plants were exposed to low temperature (18°C/12°C), to 200mM salt, a combination thereof, and compared to control plants. Leaf samples were harvested after 1, 7 and 14 days and analyzed regarding their metabolome profiles via LC-MS, and transcriptome profiles by using high-throughput sequencing. Different multivariate statistic approaches were carried out to analyze the effects of different treatments.

Metabolome profiles, for both Mazurka and CAP1035, differ significantly between treatments. Additionally, these differences increased clearly with time. Furthermore, some compounds were only affected by specific treatment, in other words, a combination of salt and cold stresses influenced some compounds that were not influenced by only one of them. The transcriptome profiles of all treatments after 14 days were drastically different from the control group. However, the effects of combination treatment were clearly obtained after 7 days. More precisely, low temperature influenced the synthesis of secondary metabolism significantly. Further, the applied salt stress affected the organization of cell cycle and protein modifications. To summarize, pepper responded differently to salt and temperature stress and the combination of these stresses instigated a unique response.

## **519 - Identification of novel miRNAs target genes in the response of abscisic acid in Arabidopsis**

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miRNAs identification at the global genome-level by high-throughput sequencing is essential to functionally characterize miRNAs in plants during different environment. Small non-coding RNAs such as miRNAs function in post-transcriptional regulation of gene expression and these play very important roles in plant development. The phytohormone abscisic acid (ABA) is involved in a plant's response to environmental stresses and plays crucial roles in regulating stomatal movement, seed germination, vegetative growth, and development. To understand the role of miRNAs in plants response to ABA, ABA-responsive miRNAs were identified by high-throughput sequencing in Arabidopsis Wild-type after ABA induction for four hours. After further analysis, in WT Col-0 among 117 known and novel miRNAs, there were 33 miRNAs up-regulated and 58 miRNAs were down-regulated after ABA induction as compared to mock. Potential up-regulated and down-regulated target genes of known and novel miRNAs were identified through psRNATarget Database. The predicted targets of novel miRNAs, were further validated through 5' RLM-RACE. Through GeneOntology analyses, the predicted potential target genes of the ABA-responsive known and novel miRNAs were found to be involved in diverse cellular processes in plants, including development, transcription, phosphorylation, stomatal movement and nutrient status. These outcomes suggest that many of these identified miRNAs might have crucial roles in plant responses to environmental stresses as well as in development of plants and identified miRNAs in these genotypes might have also common regulatory roles in core ABA signaling pathway.

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**525 - The fundamental role of plant mitochondria in stress responses is linked to the functionality of the mitochondrial nucleoid binding protein WHIRLY2.**

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**SIBV, University of Padova, Padova, Italy** <sup>(1)</sup>

In the last few years, the importance of organelles as central coordinators of plant responses to internal/external stimuli has become increasingly important. Mitochondria play a role as stress sensors of environmental stimuli, being a component of a complex communication network involving different organelles and the nucleus. WHIRLYs are plant specific proteins that have been characterized as ssDNA-binding proteins because of a characteristic conserved DNA-binding domain. In Arabidopsis, the WHIRLY family includes three members: WHIRLY1, WHIRLY2 and WHIRLY3, presenting specific target sequences that localize them in the plastids and nucleus (WHIRLY1, WHIRLY3) or the mitochondria (WHIRLY2). WHY2 among the proteins involved in mtDNA repair is the most abundant, evidences suggest an important role of WHY2 in mitochondrial genome replication and that permit a complete mtDNA activity. Recent results on WHY2 show a link between mtDNA stability and proper mitochondrial morphology, kinetics and functionality, indicating a fundamental role in mitochondrial activity during different conditions, such as development and stress responses. Failure in maintaining the mitochondrial genome stability results in the accumulation of mutations and genomic rearrangements that can become deleterious. In the present work data will be reported showing the connection between mtDNA maintenance, high levels of WHY2 and the response to abiotic stress. Recent experimental evidences show that WHY2 plays a role in the response to different abiotic stresses. The results also suggest an involvement of WHY2 protein in retrograde signaling in response to abiotic stresses. The study of mitochondrial proteins is opening up to a new conception of the role of mitochondria as a stress response center and the molecular mechanisms underlying the communication between mitochondria and nucleus in response to stress.

## **526 - In vivo imaging and quantification of carbon tracer dynamics in nodulated root systems of pea plants**

**Ralf Metzner**<sup>(1)</sup> - Antonia Chlubek<sup>(1)</sup> - Jonas Bühler<sup>(1)</sup> - Daniel Pflugfelder<sup>(1)</sup> - Ulrich Schurr<sup>(1)</sup> - Gregor Huber<sup>(1)</sup> - Robert Koller<sup>(1)</sup> - Siegfried Jahnke<sup>(1)</sup>

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Legumes associate with root colonizing rhizobia that provide atmospheric nitrogen to its plant host in exchange for recently fixed carbon. While much of the nodulation process and its regulation is now understood, there is a lack in understanding how plants modulate carbon allocation to a nodulated root system as a dynamic response to abiotic stimuli. One reason is that most approaches are based on destructive sampling, making investigation of localized carbon allocation dynamics in the root system difficult. We employed non-invasive Positron Emission Tomography (PET) to follow the allocation of leaf-supplied <sup>11</sup>C tracer towards individual nodules in a three-dimensional (3D) root system of pea (*Pisum sativum*). Nitrate was applied to the root system to rapidly shut down biological nitrogen fixation and follow the effect on carbon allocation dynamics. This treatment lead to a reduction of <sup>11</sup>C tracer allocation to nodules by 40% - 47% in 5 treated plants within 42h while the change in control plants was less than 11%. Our study demonstrates the strength of using <sup>11</sup>C tracers in a PET approach for non-invasive quantification of dynamic carbon allocation in growing plants over several days. A major advantage of the approach is the possibility to investigate carbon dynamics in small regions of interest in a 3D system such as nodules in comparison to whole plant development.

## 540 - Does glycation of plant proteins impact on ageing and response to environmental stress?

**Andrej Frolov** <sup>(1)</sup>

**Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Leibniz Institute of Plant Biochemistry** <sup>(1)</sup>

Protein glycation is usually defined as an array of non-enzymatic post-translational modifications formed by interaction of their amino and guanidino groups with carbonyl compounds - reducing sugars and  $\alpha$ -dicarbonyl products of their degradation. Resulting advanced glycation end products (AGEs) represent a diverse group of protein adducts, which are formed during thermal processing of foods and are clearly pro-inflammatory and atherogenic in mammals. Their formation accompanies aging and pathogenesis of life-style and age-related diseases, like atherosclerosis, diabetes mellitus, Alzheimer's disease. Although the role of protein glycation in human physiology is known for decades, only recently it was reported for plants. We could show, that the constitutive levels of AGE formation in plants are essentially higher than in animal blood and tissues - more than 1000 glycation sites were found in Arabidopsis and rapeseed proteins. Thereby, in comparison to mammals, plant glycation are featured with different mechanisms, patterns and intermediates. Thus, in contrast to mammals, glycation products dominate by arginine-derived AGEs, mostly originating from glyoxal. Moreover, environmental stress (e.g. short-term drought) resulted in enhanced formation of such AGEs in specific proteins, mostly enzymes and transcription factors. Such specificity of glycation was even more characteristic for ontogenetic changes and ageing. Thus, we have shown that the levels of glycation at specific protein sites - so-called glycation hot spots, selectively increased with the age of plant organs. Moreover, although stress application resulted in limited changes of glycation patterns, post-stress recovery was accompanied with accelerated ageing, manifested by characteristic glycation hotspots. Importantly, to a large extent, such glycation sites were found in regulatory proteins - transcription factors, receptors, regulatory enzymes. This suggests the role of glycation in the regulation of plant functions, which certainly opens a new page in plant physiology. The work was supported by grant no. 20-16-00086 from the Russian Science Foundation.



## TOPIC:

### Phenotyping plant performance under abiotic stress

#### Posters

##### 559 - Dynamic root study in wheat–pea intercrops by minirhizotron method

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Intercropping systems, including cereal–legume mixtures are increasingly important in sustainable agriculture. The minirhizotron (MR) technique was used to track root development in winter wheat (*Triticum aestivum* L. 'Mv Nádor', 'Mv Kolompos', YQCCP composite cross population) grown as sole crops and as additive intercrops with pea (*Pisum sativum* L. 'Aviron'). Cubic greenhouse containers (1000 L) were filled with chernozem topsoil. Crops were grown under well-watered (70% field capacity) and drought stressed (near to the wilting point) conditions. Three MR tubes were placed horizontally in each container at depths of 20, 50 and 80 cm. The MR-based root length (RL) and root surface area (RSA) were monitored from the early vegetative to full maturity stages. The root images were recorded with CI-600 rotary scanner, and then were analyzed using RootSnap! software. The leaf chlorophyll content in SPAD value was detected instrumentally. Wheat shoot dry mass (SDM) and grain yield (GY) were determined at maturity. Image analysis revealed that RL and RSA sharply increased until flowering, followed by a moderate decrease during maturity. The presence of pea reduced the maximum RL and RSA for each wheat cultivar. Drought significantly increased the MR-based root size. The intercropping and drought treatments reduced wheat SDM and GY. The water deficit significantly reduced the SPAD value in each treatment. Our results demonstrated that the biomass and grain yield in additive intercrops tended to decrease under stressed conditions due to the strengthened intra- and interspecific competition by the overcrowded plant density. The application of in situ MR method allows us an improved evaluation of root dynamics and plant responses under various cultivation and environmental conditions.

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## 587 - Different stress response of hyperaccumulator and non-hyperaccumulator *Pteris* species to arsenic toxicity

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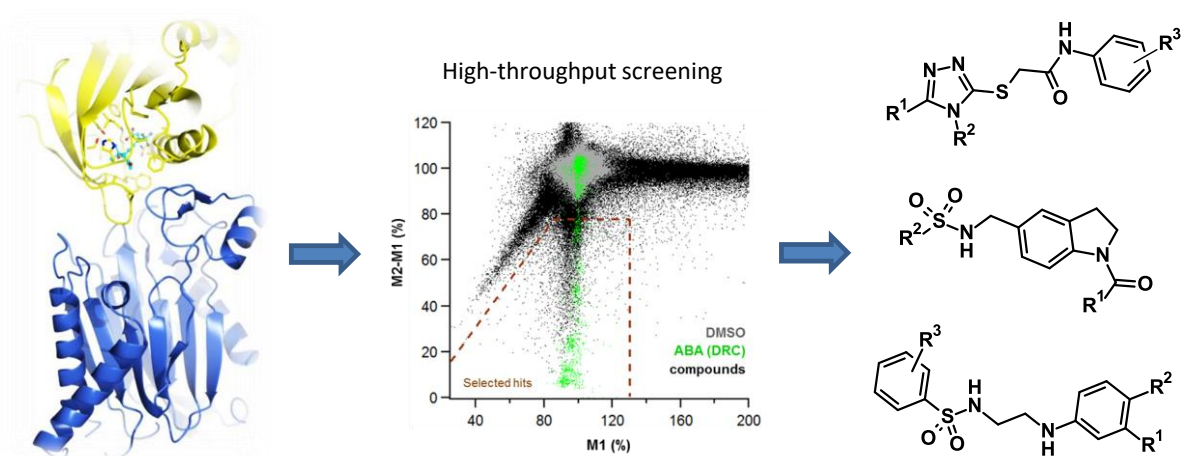
One of the major pollutants in global soil pollution is arsenic (As). Plants on these soils accumulate As and are exposed to stress. In this study, we compared the stress responses of the hyperaccumulator *Pteris cretica* 'Albo-lineata' and the non-hyperaccumulator *P. straminea*. A pot experiment was used for this study, where ferns were cultivated for 83 days in haplic chernozem with applied arsenate treatments – 0, 100, and 500 ppm. In addition to the content of As in biomass, the content of stress phytohormones (abscisic, salicylic, and jasmonic acid) and free amino acids, which can serve as an indicator of stress, was also analysed. Both species had an increasing trend in the accumulation and translocation of As into the fronds. The difference in the As content between ferns fronds was more than nine times greater. *P. cretica* was demonstrably more effective at accumulating As than *P. straminea*. As content in *P. cretica* and *P. straminea* ranged from 43.8 to 9095.6 ppm and 5.4 to 723.9 ppm, respectively. In the fronds of hyperaccumulator with applied As, a demonstrable increase in the total content of amino acids was observed. At 100 and 500 ppm As variant, it was increased by 436% and 79%, respectively, compared to control. In contrast, the non-hyperaccumulator had a significant increase only in the variant with 500 ppm As (by 104%). Salicylic and abscisic acid analyses proved the difference between the ferns in response to As stress. Apart from salicylic acid in the fronds of both ferns, no rising trend was observed in stress phytohormones. In *P. cretica* at 100 and 500 ppm As, salicylic acid was 1.2 and 3.1-fold higher, respectively, compared to control. A higher increase was shown in *P. straminea*, 1.6 and 4.7-fold higher compared to control.

#### 4 - A quantum of solace for crops - New lead structures from high-throughput screening against drought stress interacting with ABA receptor proteins

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Agricultural crops face tremendous losses due to biotic stress such as pests, diseases, and weed damages, but abiotic stress adversely affects crop production even further in various parts of the world, decreasing average yields for most of the crops significantly. Among various abiotic stresses affecting agricultural production, drought stress is considered to be the main source of crop losses around the globe. We have identified and confirmed three unprecedented lead structures against drought stress interacting with RCAR/(PYR/PYL) receptor proteins in wheat via high-throughput screening of our corporate compound library and subsequent dedicated SAR studies. Receptor protein TaRCAR14 and the PP2C phosphatase TaABI1 were isolated from wheat and chosen for the screening campaign. Whilst phenylsulfonyl ethylenediamines showed consistent strong target affinities in vitro on the same level or even better than plant hormone abscisic acid (ABA), several thiotriazolyl acetamides exhibited promising efficacy against drought stress mainly in wheat combined with good target interaction. In all three compound classes particular structural features such as 4-cyanoaniline or 5-halogenthiofen-2-yl moieties were explored which had an essential impact on target affinity in vitro and on the in vivo efficacy. Our results demonstrate that p-cyano aniline moieties are effective bioisosters of the carbonyl group in ABA's cyclohexenone headgroup and in tetrahydroquinoliny sulfonamides. Furthermore, indolylmethyl sulfonamides and phenylsulfonyl ethylenediamines represent two novel classes of sulfonamides nicely complementing earlier work in this field.



## 32 - The effects of drought and nitrogen resources on soybean's physio-morphology, yield components and quality

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The influence of drought and inoculation on soybean (cv. Boglár) under two N-fertilizer rates; 0 and 105 kg ha<sup>-1</sup> (0N and 105N, respectively) was studied in 2017, 2018 and 2019 in Debrecen, Hungary. The experimental design was split-split-plot with 4 replications. Results showed that drought decreased SPAD, NDVI and LAI in 0N, but increased these traits in 105N treatment. Compared to inoculated counterparts, non-inoculated plants had higher SPAD, regardless of irrigation, whereas had higher NDVI and LAI under drought stress conditions only. Fertilization enhanced SPAD, NDVI and LAI of both inoculated and non-inoculated plants; its positive effect was more measurable on NDVI and LAI traits of inoculated plants. Drought measurably reduced plant height, flower and pod number<sup>-1</sup> of inoculated plants and, to a smaller extent, non-inoculated counterparts. Inoculation increased plant height in all fertilization and irrigation treatments, whereas increased flower number plant<sup>-1</sup> in 0N treatment under both irrigation regimes and pod number plant<sup>-1</sup> in 0N under fully-irrigated regime only. Fertilization enhanced plant height, and also flower and pod number plant<sup>-1</sup> with more noticeable effect on non-inoculated plants. The 100-seed weight decreased by drought in 0N, but increased in 105N treatment. This trait increased by inoculation in 0N, but not in 105N treatment. Fertilization enhanced the 100-seed weight and the final seed yield, with higher effect on non-inoculated plants. Yield and protein concentration were reduced by drought. Interestingly, non-inoculated plants had higher protein concentration, however, fertilization increased protein concentration, regardless of inoculation. Oil concentration followed the opposite trend of protein concentration. It could be concluded that inoculation has more positive effects on yield components under favorable water conditions. N-fertilizer enhances soybean physiology under drought conditions, and enhances yield components, regardless of available water. Drought negatively affects yield, yield components and protein concentration, regardless of inoculation and fertilization.

### 33 - Silicon and salicylic acid applications alleviate the damage effects caused by boron toxicity in field pea plants

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Field peas are widely cultivated throughout the world as a cool season grain and forage crop. Boron (B) toxicity is caused by high B concentration in the soil or irrigation water, and is particularly problematic in medium or heavier textured soil types with moderate alkalinity and low annual rainfall. Previous studies have indicated that B toxicity increases the oxidative stress in plants, and B tolerance has been considered an important target in field pea plant breeding programmes. In this way, inducers of tolerance may be a promising alternative for plant breeding. Little or no research has been conducted on the combined use of silicon (Si) and salicylic acid (SA) to remediate B toxicity on field peas. The present study revealed the effects of Si+SA on field pea plants physiological and biochemical responses under B toxicity (15 mg B L<sup>-1</sup>). A semi-hydroponic experiment was conducted under a completely randomized design in a factorial scheme (2 x 5): two field pea cultivars and five treatments which were formed by combining and not Si and SA under B toxicity plus the control plants (control, B, B+Si, B+SA, and B+Si+SA). Si (2 mmol L<sup>-1</sup>) was applied to plants in two forms (root and leaf), while for SA (36 µmol L<sup>-1</sup>) only foliar applications were performed. For instance, our results demonstrated that the combined use of exogenous Si+SA in field peas increased tolerance to B toxicity through an intensified antioxidant plant defence system, resulting in a better regulation of reactive oxygen species (ROS) production and degradation. It significantly increased the activity of major antioxidant enzymes, and reduced malondialdehyde (MDA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content, resulting in increased shoot fresh and total plant dry biomass. Therefore, the combined use of Si+SA is an important and sustainable strategy to alleviate B toxicity in field pea cultivation.

#### **40 - Plasma activated water: the next generation eco-friendly stimulant for enhancing plant seed germination, vigor and increased enzyme activity, a study on black gram**

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Chemical fertilization in agriculture is threatening to the ecosystem. Therefore, the use of eco-friendly stimulant for crop revolution is highly desirable. This study investigates the effects of plasma activated water (PAW) created by treating de-ionized water with high voltage discharge on black gram. Results showed significant improvements in different agronomic traits. Significant changes in H<sub>2</sub>O<sub>2</sub>, a reactive-oxygen-species (ROS) were observed in seeds, leaves, and roots. Increase in catalase (ROS scavenger) was also observed in roots of plants grown from 3 and 6 min PAW treated seeds which was consistent with the upregulation of VmCAT gene. This reveals that PAW is associated with elevated H<sub>2</sub>O<sub>2</sub> in black gram in a tightly regulated manner by the upregulation of CAT activity resulted in increased germination, growth, and development. Non-covalent binding pattern of H<sub>2</sub>O<sub>2</sub> with CAT through molecular docking strongly supports this phenomenon. It does suggest that the increase of H<sub>2</sub>O<sub>2</sub> tightly regulated by the upregulation of CAT provides optimum conditions for improved seed germination and growth in black gram. These findings reveal the benefits of PAW for enhanced crop production.

#### **41 - Manganese deficiency tolerance is associated with the upregulation of TaNRAMP1 transporter gene along with antioxidant defense in wheat**

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Manganese (Mn) deficiency is common abiotic stress causing yield loss in crop plants. This study elucidates the mechanisms of Mn deficiency tolerance in wheat. In this study, Mn deficiency showed no significant effect on root parameters in BARI-28 and BARI-29 but showed a substantial reduction in shoot parameters in BARI-28 under Mn deficiency compared to controls. Atomic absorption spectroscopy (AAS) showed a significant decrease in Mn concentrations in both roots and shoots of BARI-28 but not in BARI-29 under Mn deficiency, implying that Mn deficiency tolerance mechanisms do exist in BARI-29. Further, electrolyte leakage, cell death, H<sub>2</sub>O<sub>2</sub>, and reactive O<sub>2</sub> significantly increased in roots of BARI-28, while these stress indicators showed no changes in BARI-29 subjected to Mn deficiency. Interestingly, chlorophyll score and Fv/Fm (maximum quantum yield of PS-II) severely affected in BARI-28, while these were unchanged in BARI-29 subjected to Mn deficiency. We performed targeted identification of wheat NRAMP1 gene (natural resistance associated macrophage protein 1), known as high-affinity Mn transporter through ENSEMBL database and further aligned for designing genome specific primers based on SNP (single nucleotide polymorphism). The qPCR analysis showed that expression of the TaNRAMP1 transporter gene significantly induced in roots of BARI-29 in both genome B and D, while BARI-28 showed no upregulation in any of the genomes due to Mn deficiency. It suggests that differential expression of TaNRAMP1 is associated with genotypic variations in BARI-29 and BARI-28 in response to Mn deficiency in wheat. In addition, Mn-tolerant BARI-29 showed a significant increase in proline, CAT and APX activity due to Mn deficiency, suggesting that CAT and APX-mediated antioxidant defense might provide tolerance, at least in part, in response to Mn deficiency in BARI-29. This study provides an essential background for improving Mn biofortification in wheat.

## 42 - The role of DELLA protein in tomato plant response to water deficit stress

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Several studies have shown that reduced gibberellin (GA) activity promotes plant tolerance to water-deficit stress. We found that exposure of tomato plants to water-deficit conditions inhibited the expression of the GA biosynthesis genes GA20 oxidase-1 (GA20ox-1) and GA20ox-2 in leaves, induces the expression of the GA-deactivating gene GA2 oxidase-7 (GA2ox-7) specifically in guard cells, and reduces the levels of the bioactive GAs, GA1 and GA4. Reduced level of GA leads to the accumulation of the general growth suppressor, the DELLA protein. Transgenic tomato plants over expressing non-degradable tomato DELLA protein, proceras (pro)Δ17, exhibited increased tolerance to water-deficit stress. These plants maintained higher leaf relative water content (RWC) under water-deficit conditions, due to reduced whole-plant transpiration. Overexpressing proΔ17 under the regulation of guard cell (KST1) specific promoter was sufficient to increase leaf RWC under water-deficit conditions. We found that PRO activity in guard cells promotes stomatal closure in both irrigated and drought-exposed plants. RNA-seq analysis of isolated tomato guard cells identified the putative ABA transporter SINPF4.6-1 as upregulated by PRO. We generated a CRISPR-Cas9 derived slnpg4.6-1 mutant. The loss of SINPF4.6-1 caused increased stomatal aperture and reduced stomatal closure in response to ABA. Moreover, slnpg4.6 suppressed the effect of proΔ17 on stomatal closure and transpiration. Taken together, our study suggests that water-deficit conditions reduce GA levels in guard cells and promotes PRO accumulation. PRO promotes the expression of the ABA importer NPF4.6-1 that enhances ABA uptake into the guard cell, leading to stomatal closure.



## **56 - Water deficit-dependent changes of the endogenous content of phytohormones and transcript-related genes in two contrasting conifer species**

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In due to the attached lifestyle of plants drought stress has a strong inhibitory effect reduces the intensity of photosynthesis and productivity. Conifer plants, especially in the early ontogenetic stages, very sensitive to osmotic stress caused by lack of moisture. Plant hormones play critical roles in plant growth and development and physiological responses to biotic and abiotic stresses, including drought. To regulate all drought-induced adaptive responses, plants rely on a complex network of hormonal signals interacting with each other. ABA is recognized as a key hormone for plant adaptation to water deficit, however, plant drought responses are regulated by an intricate network of plant hormones, which, in addition to ABA, implicates cytokinins, auxins, ethylene, etc. We found that Scots pine and Norway spruce showed pronounced stress-dependent dynamics of ABA under conditions of water deficiency. ABA changes were presumably due to an increase in de novo ABA synthesis in the shoots of both species. We also found less expected phenomena, namely an increase of active CK content in needles of both plant species, as well as clear stimulating effects of water stress on ACC content and expression of ethylene biosynthesis genes. The increase in ABA in spruce plants can probably be associated with a more water-saving strategy of this plant, while the detected activation of cytokinins in pine can due to the need of growth maintain under stress. It is clear that the reaction of hormone pools of Pinaceae plants to drought could differ substantially from better studied angiosperm species, and further studies are required to evaluate the extent and significance of these differences to the adaptation to water deficit conditions.

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## **76 - Drought stress and recovery effects on morpho-physiological and biochemical responses in *Impatiens walleriana* grown ex vitro**

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*Impatiens walleriana* (Balsaminaceae) is a worldwide popular horticultural plant and commercially produced in Serbia for many years. Plants have high requirements for the presence of water in the substrate, which deficiency leads to a rapid drop in the cells turgor pressure and tissue dehydration. This is the main problem in commercial production of these plants, especially during transport and sale process when plants are not always sufficiently supplied with water. We assessed the drought and recovery effect on growth, physiological and biochemical parameters in *I. walleriana* (Xtreme Scarlet) grown in chamber under controlled physical conditions (ex vitro). Plants were 74 days old when the drought stress was imposed. We examined: control plants – grown under optimal watering (37%-40% of soil moisture content), drought stressed plants – (25%, 15%, 10% and 5% of soil moisture content), and recovery plants – rehydrated for four days to reach optimum soil moisture content (37%-40%). Drought reduces fresh (FW) and dry weight (DW) of shoots, as well as total leaf area and shoots water potential. Drought significantly increased abscisic acid (ABA) content in the leaves in order to reduce transpiration rate and preserve water in it's tissue. The concentration of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA) were increased in drought-stressed plants, as a consequence of drought induced oxidative stress in the plant cells. The activity of antioxidant enzymes (superoxide dismutase - SOD, peroxidase - POX and catalase - CAT) contributed to neutralizing negative effects of oxidative stress on plant growth, while the different SOD and POX isoforms were detected on gel. Recovery treatment neutralised negative effect of drought on growth, physiological and biochemical parameters analysed in *I. walleriana* to a certain extent.

## 88 - ER stress response of Atgpxl3 plants

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The plant glutathione peroxidase-like (GPXL) enzymes are closely related to animal phospholipid hydroperoxide glutathione peroxidases, that catalyse the reduction of H<sub>2</sub>O<sub>2</sub> or organic hydroperoxides to water or the respective alcohols. Contrary to most of the animal glutathione peroxidases, GPXL isoenzymes contain cysteine instead of selenocysteine in their active site, and they may prefer the thioredoxin (TRX) regenerating system rather than the glutathione. Although GPXLs are important antioxidant enzymes, their relatively low activities compared to animal GPXs indicate that they might have other specific roles. The *Arabidopsis thaliana* AtGPXL3 is localised in the ER/Golgi membrane. The ER does not contain TRXs, thus it can be supposed that AtGPXL3 either use other electron donors in the peroxidase reaction or acts as a thiol oxidase and in this way it could oxidise directly nascent proteins in the oxidative folding machinery. In our experiments we aimed to investigate the relation between the AtGPXL3 and ER stress.

The stress responses of 6-week-old *Arabidopsis thaliana* Col-0 wild type and Atgpxl3 T-DNA insertional mutant plants were compared. ER stress was induced by tunicamycin and 3-phenylbutyric acid was used as ER stress inhibitor. Beside detecting some stress-related parameters, the glutathione and ascorbate levels and their reduced state were measured. The differences in some physiological parameters and changes in the expression pattern of selected ER stress-related genes indicate that AtGPXL3 is involved in ER-related stress responses.

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## 122 - Sensitivity of water loss and nitrogen fixation to soil drying in the Vigna genus

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Studying drought tolerance of pulses will contribute to improving overall food security. Drought strongly impact symbiotic N fixation but there is room for improvement as legumes show significant genetic variation in their ability to fix N<sub>2</sub> under drought. Our study focused on two key physiological traits under drought conditions: early decrease in transpiration rate and maintained N fixation activity under low soil-water conditions. We compared the responses of these two traits to soil drying among 9 genotypes of *Vigna radiata*, *Vigna unguiculata*, *Vigna vexilata*, that are of significant agricultural interest. Maximum water stress was obtained at the very beginning of anthesis, i.e. at peak phase for biological N fixation. The fraction of transpirable soil water upon which transpiration rate declined ranged from 0.39 to 0.21. The variability for this trait is very small compared to other studies but allows to identify significant differences among genotypes. The fraction of transpirable soil water at which N fixation rate began to decline ranged from 0.48 to 0.07 with one line where there was no threshold. No correlation was observed between the thresholds for decline in transpiration and those for N fixation across genotypes. Variability of genotype ability to maintain N fixation under low soil-water conditions will be discussed with regard to leaf initiation rate and other leaf functional traits known determine N acquisition and storage strategies.

## 125 - Uncovering the role of three uORFs of an arabidopsis zinc transporter on translation

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Upstream Open Reading Frames (uORFs) are protein-coding regions in the 5' untranslated region (UTR) of mRNAs. They are known to affect translation of the main ORF (mORF), usually reducing the levels of protein production due to a decrease in translation efficiency. Around 35% of *Arabidopsis thaliana* genes contain uORFs, but in plant genes very few uORFs have been examined for their functional significance. Here, we identified three uORFs, named uORF1, uORF2 and uORF3, in the *Arabidopsis thaliana* ZIF2 (Zinc-Induced Facilitator 2) gene. ZIF2 is a membrane transporter that mediates vacuolar compartmentalization of zinc in root cortical cells, thereby conferring plant tolerance to the heavy metal. Using a reporter system in isolated *arabidopsis* protoplasts, we show that simultaneous disruption of all ZIF2 uORFs results in a marked increase in the activity of the luciferase gene, demonstrating the ability of at least one uORF to inhibit mORF translation. Individual disruption of the ZIF2 uORFs revealed that uORF2 is the main player in the observed translational repression and uORF1 does not affect translation, while uORF3 is translated and inhibits mORF translation only in the absence of uORF2. We therefore postulate that uORF3 acts as a fail-safe mechanism to repress translation, to our knowledge the first observation of such a mode of regulation in plant systems. Our results also indicate that uORF2 and uORF3 exert their inhibitory effect even under a weak AUG context and that uORF2 acts in a peptide sequence-dependent manner. Moreover, the ZIF2 mRNA is not targeted to degradation by Nonsense-Mediated Decay (NMD). Together these data point to ribosome stalling as the mechanism underlying ZIF2 uORF-mediated translational repression.

## **127 - CaCl<sub>2</sub> alleviates Cd-induced oxidative stress in chickpea (*Cicer arietinum* L.)**

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We studied the priming effects of calcium chloride on acquisition of cadmium (Cd) stress tolerance in chickpea (*Cicer arietinum* L.). The Cd stress reduced various morphological (length and biomass) and biochemical parameters like lipid peroxidation and antioxidant enzyme activities, but alleviated by exogenous application of calcium chloride. In our experiment, sterilized seeds of Chickpea (Pb-2002) were imbibed in distilled water (DW) and Calcium chloride separately for 24 h, later sown in pots containing 2 kg of soil and allowed to grow. Eighteen days after germination, two Cd treatments were given as i) 0.5mM and ii) with gradual increase in Cd concentration from 0.5 mM, 0.75mM, 1 mM and then 2 mM (0.5-2.0 mM). Results showed that at high concentration of cadmium, morphological parameters decreased with increased activities in malandialdehyde (MDA) content and activity of antioxidant enzymes such as guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and catalase (CAT) as compared to control. However there was improvement in morphological parameters and reduced activities in the above biochemical parameters after priming with CaCl<sub>2</sub> when compared with Cd alone. Our results showed that exogenous application of CaCl<sub>2</sub> enables chickpea plants to combat the adverse effect of cadmium through regulation of antioxidative defense mechanisms. Mitigation of cadmium induced stress by calcium application was strongly suggested and can be applied as an easy strategy to grow plants later in cadmium contaminated lands.

## 128 - Assessment of ozone risk to *Moringa oleifera*

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Ozone (O<sub>3</sub>) is a toxic oxidative air pollutant, with significant detrimental effects on plants. *Moringa oleifera* Lam. (Moringaceae) is a native species from the sub-Himalayan mountains of northern India. Nowadays it is widely cultivated in tropical and subtropical regions and its leaves are used for human and animal nutrition as well as in traditional medicine. This study aimed to assess O<sub>3</sub> risk to *M. oleifera* genotypes using O<sub>3</sub> flux-based index (POD<sub>γ</sub>, where γ is a threshold of stomatal uptake) that considers leaf O<sub>3</sub> uptake and the influence of environmental conditions on stomatal conductance (g<sub>sto</sub>). Four genotypes of *M. oleifera*, coming from different countries: Burkina Faso (BF), Ghana (G), Mozambique (M), Pakistan (P) and India (PKM1) were subjected to Free-Air Controlled Experiment (FACE) exposure at three levels of O<sub>3</sub> concentrations: ambient (Amb); Amb x1.5; and Amb x2.0. Total leaf biomass (LB) was evaluated and the potential biomass production in clean air was estimated by assuming a theoretical clean atmosphere at 10 ppb as 24 h O<sub>3</sub> average. The Jarvis-type multiplicative algorithm was used to parametrize g<sub>sto</sub> including environmental factors i.e. air temperature, light intensity, air vapor pressure deficit, and AOT40. Our results show that *M. oleifera* was very sensitive to O<sub>3</sub> stress. The onset of visible injury occurred abruptly and was characterized by adaxial silvery stipples homogeneously distributed in the leaflets. We recommend a γ threshold of 4 nmol m<sup>-2</sup> s<sup>-1</sup> to incorporate O<sub>3</sub> effects on different *M. oleifera* genotypes. In order not to exceed 4% reduction in the growth we recommend a CL of 1.08 mmol m<sup>-2</sup> POD<sub>4</sub>. Our study improves the limited knowledge of current O<sub>3</sub> impacts on tropical species.

### **132 - Room for improvement: high regulatory diversity of drought tolerance strategies in winter oilseed rape**

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Spring droughts are expected to become more frequent in Central Europe as a result of climate change. Their coincidence with flowering of biennial crops like winter oilseed rape (*Brassica napus*) can cause major impact for yield development. However, no data is available on the diversity of genetic regulation of drought tolerance during this stage under realistic conditions. Here, we assessed the phenotypic plasticity of drought response for eight diverse *B. napus* accessions under field-like conditions and linked their stress response to gene and miRNA expression during early and late stress. We observed highly diverse responses, both on the phenotypic and on the genetic level. Our data suggest that drought tolerant accessions have more effective molecular protection mechanisms like ROS scavenging, source/sink ratio and regulation of developmental timing, compared to otherwise phenotypically similar accessions. Interestingly, all tolerant accessions showed increased expression of *Bna.MAP3K13.C05*, a MAP3 kinase involved in nitrogen starvation stress, indicating that improved nitrogen uptake may also improve drought stress tolerance.



## 210 - Adaptation of roots to limiting water supply in *Brachypodium distachyon*

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*Brachypodium distachyon* became widely accepted model plant for monocots. The characterization of thirty-one *Brachypodium* genotypes differing in their drought tolerance was completed in terms of root growth and architecture as well as general growth parameters. Principal component analysis revealed that the primary component mainly derives from fresh and dry weight data and shoot length while the secondary component derives from primary root length and root/shoot length ratio out of more than twenty parameters measured. Three group of genes were studied in this model.

LOB-domain (Lateral Organ Boundary) transcription factors are important regulators of plant architecture. Twenty-eight genes code for this protein family in *Brachypodium* genome. Various plant part/organ specificities were found in the expression of these genes, one of them possesses exceptionally high root-specificity. Ectopic expression of two of them in *Brachypodium* lines resulted in severe developmental phenotypes. Some of these proteins may be substrates of cdk-cyclin complexes suggesting link between cell cycle and developmental regulation.

Scavenging toxic stress by-products during osmotic stress adaptation is catalysed by members of the glutathione transferase (GST) enzyme superfamily. Osmotic stress treatment resulted in increased GST activity indicating severe defence reaction against the harmful imbalance of the redox environment. Screening for the gene sequences led to the identification of 91 full-length or partial GST sequences. Although *Brachypodium* has small genome, the number of identified GST genes was like in wheat. The estimation of GST expression showed stress-induced differences: higher expression levels or the fast induction of BdGSTF8, BdGSTU35 and BdGSTU42 gene products presumably indicate a strong detoxification under osmotic stress.

Transcriptions of core components of the circadian clock were also followed during drought adaptation in roots. Cyclic expression of some of the clock genes were modulated by intensifying drought stress under constant illumination. Circadian regulation may help proper timing of water uptake.

## **217 - JASMONATES REGULATED GENOME-WIDE CHARACTERIZATION AND TIFY GENE FAMILY EXPRESSION PROFILING IN CAJANUS CAJAN UNDER COPPER STRESS**

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Methyl-Jasmonate (Me-JA) and Free Jasmonic Acid (JA) are oxylipins produced in thylakoid membrane from OPDA and isomerized to give different metabolites of jasmonic acid like Free JA, Me-JA, JA-Ile, etc. It has been well known fact that JA biosynthesis required prior presence of JA or signal like wounding to initiate. Both Me-JA and JA are known to play protective role against abiotic stress. Being signalling molecule, JA signalling is mediated by number of transcription factors such as JAZ and MYC2. Some of these TFs like JAZ play the role of negative regulator in JA signalling to regulate almost all biological processes of cell metabolism under normal as well as stress conditions. In the present study we have identified and characterize 18 TIFY family proteins under Cu stress which were up- and/or down- regulated by either Me-JA or JA exogenous treatments. These 18 TIFY proteins are further classified into TIFY, JAZ and ZML subfamilies. Gene expression profiling of 12 CcTIFY genes in *C. cajan* were study under copper stress in presence or absence of JA and Me-JA. Our results showed that presence of Cu in growth environment up-regulated 5 CcTIFY TFs named CcTIFY3, CcTIFY4, CcTIFY5, CcTIFY9, and CcTIFY16. As TIFY gene family is reported to be the integral part of jasmonate receptor proteins, hence it might be that JA and Cu stress signalling have some cross-talk at the molecular level for managing stress tolerance in *C. cajan*. The presence of defense and stress responsive cis-regulatory elements in the promoter regions of these genes further confirmed their involvement in Cu related stress responses. CcTIFY3 and CcTIFY9 were found to up-regulate by the exogenous application of Me-JA and JA. These findings suggested that apart from feedback regulation of JAZ proteins, they are also involved in alteration of JA and Me-JA signalling.

## 250 - An investigation of submergence tolerance in the major weed *Echinochloa crus-galli*

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**Weed infestation** is a major cause of **yield** loss in **rice production**. To cope with this problem, a significant amount of rice farming in Asia involves transplantation of rice seedlings to flooded paddy fields, where the flooded and anaerobic soils prevent establishment of most weeds. The efficacy of this method however, is undermined by the emergence of several flood tolerant weed varieties. It is of interest to understand the mechanistic basis of this tolerance as a means to identify novel flood tolerant mechanisms and potentially identify alternative ways to suppress weed proliferation in paddy fields. *Echinochloa crus-galli*, reported as one of the worst weeds worldwide, was harvested in a lowland rice field in The Philippines and screened for its tolerance to complete submergence conditions. The weed was compared to two major crops: *Zea mays* (maize) a flooding sensitive species and two different varieties of *Oryza sativa* (rice) described as tolerant and intolerant to flooding. Tolerance was evaluated based on various morphological and physiological parameters during both the submergence and post-submergence periods. To investigate the molecular basis of flooding tolerance in this weed, we evaluated the whole transcriptome response of the four different species during submergence and recovery conditions, using an RNAsequencing approach. Analyses of the submerged *E. crus-galli* responses and of the differences between species will be presented in the PBE2020. This dataset will give an insight into the mechanisms underlying the flooding tolerance in *E. crus-galli* and distinctions in the response compared to maize and rice. The findings here could be a promising for crop breeding programs for improved flood tolerance.

**254 - VIVEMA® TWIN, a new tannin-based biostimulant, improves root growth and plant health in tomato cultivated under salt stress conditions.**

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VIVEMA® TWIN, a new biostimulant (Green Has Italia S.p.A.) based on an original mix of hydrolysable and condensed tannins extracted by hot water from wood, tested in two years on different horticultural crops, showed positive effects on roots and therefore higher yield in presence of multi-stress environmental conditions.

The aim of this research was to study the effects of VIVEMA® TWIN on tomato (*Solanum lycopersicum* Mill. cv. Heinz1706) grown in greenhouse under optimal and salt stress conditions, by using agronomical and transcriptomic approaches.

VIVEMA® TWIN treatment promoted a significant increase of root fresh weight (+57%) and length (+28%) compared to control. These effects were confirmed by imaging analysis (Root System Analyzer) carried out on seedlings cultivated in quartz sand at different growth stages.

To determine the metabolic targets of this biostimulant, RNAseq analyses were performed on root tissues of control and VIVEMA® TWIN-treated plants grown in salt stress conditions. VIVEMA® TWIN application promoted the upregulation of 280 genes, mainly involved in stress response pathways (63%) and root growth (18.5%) and correlated to an improved salt stress tolerance and in general to a better performance of the treated plants compared to control. This aspect was also confirmed by the NDVI index, used as indicator of plant health.

Moreover, Orbitrap LC-MS analysis of VIVEMA® TWIN revealed the presence of several active compounds, such as gallic acid, known to be involved in root growth stimulation and antioxidant activity. Gallic acid was also tested separately on plants to evaluate the effect of the single components compared to the whole matrix.

In conclusion, the overall results provide insights on the mechanism of action of VIVEMA® TWIN and on its application in agriculture as a valid tool to improve root growth and plant health, especially in the widely spread soils subjected to salinity.

**266 - Relation of leaf structural traits to leaf specular and total reflectance in Hieracium genus and Arabidopsis thaliana mutants**

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Leaf optical properties (reflectance, absorptance and transmittance) are defined as the ratio of the incoming radiation, reflected, absorbed or transmitted by a leaf. Reflectance is dependent on wavelength and angle of incoming light, leaf chemistry, and internal and epidermal structural traits. Total leaf reflectance (TR) is a combination of 1) diffuse reflectance (DR) a spectrally changed signature affected by leaf biochemical and structural traits and, 2) specular reflectance (SR) derived exclusively from leaf surface structures. The specular reflectance component is reflected from a leaf surface directly, without spectral change and at an angle equal to the angle of light incidence, like a mirror. Determining photosynthetic pigments (PPs) based on leaf TR is quite precise, however the role of SR is still undefined. We hypothesized that SR is mainly affected by leaf epidermal structure.

We studied biochemical, optical and anatomical properties of selected species of the Hieracium genus with various modifications in epidermal structure: trichome length, density, presence of epidermal waxes, leaf internal structure; and A.thaliana mutants with different shape of trichomes. We applied machine learning to study the relation of leaf biochemical and structural traits with their reflectance properties divided into DR and SR. Leaf optical properties were measured by a spectroradiometer (ASD FieldSpec4) with an integrating sphere. Anatomical study of leaf epidermal surface structure (trichome size and type, presence of cuticular wax deposition) was visualised by ESEM and internal structure was examined via hand cross sections and light microscopy.

PPs detection was more precise when using TR without SR component, while surface properties, such as trichome density, were better corresponding to TR including SR. This was compatible with our hypothesis. Our study indicates that SR can be used for estimation of leaf epidermal structural traits.

## **273 - Physiological effects induced in tomato plants grown in salt stress conditions by a facultative endophytic bacterium expressing ACC deaminase**

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**upo, Disit, alessandria, Italy** <sup>(1)</sup>

Global climate changes strongly affect crop growth conditions: they are, therefore, a significant constraint to the world food request. Soil salinization and aridity are among the most significant problems.

It is well known that Plant Growth-Promoting Bacteria (PGPB) improve plant health status under both biotic and abiotic stresses. In this research work, we investigated the possible plant protection effects induced in tomato by a PGPB, the endophyte *Pseudomonas migulae* 8R6, in salinity conditions. We choose this bacterium for its capability of synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which cleaves ACC to  $\alpha$ -ketobutyrate and ammonia and thereby decreases ethylene levels in the host plants. A low level of ethylene can alleviate plant stress symptoms.

In a first, short time experiment, the degree of colonization by the mutant 8R6 expressing GFP and its localization in the plant tissues of tomato by fluorescence, confocal, and image analysis techniques were evaluated. Our results confirmed that 8r6 is a facultative endophyte. In the first 30 days, plants elicited with 8r6 (both mutant expressing GFP, both wild-type) showed a decreased growth. Histological analysis on stems revealed a reduction of the diameter of epidermal and parenchymatic cells.

In a second experiment, we assessed the effects of 8R6-GFP and 8r6-wild type on the growth and the health of tomato in the presence/absence of salt stress by microbiological and physiological techniques. Under salt stress, 8r6 wild type stimulated plant growth with a significant reduction of symptoms. Moreover, the OJIP test of tomato leaves showed a positive effect of 8r6 wild type under salt stress, compared to control plants. Finally, we explored the production of seeds containing 8R6-GFP to allow a simple and direct use of this endophyte: tomato seeds contained 8R6-GFP and raised new plants, already colonized by 8R6-GFP.

## 290 - Meta-analysis of RNA-seq data to gain insight into crop responses to environmental stresses

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Transcriptomic studies are usually conducted in a singular time, they do not provide any repetition across different seasons and frequently they are performed in field conditions where environmental variability is high and disturbing factors are frequently present. Meta-analysis of transcriptomic data is a powerful technique to identify more reliable biomarkers for: 1) early stress-specific responses, 2) resistance and tolerance. The objective of this work is to identify specific and common molecular features (genes, proteins, gene sets, pathways), linked to both abiotic (drought, salinity, cold) and biotic stress resistances among key crops (fruit tree crops, cereals, horticultural species). In *Malus*, different cell wall-related categories were modulated by bacterial pathogens compared with viral and fungal agents. These latter pathogens upregulated aminoacid-related pathways and WRKYs. While gibberellins and ABA were repressed by fungal pathogens, jasmonic acid-response was downregulated by apple stem grooving virus. Cell-wall related genes were commonly modulated by Huanglongbing disease among different studies and protein-protein interaction networks highlighted the link between heat shock proteins and symptoms in leaves. Relating to drought stress, we identified 21 genes in common among different crops. These genes are known to be key players in resistance/tolerance mechanisms conserved across different plant species (SnRK2, Calcineurin B, CBL interacting protein, Homeobox 7, LTP3, Potassium transport 3, SOS, cellulose synthase, WRKY20, MYC4, MET1). Chromosome 1 in maize and potato showed to be with a high density of genes linked with drought response. A model of transcriptional activation cascade was proposed for drought response. Finally a meta-analysis across different plant species was conducted in a similar way for cold response. These analyses will help in monitoring stressed plants to start early specific management procedures for each stress and develop sustainable strategies to develop resistant and tolerant genotypes to harsh environmental stresses.

**Keywords:** abiotic stresses, biotic stresses, crops, meta-analysis, RNA-seq, transcriptomic.

## 296 - Non-intrusive monitoring of root dynamics in wheat–pea intercrops

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Cereal–legume intercrops are particularly important in low-input sustainable agriculture. Their performance strongly depends on root dynamics and interactions, which is difficult to study in situ. The root electrical capacitance method is based on correlation between the absorptive root surface area (root activity) and its electrical capacitance ( $C_R$ ), measured between a ground electrode embedded in the soil and a plant electrode clamped to the stem-base. We aimed to use this non-intrusive technique in a container experiment for monitoring root growth pattern of three winter wheat cultivars grown as sole crops and intercropped with pea (additional design) under well-watered and drought-stressed conditions. The aboveground biomass and grain yield of wheat was determined after harvest.

The wheat root activity ( $C_R$ ) continuously increased during the vegetative stages, peaked at anthesis (different times in the cultivars) and declined during maturity. Drought stress promoted wheat flowering and accelerated the root senescence. Pea intercropping caused 5–18% decrease in the maximum wheat  $C_R$ , depending on cultivar and irrigation. Drought stress led to substantial (32–59%) reduction in the maximum wheat  $C_R$ . Intercropping and water shortage reduced the wheat shoot dry mass by 7–23% and by 40–68%, respectively. Grain yield decreased by 17–54% and by 29–73% in the same comparison. The relative change in maximum  $C_R$  was a reasonable predictor of cultivar-specific response to intercropping and drought.

The root capacitance method proved to be adequate for monitoring the activity of intact root systems in different soil environments. Consequently, it is potentially useful for various fields of agricultural research, including the studies on intercropping systems.

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## 297 - Wheat, barley and cucumber germination in the presence of imidazolium based ionic liquids and further seedling growth

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The search for environmentally friendly solvents, substitutes for volatile organic compounds, leads to development of solvents tailored for specific purposes. However, even though they have many desirable properties, like low volatility, non-inflammability, and good chemical and thermal stability, their environmental safety (their “green” nature) is still being questioned and explored. In this regard, a series of experiments was set up, using five different synthesized imidazolium based ionic liquids (IL): 1-(2-oxybutyl)-3-methylimidazolium chloride, [C<sub>2</sub>OC<sub>2</sub>mIm][Cl]; 1-(2-oxypropyl)-3-methylimidazolium chloride, [C<sub>1</sub>OC<sub>2</sub>mIm][Cl]; 1-(3-hydroxypropyl)-3-ethylimidazolium chloride, [OHC<sub>3</sub>eIm][Cl]; 1-(3-hydroxypropyl)-3-methylimidazolium chloride, [OHC<sub>3</sub>mIm][Cl]; 1-(2-hydroxyethyl)-3-methylimidazolium chloride, [OHC<sub>2</sub>mIm][Cl], together with commercial 1-butyl-3-methylimidazolium chloride, [bmim][Cl] and synthesized protic imidazolium chloride, [Im][Cl], in order to test their effect on germination and early growth of wheat, barley and cucumber. Seeds were exposed to either 0 (control), 10, 100 or 1000 mg/l of selected ionic liquids during germination. Number of germinated seeds was counted after 24, 48 and 72 h. Seedlings were then transferred to pots filled with Hoagland nutrient solution and grown in a growth chamber under controlled conditions. The root and shoot length and dry weight of wheat and barley seedlings were recorded 8 days after transplanting. In cucumber, besides on germination the effect of ILs on seedling growth was pronounced and therefore fresh weight of seedling’s roots and shoots was recorded 72 h after sowing. Forty days following transplanting, fresh weight of cucumber, concentration of photosynthetic pigments and MDA in leaves were assessed. Substantial differences between the effect of the selected ILs on wheat, barley and cucumber were found. The tuning of the lipophilicity of imidazolium cations by introduction of polar groups in the alkyl side chain reduces toxicity with respect to the unsubstituted [bmim][Cl]. Although investigated ILs affected root and shoot growth of cucumber, the effect on stress marker (MDA) as well as on biosynthesis of photosynthetic pigments was negligible.

### **301 - Relative contribution of pre- and post-anthesis phosphorus uptake to grain P in durum wheat plants grown under contrasting P levels**

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Grain phosphorus (P) in wheat originates from the post-anthesis P uptake and the remobilization of P from different plant tissues. The supply of P and its use by the plant are important processes that can affect the contribution of each sources to the accumulated P into grains. Thus, the aim of this experiment was to determine the contribution of exogenous P to the grain in durum wheat plants with different P nutritional status. Plants were grown hydroponically under high P (0.125 mM) or low P (0.025 mM) supply in greenhouse conditions. Using <sup>32</sup>P isotopic tracer technique, we quantified the proportion of post-anthesis P uptake and its partitioning into plant tissues and grains. We also determined traits related to P use efficiency in durum wheat plants. The two P supply resulted in two contrasting nutritional plant P status. Plants with low-P status remobilized most of their stored P in all organs and allocated more than 72% of post-anthesis P uptake to grain P nutrition, whereas in the high-P plants this was only 56%. In low-P plants, P remobilization from different plant tissues represented 81% of grain P while its represent 65% for high-P plants. Low-P plants inhibited post-anthesis tiller development suggesting a tight control of P allocation to the grain under this treatment. Enhanced remobilization of P and the efficient allocation of newly acquired P to grain were crucial for durum wheat grain P nutrition grown under low-P supply. Our study demonstrated that even with high external P supply, most of the P in the grain is derived from the remobilization of internal P pools. These findings highlight the importance of P remobilization as a target for the improvement of more P-efficient crops.

### **319 - Increasing plant cell tolerance to heavy metals by inhibition of programmed cell death pathway**

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Plant and animal cells undergo environmental programmed cell death (ePCD) under biotic or abiotic stress. Heavy metals induce ePCD in suspended cells of *Viola tricolor*, which is a pseudometallophyte of calamine (Zn/Pb) soils. However, the mechanism of ePCD in the plant is poorly understood. The current studies aimed to identify the role of caspase-like enzymes in plant ePCD and to increase cell tolerance to heavy metals by blocking ePCD.

The cell suspension was treated with 2000 µM of Zn or Pb for 72 h, non-treated culture served as a control. We estimated the frequency of PCD events with TUNEL assay and found that Zn- and Pb-treated cells underwent PCD with a significantly higher frequency than non-treated cells. A ligand blotting using biotinylated inhibitor of caspase-like proteases (biotin-xVAD-fmk) was conducted to determine the enzymes with caspase-like activity in ePCD. The ligand blotting revealed that papain-like cysteine proteases (PLCPs) were involved in heavy-metal-induced PCD. Next, we examined the effect of specific protease inhibitors on ePCD under heavy metal stress. Exogenous application of E-64d (50 µM), a PLCPs inhibitor, together with Zn or Pb for 24, 48 and 72 h significantly increased cell viability estimated by alamarBlue assay in comparison with the treatment without inhibitor.

In conclusion: Both Zn and Pb treatments induced ePCD by activating PLCPs with caspase-like activity. The E-64d treatment could successfully inhibit the PCD pathway, thereby increase plant cell tolerance to heavy metals.

### **320 - Salt-induced ultrastructural changes in durum and soft wheat seedlings**

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Under natural conditions, plants are often subjected to various stresses, which can seriously affect their growth and development. Cellular toxicity caused by a high content of Na<sup>+</sup> ions is the predominant ionic toxicity. In plants, Na<sup>+</sup> transporters are responsible for the elimination of ions, which reduce the high concentration of Na<sup>+</sup>. Salt stress (150 mM NaCl) leads to an increase in the level of expression of the gene HKT1; 4. The expression level in the roots of wheat varieties Oreburskaya 10 and Oreburskaya 22 increases by more than 2 times compared with leaves and 3-4 times compared with control variants.

Assessment of the state of the mitochondrial network using Mitotracker Green showed that the cells of the epidermis and cortex from the cap and dividing cultivars of the Orenburg region 10 are most susceptible to the negative effects of salt. Transmission microscopy revealed the formation of autophagosomes containing cytoplasmic fragments and cellular compartments localized in them - mitochondria and plastids in vacuoles of wheat cells. In coleoptile cells, there was an increase in programmed cell death and an associated increase in the activity of restriction endonucleases, leading to DNA fragmentation. Abiotic stress is accompanied by an increase in methylation in wheat cells. The formation of micronuclei caused by the destruction of the cytoskeleton of polyploid nuclei is shown. The ultrastructure of the plant micronucleus is characterized in connection with the programmed cell death in the vascular cells of the central cylinder.

The influence of stress factors on the induction of the expression of genes encoding enzymes involved in the neutralization of ROS is studied. A high level of MnSOD expression in common wheat roots may indicate high protective properties against ROS.

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### **324 - Rhizospheric bacteria isolated from paddy fields can promote cold tolerance in rice plants**

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Rice is the basic food for over half of the global population. Paddy cultivation is limited by environmental stressors, which tend to worsen with climate change. Also, an increase in food demand is expected soon. Therefore, innovative agricultural technologies capable of increasing food production without increasing agricultural frontiers are needed. Some microorganisms can confer plant tolerance to environmental stresses, however, research with rhizospheric bacteria inducing cold tolerance in rice plants is scarce. In this work we evaluated the prokaryotic community of cold-impacted rice paddies, and the ability of selected rhizospheric bacteria increase the rice tolerance to cold without promoting yield penalty. The most abundant phyla identified (Proteobacteria, Acidobacteria, and Actinobacteria) are common soil bacteria, which harbor most of the plant growth promoting bacteria (PGPB) and should be responsible for plant protection to abiotic stresses, as low temperature. From these soils, nine PGPB were selected and inoculated in cold stressed rice plants, and two (*Kosakonia* sp. CIR2 and *Staphylococcus* sp. CSR1T2) were able to confer cold tolerance to rice plants. These cold stressed plants inoculated with CIR2 and CSR1T2 presented higher survival rates (69% and 85%, respectively) than non-inoculated plants (33%). In greenhouse, cold stressed inoculated plants reached the reproductive cycle approximately 25 days earlier than non-inoculated plants, besides presenting increased fertility (percentage of full seeds/full seeds per plant) and improvement in yield parameters (weight of 1,000 full seeds, grain length, seed weight per plant, and seed yield). These data can contribute to the improvement of inoculation practice in rice plants and to the maintenance of rice production in environments impacted by low temperature stress in the early developmental stages.

**328 - Morpho-physiological response and influence of *Heliconia psittacorum* on enzymatic activity at rhizosphere level under waterlogging.**

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Plants require water for growth and development, but excessive water negatively affects their productivity and viability. This problem is magnified with climate change that has been and will be increasing the frequency and severity of extreme climate events and natural disasters like floods in many ecosystems. A lot of studies about the stress response of crop plants and model plants under flooding have been reported. However, information on other kinds of plants, like ornamentals, is limited. *Heliconia psittacorum* is an ornamental plant with a high demand around the world and currently grown in regions that have tropical climatic conditions, such as the Soconusco in Chiapas, Mexico. It is exported in large quantities to the United States, Canada, and many countries of the European Union due to their long-lasting inflorescences, making them an important factor in the agricultural economy of many countries. The aim of this investigation was to study the level effect of flooding on *Heliconia psittacorum* and the interactions between morpho-agronomic, physiological and enzymatic parameters by six months in three different conditions: soil, wet support and flooding support. The treatments showed significant differences ( $P < 0.05$ ) in morpho-agronomic and physiological tests. Plants planted in the soil had the highest values in all morpho-agronomic parameters. The photosynthetic pigments (Chl-a, Chl-b, Chl-T and Car-T) showed a decrease in the waterlogged and wet treatments, while the concentration of proline and electrolyte leakage to soil treatment. In relation to rhizospheric enzymatic activity, the waterlogging treatment showed the lowest enzyme activity. The alterations in the morpho-physiology and enzymatic activity of *H. psittacorum* demonstrate that waterlogging conditions cause stress to the plant. Plants planted in the soil had the highest values in all morphoagronomic parameters.

### **340 - Redox capacity of Bryophytes submitted to the increase of UV radiation and water deficit**

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Studies focused on the chemistry of Bryophytes are relatively recent compared to other groups, especially Angiosperms. Bryophytes have the ability to adapt to strict ecological ranges, are very sensitive to environmental changes and can be used as bioindicators of climatic and microclimate conditions, characterizing an efficient application for experimental studies in stressed environments. Thus, our aim is to contribute to the knowledge of the mechanisms of formation of reactive oxygen species (ROS), defense and metabolomics mechanisms in species of bryophytes present in forest fragments in Southeast Brazil, as well as the understanding of cellular events related to control of the survival of these species when exposed to gradients of environmental stresses caused by UV-B radiation and water deficit. The experiment was carried out in two biochemical oxygen demand (BOD) chambers, with programmable photoperiod and temperature (12/12 h photoperiod and 25 ° C). The first chamber was used as a control, with no UV radiation and a regular water supply. The second chamber simulated UV radiation (2h/day for 1 week) and water deficit, with trays without a water supply. The species used were *Atrichum androgynum*, *Dumortiera hirsuta* and *Symphyogyna brasiliensis*. Preliminary results indicated that at the end of the experiment, the enzymes present in the antioxidative defense system - such as catalase, ascorbate peroxidase, superoxide dismutase and glutathione reductase - showed a reduction in their contents in the experiment with exposure to UV-B radiation in the three species analyzed. The non-enzymatic compounds - ascorbic acid and glutathione - also showed a reduction in UV-B treatment. Metabolomics analyzes - carbohydrates, starches and phenolic substances - and formation of EROS - such as the superoxide anion, hydrogen peroxide and hydroxyl radical - will be performed to better understand the results.

### 355 - Exogenously applied sugar effect on silver birch (*Betula pendula*) in vitro shoot rejuvenation

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Tissue recalcitrance is a major factor limiting mature woody plant micropropagation. Sugars act as signals that mediate vegetative phase change in plants. To obtain a better understanding of the role of sugars in birch in vitro shoot rejuvenation, we investigated the effect of sucrose and glucose on rejuvenated proliferating and mature recalcitrant birch in vitro shoots. Shoot cultures initiated in vitro from 20 years old silver birch were used in this experiment. The effect of 2 %, 4 % and 6 % sucrose, 2 % and 4 % glucose, as well as 2 % sucrose + 2 % glucose on main shoot length, lateral shoot formation, peroxidase and polyphenol oxidase activity in leaves and stems was assessed. Elevated sucrose concentration (4 %, 6 %) in juvenile in vitro birch shoots inhibited shoot growth and proliferation and caused a decrease in peroxidase activity in leaves and stems. Active plant growth, as indicated by high peroxidase activity was observed with 2 % sucrose as well as 2 % and 4 % glucose. Polyphenol oxidase activity increased in stems in the presence of high concentrations of both sugars, enhancing synthesis of phenols, that contributes to recalcitrance. Thus, high sucrose concentration plays an important role in rejuvenated birch in vitro shoot maturation processes. Mature birch in vitro shoots grown in medium with 2 % and 4 % glucose showed high shoot proliferation by promoting rejuvenation but 4 % and 6 % sucrose had a negative effect on main shoot length and lateral shoot formation. Peroxidase activity in stems of mature shoots increased with 4 % and 6 % sucrose concentrations, contributing to lignification processes. In mature shoot leaves and stems, polyphenol oxidase activity was higher compared to rejuvenated shoots, contributing to possible higher phenol synthesis and oxidative stress in mature shoots, leading to recalcitrance.



### **356 - The effect of different light spectrums on growth and development of Silver birch (*Betula pendula* Roth) in vitro cultures**

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Light emitting diodes (LED) offer many advantages over conventional fluorescent lighting as a light source for in vitro cultures. LEDs are energy efficient, produce less heat and the spectral composition can be adjusted to specific requirements. Currently indoor farming systems utilise adapted LED lighting spectrums for various crop species, whereas there are limited solutions for micropropagation of woody tree species. Our aim was to develop an innovative LED lighting system specifically adapted for in vitro propagation of silver birch (*Betula pendula* Roth) clones. Cultures were grown under LED lighting with three different spectral compositions: 1)Red+Blue (RB) 2)Red+Green+Blue (RGB) 3)Red+Orange+Yellow+Green+Blue (RGBYO) and fluorescent tubes (FL) as control lighting. Photon flux density was constant at  $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ . To evaluate the effect of different light spectrums, we compared plant growth parameters and propagation ability. We did not observe significant differences of growth parameters (main stem length, total shoot length, number of internodes, length of third internode) nor multiplication index between LED and control lighting (FL). However, differences were observed where both the main stem length and total shoot length were significantly higher for plants when grown under RGBYO as compared to RB LED lighting. The average area of a single leaf and total leaf area of one plant was significantly lower for plants grown under RB as compared to FL, RGB and RGBYO lighting. We also observed a significant decrease in chlorophyll a, fluorescence parameter Fv/Fm values for plants grown under RB compared to FL control lighting. Overall the use of a narrower spectral composition (RB) resulted in a reduction of plant growth, compared to the control lighting (FL) and LED with a broader spectral composition. The energy efficiency of LED lighting and the possibilities to arrange specific light spectrum combinations promotes the replacement of traditional fluorescent tubes.

### **363 - Phenotyping for drought tolerance traits in common bean (*Phaseolus vulgaris* L.)**

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Various plant phenotyping platforms which combines sophisticated imaging techniques with automatic data analysis have been developed in order to assess plant performance under different environments. Detection of the phenotypic traits in response to drought is the basis of conventional and alternative plant-based methods for crop water status monitoring as well for identification of potential traits for drought tolerance improvements by breeding. The long tradition of common bean cultivation in Croatia has enabled the evolution of many landraces which poses considerable genetic variability and extreme adaptation to different environmental conditions. Aims of this experiment were to quantify changes in morphological and physiological traits in common bean landraces under drought stress and to determine the most sensitive trait to drought stress by using chlorophyll fluorescence and multispectral imaging. Five Croatian common bean landraces, 'Trešnjevac', 'Tetovac', 'Biser', 'Zelenčec' and 'Puter' were grown in controlled conditions in solutions with different concentrations of polyethylene glycol 8000 forming drought treatments: control (0 MPa), -0.5 MPa, -1 MPa and -1.5 MPa. Physiological and morphological traits were monitored on daily basis for one week, using CropReporter™ chlorophyll fluorescence and multispectral imaging cabinet and PlantEye F500 multispectral 3D scanner.

Drought treatments decreased all measured morphological traits, with highest reduction and earliest response in digital biomass, total leaf area and leaf area index. Color and multispectral parameters shown different trends regarding drought treatment. Namely, drought treatments decreased normalized difference vegetation index, normalized pigments chlorophyll ratio index, but increased HUE, chlorophyll content index and anthocyanin index, on from fourth day of drought treatment. Chlorophyll fluorescent parameters has been shown to be less sensitive to drought in comparison with multispectral and morphological parameters, with electron transport rate and non-photochemical quenching showing to be most sensitive. As was shown by all measured traits 'Tetovac' could be considered as most tolerant landrace.

**Guidelines for abstract submission**

### 366 - The molecular mechanism integrating hypoxia signaling with sugar availability

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During flooding plants are exposed to low oxygen stress. Hypoxia activates expression of several genes, including those encoding for fermentation enzymes. As ATP production via the mitochondrial electron transport chain requires oxygen, plants activate ethanol and lactic fermentation to sustain glycolysis to provide a basal level of cellular energy. This requires the input of sugars and therefore carbon availability is crucial for survival in low oxygen conditions. However, the strength of the anaerobic response requires fine-tuning so it does not deplete the carbohydrates reserves of the plant before flooding recedes. This hypothesis is supported by the observation that plants subjected to sugar starvation under low oxygen conditions show a reduced expression of hypoxia responsive gene ALCOHOL DEHYDROGENASE (ADH). However, the molecular mechanism that integrates sugar reserves and anaerobic response remains elusive. Previously, we showed that the repression of the anaerobic response due to sugar starvation is downstream of the hypoxic stabilization of the ethylene responsive factor VII (ERF-VII) proteins. Interestingly, the analysis of a hypoxia-inducible pPCO1:GUS reporter line revealed that the response dampening take place at the gene expression level. Taken together, these data indicate that the fine-tuning of anaerobic response occurs upstream of interaction of ERF-VII transcription factors with hypoxia responsive genes promoters. In this context we aim to understand the regulation of transcriptional activity of ERF-VII proteins during sugar starvation in *Arabidopsis thaliana*. Remarkably, we identified two phosphorylation sites in RAP2.12 which are involved in the dampening of anaerobic response upon carbohydrate starvation, providing a starting point to unravel the molecular mechanism.

### 370 - Response of the circadian clock to drought stress in *Brachypodium distachyon*

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As synchronizing biological processes to periodic environmental changes has a great contribution to fitness, living organism evolved inner chronometer called circadian clock for measuring time and timing of physiological, metabolic and developmental processes. Composition of plant circadian clock has been established in the model dicot *Arabidopsis* and its crucial role in time-based patterning of development and stress responses in green parts is widely known. However, our knowledge on circadian clock in plants other than *Arabidopsis* is still rudimentary and literature is merely restricted to shoots. Here we present a comprehensive study of core clock genes and their response to modest drought stress in shoots and roots of the model monocot *Brachypodium distachyon*. Changes in relative transcript amounts of clock components (PRR95 – Bd4g36077; LHY/CCA1 – Bd3g16515; TOC1 – Bd3g48880; GI – Bd2g05226; LUX – Bd2g62067; ELF3 – Bd2g14290; ELF4/1 – Bd4g13227; ELF4/2 – Bd1g60090; ELF4/3 – Bd4g29580) were monitored for three and a half days by qRT-PCR in green plant parts and in roots under three different light regimes [standard long-day (18:6 light:dark photoperiod), continuous light and continuous dark] after two weeks of mild water depletion (40% soil water content). We show that amplitude and phase of clock gene expression in root differs markedly from that in the shoot, even under control conditions. Evening loop genes (LUX, GI and ELFs) did not show rhythmic expression in root neither under well-watered nor water-limited conditions indicating a simplified form of circadian clock in the root. In addition, ELF3 and ELF4 family did oscillate neither in shoot nor in root implying differences in clockwork of *Arabidopsis* and *Brachypodium*. Under drought stress expression levels of PRR95 and LHY/CCA1 were slightly elevated while expression of TOC1 showed slight phase advance in roots but not in green plant parts suggesting organ specific differences of the circadian clock in response to water depletion.

### **373 - Characterization of Fe-transporting proteins AtMIT1, AtMIT2 and AtMFL1 from Arabidopsis thaliana**

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Arabidopsis thaliana AtMIT1 and AtMIT2 (Mitochondrial Iron Transporter 1 and 2) as well as AtMFL1 (Mitoferrin-like 1) belong to the Mitochondrial Carrier Family (MCF) of proteins implicated in the transport of very diverse compounds, including iron ions. AtMIT1 and AtMIT2, also called mitoferrins, form a functional and separated cluster within MCF carriers, in which AtMFL1 is an additional member, with a more distant relationship to the other two proteins. When expressed in yeast and protoplasts isolated from A. thaliana cells, AtMIT1 and AtMIT2 localized in mitochondria and contributed to the influx of Fe into mitochondria. In contrast, AtMFL1 localized in mitochondria when expressed in yeast, but it didn't restore the growth of low Fe-sensitive yeast mutant on Fe-deficient medium. Analysis of expression in A.thaliana protoplasts showed that AtMFL1 is a chloroplast protein with a N-terminal targeting peptide (92 aa). The protein coding transcripts of all three genes were detected both in the roots and shoots of 8 weeks-old plants. Under control conditions, the expression of AtMIT1 and AtMFL1 was highest in shoots while AtMIT2 showed the highest transcription in roots. In plants grown under Fe excess or deficiency, the expression profile varied depending on analyzed gene and organ, exhibiting lower levels of transcript in roots (AtMIT1, AtMIT2) or shoots (AtMFL1) under Fe-deficiency conditions. Overall, these results indicate that AtMIT1 and AtMIT2 function as the mitochondrial iron importers contributing to the distribution of iron between cytosol and mitochondria of plant cells. On the other hand, AtMFL1 is the chloroplast protein with 92 amino acid N-terminal signal peptide. Additionally, obtained results show that mitoferrin gene expression is organ-specific and is regulated by Fe availability to maintain both cellular and whole plant iron homeostasis.

### **384 - Effects of graphene-related materials on the sexual reproduction of Cucurbita pepo L.**

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Products containing graphene-related materials (GRMs) are spreading in our society raising at the same time concerns for environmental safety. GRMs have widely varying effects on the vegetative body of plants thus they might also influence their sexual reproduction process. The first in-vitro experiments on the pollen of *Nicotiana tabacum* L. and *Corylus avellana* L. demonstrated that graphene oxide (GO) and few-layer graphene (FLG) impair pollen performances, i.e. pollen germination and pollen tube elongation. This was due to the pH and Ca<sup>2+</sup> content decrease in the germination medium (GO) and to an impairment of the pollen tube ROS distribution (FLG and GO). In this work, we verified the effects of GRMs and muscovite, a phyllosilicate structurally similar to graphene, on pollen, stigma and on their interaction in *Cucurbita pepo* L., whereas GO acidity in comparison to other GRMs (FLG, reduced-GO) was tested on the stigma. Pollen was mixed or not (controls) with FLG or muscovite (treated) to concentrations of 0.5 and 2.0 µg/mg and its viability was monitored for six hours. Stigmas were exposed or not (controls) to FLG or muscovite for three hours and then observed at E-SEM to verify possible alterations to the receptive surface. Control and treated stigmas were then hand-pollinated to verify possible effects on pollen adhesion and in-vivo pollen germination. The GRMs acidification capacity was verified increasing progressively their concentrations from 0 to 100 µg/mL. FLG and muscovite did not impair pollen viability nor affect the stigmatic surface which was able to buffer the acidity of GO. Accordingly, pollen could still germinate at a normal rate. However, both materials increased pollen detachment by 126% compared to controls. Considering this, it seems that only strong GRMs depositions might decrease the pollination yield which could result in an alteration in fruits and seed production and development.

### **393 - Vacuolar H<sup>+</sup>-ATPase activity under cadmium stress conditions depends on the form of nitrogen fertilizer**

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Soil contamination with cadmium is one of the most serious environmental problems. It is estimated that 30,000 tons of this heavy metal is released into the atmosphere each year. Because cadmium is not degraded, its concentration in the soil will continue to increase.

Although Cd toxicity in plants was broadly studied, only few reports showed that plant stress response may depend on the form of nitrogen fertilizer. Unfortunately, the molecular bases of this process are mostly unknown. Presented studies revealed that nitrate versus ammonium nutrition induce quite contrary changes in activity of the main tonoplast proton pump – vacuolar H<sup>+</sup>-ATPase (V-ATPase, EC 3.6.3.14).

V-ATPase, using ATP as a substrate, translocates H<sup>+</sup> ions to the vacuolar lumen and generates the proton gradient across the tonoplast. This gradient is used as a power for secondary transport of ions and small metabolites into vacuole. V-ATPase regulates intracellular pH and ionic homeostasis, including regulation of Ca<sup>2+</sup> level and sequestration of harmful compounds, like Cd<sup>2+</sup> or Na<sup>+</sup>, in vacuole. In addition, it influences growth processes by regulating the expansion of the central vacuole, and in consequence the cell turgor as well as is directly involved in the stomatal opening. Moreover, V-ATPase is localized not only in vacuole but also in Golgi apparatus, endoplasmic reticulum, endosomal vesicles or lysosomes and is responsible for acidification of these organelles, endocytosis and vesicle trafficking (including possibility of Cd<sup>2+</sup> trafficking).

Taking into account that V-ATPase participates in many process essential for cell functioning, changes in enzyme activity may be crucial for plant heavy metal tolerance. To the best of our knowledge these data represent the first study of V-ATPase regulation in Cd stress condition under different nitrogen supply.

**396 - Phytoextraction efficiency of *Pteris vittata* grown on a naturally As-rich soil and characterization of As-resistant rhizosphere bacteria**

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This study evaluated the phytoextraction capacity of the fern *Pteris vittata* grown on a natural arsenic-rich soil of volcanic-origin from the Viterbo area in central Italy. This calcareous soil is characterized by an average arsenic concentration of 750 mg kg<sup>-1</sup>, of which 28% is bioavailable. By means of micro-energy dispersive X-ray fluorescence spectrometry ( $\mu$ -XRF) we detected As in *P. vittata* fronds after just 10 days of growth, while a high As concentrations in fronds (5,000 mg kg<sup>-1</sup>), determined by Inductively coupled plasma-optical emission spectrometry (ICP-OES), was reached after 5.5 months. Sixteen arsenate-tolerant bacterial strains were isolated from the *P. vittata* rhizosphere, a majority of which belong to the *Bacillus* genus, and of this majority only two have been previously associated with As. Six bacterial isolates were highly As-resistant (>100 mM) two of which, homologous to *Paenarthrobacter ureafaciens* and *Beijerinckia fluminensis*, produced a high amount of IAA and siderophores and have never been isolated from *P. vittata* roots. Furthermore, five isolates contained the arsenate reductase gene (*arsC*). We conclude that *P. vittata* can efficiently phytoextract As when grown on this natural As-rich soil and a consortium of bacteria, largely different from that usually found in As-polluted soils, has been found in *P. vittata* rhizosphere.



#### **414 - Arsenic uptake and translocation to caryopses in durum wheat**

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Heavy metal in soil are dangerous for crop quality and productivity. Among these pollutants, Arsenic (As) is one of the most widespread in Italy. To evaluate the health risk to humans due to the consumption of products derived from durum wheat (*Triticum turgidum* L. subsp. durum) cultivated in contaminated soils, it is necessary to collect information regarding the As plant uptake and the related accumulation in grains.

Sixteen commercial varieties of durum wheat were exposed to a nontoxic level of As (V) (500 ng/l) to study its final concentration in grains, shoots, and roots as well as the heavy metal effects biomass production. Three genotypes (low, high and intermediate As accumulators in grains) were selected and used for further analyses.

Arsenic treatment modified the lateral root development and organization with adverse consequences on root system. The roots of As-treated plants appeared shorter, with more lateral ramification and with an increased cell wall thickness.

A transcriptome analysis of root tissues has highlighted the up-regulation of several transcription factors such as bHLH29, bHLH38 and bHLH47 determining a reduced translocation of As to leaves and grains (low-As plants). The As treatment also induced a strong activation of metal transporters (even 10-fold for some genes) like HMA, ABC transporter, ZIF and YSL which is specific for nicotianamine. Moreover, by HPLC-MS, we determined that the level of such metal chelator was really high in roots of low-As plants in comparison to high-As plant, suggesting a key role of nicotianamine in As chelation and root storage.

Finally, we have determined the localization of As in the grain tissues using TEM-EDX, observing an high level of As in germ and bran (and not in endosperm), suggesting that the use of wholemeal wheat could introduce more As into human diet compared to the use of semolina flour.

### **433 - Physiological phenotyping of tomato plants performances under combined abiotic stresses and biostimulant application**

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Abiotic stresses, including drought and heat stress, are amongst the main limiting factors for plant growth and productivity in crops, such as tomato (*Solanum lycopersicum*) that is regarded as a heat sensitive crop. In the framework of an EPPN2020 plant phenotyping program, we investigated the effects of one plant-based biostimulant (CycoFlow - Agriges) on physiological responses of two tomato genotypes (E42 and LA3120) subjected to heat stress, drought and combined stress. In general, the effect of the biostimulant was linked to the different stress applied and to the investigated genotype. The application of CycoFlow increased plant height (up to 11.86%), number of leaves (up to 29.89%), shoot fresh weight (up to 28.12 %) and chlorophylls content (up to 12.03 %) in plants subjected to combined stress. Probably, the presence of signaling molecules in the biostimulant, such as free amino acids, promoted endogenous phytohormonal biosynthesis thus stimulating growth. An increase in net photosynthetic rate ( $P_N$ ) and in maximum quantum efficiency of photo system II ( $F_v/F_m$ ) was also registered in E42 treated plants subjected to drought. This could be related to CycoFlow capacity to maintain cell turgidity through higher water retention induced by its high content of glycine betaine. This last compound can also promote the activity of specific enzymes involved in antioxidant homeostasis in plants. Accordingly, our data indicate that biostimulant treatment induced the activation of the antioxidant defense system, as demonstrated by the higher content of ascorbic acid and the lower amount of proline,  $H_2O_2$  and malodialdehyde detected in treated plants subjected to combined stress. Altogether, these results will give an important contribution to the choice of management practices to improve plant performances and final yield under abiotic stress. Biochemical and metabolic analyses are under way to further understand the mechanisms controlling tomato responses to biostimulant application and abiotic stress.

#### **466 - The Potential Use of *C. reinhardtii* and *C. sorokiniana* as biostimulants on maize plants.**

**Giorgia Beghini <sup>(1)</sup> - Flavio Martini <sup>(1)</sup> - Anita Zamboni <sup>(1)</sup> - Matteo Ballottari <sup>(1)</sup>**

**University of Verona, Department of Biotechnology, Verona, Italy <sup>(1)</sup>**

The use of plant biostimulants (PBs) on crops is a very promising application for the future agriculture to improve crop yield, but especially to prevent the effect of abiotic stresses. Micro-algae derived biostimulants represent an efficient tool to stimulate the root development, also under nutrient deficiency. The aim of this work is to test the stimulant ability of *Chlamydomonas reinhardtii* (CR) and *Chlorella sorokiniana* (CS) cells on maize roots. We tested two different extracts for both the algae species using un-treated and physically broken cells, to analyze if nutrients were more available after the disruption of cellular wall and membrane. Both CR and CS promoted the maize root system compared to the untreated negative control, but CS seemed to increase especially the number of secondary roots. The ICP-MS analysis showed that CR mostly affects the micro-nutrients accumulation on maize roots and shoots, while physiologic analyses showed that CS enhances the tolerance to abiotic stresses. Nitrogen (N) deficiency is one of the major problems in agriculture, affecting plant development and inducing visible chlorosis on shoots. Moreover, water deficiency is another negative abiotic stress for the crop yield, due to water deficiency itself or to the high soil salinity. Thus, the two CS extracts were tested under both N deficiency and drought stress showing an improved micro-nutrients accumulation and development of the root system compared to the control, respectively. Photosynthetic parameters were analyzed for all the experiments showing significant differences only for the CS untreated cells extract compared to the control under water stress condition.

#### **484 - The Potential Use of *C. reinhardtii* and *C. sorokiniana* as biostimulants on maize plants.**

**Giorgia Beghini <sup>(1)</sup> - Flavio Martini <sup>(1)</sup> - Anita Zamboni <sup>(1)</sup> - Matteo Ballottari <sup>(1)</sup>**

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#### **485 - Redox balancing in salt stress tolerant and sensitive varieties of rice: focus on glutathione metabolism**

**Sara Cimini** <sup>(1)</sup> - **Vittoria Locato** <sup>(1)</sup> - **Valentina Giacinti** <sup>(1)</sup> - **Laura De Gara** <sup>(1)</sup>

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The knowledge of the metabolic alteration induced by stresses in tolerant and resistant plants is pivotal for identifying traits of interest for improving plant resilience toward unfavourable environmental conditions. This is of particular interest for those crops representing worldwide a main food source, like rice.

In this work, a detailed analysis of the salt stress-dependent modulation of the intricate redox network is presented. The study has been performed at different levels (phenotyping, gene expression, metabolomic profile of redox homeostasis players and kinetic activities of the main ROS scavenging and redox enzymes) in shoot and roots of two varieties of rice with different salt stress sensitivity (Baldo and Vialone nano, tolerant and sensitive, respectively). The different phenotypes, in term of biomass and growth, are coherent with a different behaviour of cell cycle and cell death patterns, analysed by cytofluorimetric assays and gene expression.

We also shown that Baldo presents a more performant antioxidative capacity, already when growth in control condition, and a more efficient capacity to activate enzymes involved in redox homeostasis and in the synthesis of redox metabolites after salt stress exposure. Consistently, Baldo stressed plants show levels  $H_2O_2$  lower than that of Vialone Nano in all the analysed tissues. Moreover, a fine modulation of the glutathione metabolism, in terms of its synthesis, is observed in tolerant Baldo plants, suggesting an efficient cross-talk between GSH metabolic network and the modulation of growth pathways as a salt stress response. Taken together our results contribute to highlight the role of ROS and antioxidative pathways as a part of a complex signalling network working in plant responses against salinity. A better knowledge of the mechanisms acting in tolerant varieties, will also allow the identification of effective strategies aimed at increasing rice resilience toward salt stress, one of the main consequences of climate change.

#### **492 - Impact of different irrigation regimes on the regulation of suberin synthesis and cork development**

**Helena Sapeta** <sup>(1)</sup> - **Pedro Barros** <sup>(1)</sup> - **Diogo Lucas** <sup>(1)</sup> - **Joana Belo** <sup>(1)</sup> - **Ana Rita Leal** <sup>(1)</sup> - **M. Margarida Oliveira** <sup>(1)</sup>

**GPlantS Unit, Instituto de Tecnologia Química e Biológica António Xavier (ITQB NOVA),, Oeiras, Portugal** <sup>(1)</sup>

Forest-based materials, such as cork, are key sources of biopolymers with added-value properties and acting as major carbon sinks. Yet, the development of sustainable strategies for growth intensification is needed to support a bio-based economy. The urgency to develop such strategies is emphasized considering the ongoing climatic alterations. In this context, we aimed to study cork development and suberin composition as function of stress (vs. control)-inducing environmental signals, with a special focus on stress-responsive genes and pathways regulating suberization. To contribute to this aim, we have studied the effect of different irrigation regimes on cork development. In detail, one-year-old potted cork oak (*Quercus suber*) plants grown in greenhouse conditions were subjected to well-watered or water-deficit conditions for a period of 6 months (March-September, 2020). Along the experimental period stem diameter growth was monitored monthly and at the end of the assay, stem samples were collected for histology analysis, suberin chemical profiling and whole transcriptomic study of dissected xylem, phloem and phellem (cork) using RNA-seq. Differences in stem diameter were detected 3 months after starting the different irrigation treatments, with larger stems observed in well-watered plants. Six months after treatment initiation, well-watered plants presented 45% larger stems as compared to plants submitted to water-deficit. These differences in stem growth were visible in the stem histology studies, with well-watered plants presenting larger phloem and xylem areas. However, despite the differences in growth, no differences were observed in cork thickness. Transcriptomic data comparing the different tissues produced in contrasting irrigation regimes are currently ongoing and will be presented at the meeting. Our work will provide an integrated view of the biosynthetic pathways regulated by environmental conditions, to further support the design of science-based strategies capable of promoting cork oak growth acceleration while ensuring cork quality.

#### **493 - The distinctive molecular reprogramming induced by exogenous polyamines in tomato plants under non-stress and salinity conditions**

**Begoña Miras Moreno**<sup>(1)</sup> - **Valentina Buffagni**<sup>(1)</sup> - **Leilei Zhang**<sup>(1)</sup> - **Biancamaria Senizza**<sup>(1)</sup> - **Luigi Lucini**<sup>(1)</sup>

**Università Cattolica del Sacro Cuore, Department for Sustainable Food Process, Piacenza, Italia**<sup>(1)</sup>

Polyamines (PAs) have putative function(s) in adaptive responses to various environmental stresses. These plant growth regulators have been proposed as secondary messenger in plant signalling. In our study, different PAs (spermine, spermidine and putrescine) were applied to stressed (100 mM NaCl) and non-stressed tomato plants. Morphophysiological parameters (biomass, SPAD, root length) were initially measured. Thereafter, an exhaustive characterization of the metabolomic profile of plants was performed to unravel the molecular and biochemical processes elicited by the application of exogenous PAs. To this aim, a UHPLC chromatographic system coupled to a hybrid quadrupole-time-of-flight mass spectrometer (UHPLC/QTOF-MS) was used. Multivariate statistics allowed discerning the metabolic changes in plants after PAs application and to identify differential metabolites. Both unsupervised hierarchical clustering and supervised OPLS-DA from untargeted metabolomics allowed discriminating the most effective treatment in stress mitigation. In order to shed light on the complexity of the physiological response to exogenous PAs, the data were then interpreted using the PlantCyc Pathway Tool Software.

Our findings revealed a broad modulation of plant metabolism following PAs application and indicated a synergic effect between the different PAs. In particular, secondary metabolism was elicited in all the cases. Nitrogen-containing secondary metabolites and phenylpropanoids appeared to play a pivotal role in plant response to the application of PAs under salinity. Notably, a distinct reprogramming of phytohormones took place when PAs are applied. These modulation of phytohormones seemed to be stronger when PAs were applied together with NaCl, appearing brassinosteroids and auxins as a major player.

## 508 - Glutathione transferases in roots as antioxidants and redox modulators

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Glutathione transferases (GSTs) participate in a broad network of catalytic and regulatory functions. Their most known role is the detoxification by catalyzing the conjugation of glutathione (GSH) to harmful electrophilic compounds, but they are also involved in numerous redox-, hormone- and stress responses. Mediating cross-talks between these signaling pathways, they have important roles in developmental processes, such as apoptosis or regulation of growth. Applying different mannitol, NaCl and salicylic acid (SA) treatments on *Arabidopsis thaliana* revealed that changes in AtGST expression pattern and GST activities are important part of the stress responses. Detailed analysis of the reactive oxygen species (ROS) levels and redox status in roots of wild type and Atgst mutants indicated that some AtGST isoenzymes function in fine-tuning the redox homeostasis both under control and stress conditions. Involvement of GSTs in salt stress responses and SA-induced priming of tomato was found also in our earlier studies. Now we aim to compare the stress responses of two tomato cultivars, particularly regarding to the relationship between the redox status and GSTs.

Four-week-old *Solanum lycopersicum* cv. Moneymaker and Ailsa Craig plants were treated by NaCl, mannitol and SA. The two tomato cultivars have different stress sensitivities and there are significant differences in ROS, ascorbate and glutathione levels and their redox potentials. The expression pattern of redox-related and SIGST genes investigated by high-throughput quantitative PCR (HTS-QPCR) showed cultivar- and stress-specific changes. The redox potential of cv. Ailsa Craig was more oxidized compared to Moneymaker even under control conditions and became more positive due to treatments. To investigate the redox status of plants the redox sensitive green fluorescent protein (roGFP2) redox probe was introduced into cv. Moneymaker.

Financial support to the project was provided by the Hungarian National Research, Development and Innovation Fund (grant numbers are K 125265, PD 131909 and PD 131884).



## **512 - ROS dynamics in Arabidopsis thaliana ecotypes with deep and shallow rooting model under salt stress**

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**Università Campus Bio-Medico di Roma, Plant Science, Rome, Italy**<sup>(1)</sup> - **National Research Council of Italy (CNR-DISBA), Department of Biology, Agriculture, and Food Sciences, Rome, Italy**<sup>(2)</sup>

Root system architecture (RSA) is controlled by different mechanisms reflecting plant evolution and different strategies of foraging in the soil and adaptation to soil-borne stresses. Salinity is known to inhibit primary root growth and to generate Reactive Oxygen Species (ROS) that further control developing and stress responsive pathways. Nine Arabidopsis thaliana natural accessions previously selected for their different RSA development (shallow or deep rooting) were grown under control and two salt stress conditions (75 and 150 mMol of NaCl). 2D phenotyping was performed to follow root development. We ranked plants according to the principal root growth rate from the longer (accession Ty-0) to the shortest (accession Sha).

We speculated that growth of the principal root may indicate tolerance to salt stress and may be correlated with further measurements of salt sensitive traits (e.g. photosynthesis, stomatal conductance) in the analyzed accessions. We are also monitoring ROS accumulation with confocal microscopy, using specific probes for superoxide and hydrogen peroxide, and measuring the expression of ROS related genes (SOD, APX, CAT, ROBHD and ROBHF) through Real Time PCR.

If ROS accumulation and ROS expression related genes are associated to root growth reduction, and indicate sensitivity to salt, the study of patterns and stress consequences in model plants where genotypic diversity is large and well characterized (like Arabidopsis) may help selecting the most performant RSA to sustainably exploit soil resources and the use of saline land for agriculture.

## **516 - Foliar application of Si and nano-SiO<sub>2</sub> improves tomato tolerance to glyphosate – oxidative damage and antioxidant responses**

**Cristiano Soares**<sup>(1)</sup> - **Pedro Nadais**<sup>(1)</sup> - **Bruno Sousa**<sup>(1)</sup> - **Ruth Pereira**<sup>(1)</sup> - **Fernanda Fidalgo**<sup>(1)</sup>

**GreenUPorto - Sustainable Agrifood Production Research Centre, Department of Biology, Faculty of Sciences of University of Porto, Porto, Portugal**<sup>(1)</sup>

Given the widespread use of glyphosate (GLY)-based herbicides, this agrochemical is becoming a concerning environmental contaminant, especially in agricultural soils, capable of affecting non-target organisms, such as crops and soil biota. Therefore, sustainable strategies to boost the plant's tolerance to GLY need to be explored. Within this perspective, and recalling the role of silicon (Si) against different abiotic stresses, the main goal of this study was to evaluate whether the foliar application of Si (1 mM), either as bulk (sodium metasilicate) or nano (nano-SiO<sub>2</sub>) form, is capable of enhancing *Solanum lycopersicum* L. (tomato) tolerance to GLY (10 mg kg<sup>-1</sup>). After 28 days of exposure, GLY-treated plants exhibited growth-related disorders in both shoots and roots, which resulted in reduced biomass and organ elongation. This macroscopic phytotoxicity was concomitant with an overproduction of superoxide anion in both organs, and an increase of malondialdehyde, indicative of lipid peroxidation (LP), in shoots. Although plants exclusively exposed to GLY activated several non-enzymatic antioxidant (AOX) mechanisms (proline, ascorbate, and glutathione) in both analysed organs, a generalized inhibition of AOX enzymes was found, leading to an unbalanced cellular redox state. However, in response to bulk Si and nano-SiO<sub>2</sub> application, most of the inhibitory effects of GLY on growth have been mitigated, being accompanied by a better redox management, with reduced levels of ROS and LP. This protective effect was presumably linked to an upregulation of the main AOX enzymes, including superoxide dismutase, catalase and ascorbate peroxidase, which were generally enhanced upon treatment with nano-SiO<sub>2</sub> and, especially, bulk Si. Although some parameters responded differently to both sources of Si, no major changes concerning the ameliorative efficacy were registered between bulk Si and nano-SiO<sub>2</sub>. Overall, the data herein presented pointed towards the alleviation of GLY-induced oxidative stress by the foliar application of Si, either as bulk or as nanomaterial.

**534 - Beneficial role of silicon (Si) on giant reed (*Arundo donax* L.) exposed to antimony (Sb) phytotoxicity.**

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Antimony (Sb) is a metalloid without any specific role in plant metabolism. However, it is a carcinogen and emerging pollutant which causes detrimental effect to environment and living beings. Silicon (Si) is an element extensively studied for beneficial effects against biotic and abiotic stress in plants. The mechanism of toxicity alleviation can, however, be different for different toxic metals or metalloids and can also vary between plant species. For instance, Si can mitigate phytotoxicity by complexation and co-precipitation with metals, by regulation and expression of transport genes, by stimulation of antioxidant systems, by various structural alterations of affected tissues, etc. Hence, we wanted to investigate the potential mechanisms of Sb toxicity alleviation by Si in giant reed (*Arundo donax* L.), a high biomass accumulating grass species. Plants were grown in a hydroponic experiment for ten weeks in controlled environment. Those exposed to 20 mg kg<sup>-1</sup> Sb were under severe stress, which was evident in reduced plant biomass and root length. However, Si treatment at 1 mM improved plant biomass and root length. In addition, the photosynthetic pigment content and the rate of net photosynthesis were significantly improved, suggesting that Si treatment improved overall photosynthetic efficiency. The phytotoxicity may have been reduced by a substantial reduction in Sb shoot uptake under Si treatment. Lower translocation of Sb from root to shoot indicated that giant reed is a root accumulator of Sb, whereas high accumulation of Si in both root and shoot indicated that it is an Si accumulator plant. However, co-localization of Sb with Si was not observed in root or shoot tissues. Results of our study showed the potential of Si to alleviate Sb toxicity and further enhance the Sb tolerance of giant reed for remediation of Sb-contaminated sites.

### 537 - Wrack application as a sustainable practice to mitigate Cu-induced stress in barley plants

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Soil contamination with metals is a worrying problem, threatening agriculture on a global scale. Copper (Cu) is a metal, whose increasing accumulation in soils is due to its widespread use in both conventional and organic agriculture. Although Cu is a micronutrient, therefore, essential for plant growth and development, it can also be phytotoxic in high concentrations, leading to photosynthetic impairment, oxidative stress and, ultimately, growth reduction, becoming imperative the use of sustainable strategies to mitigate these effects on crops. Thus, this study aimed to use wrack, an organic waste of marine biological productivity, as a soil amendment to mitigate Cu-induced stress in *Hordeum vulgare* L. (barley). For this purpose, barley plants were grown in agricultural soil prepared to test the following treatments: Cu (219 mg kg<sup>-1</sup>); wrack 2% (m/m), and Cu (219 mg kg<sup>-1</sup>) + wrack 2% (m/m). In parallel, a control without Cu and wrack was considered. After 14 days of plant exposure, Cu-induced impairment of growth-related parameters was reverted with the application of wrack, along with a lower metal accumulation in the roots. Additionally, Cu caused oxidative stress, evidenced by the high levels of superoxide anion in the leaves and roots, and, by the high hydrogen peroxide and lipid peroxidation (LP) by-product content in the roots. To overcome Cu-induced stress, the non-enzymatic antioxidant (AOX) system was activated, as shown by the increased content of glutathione (GSH), ascorbate (AsA) and phenols. However, the accumulation of proline was inhibited, favouring the increase of LP degree. The beneficial effects of wrack against Cu toxicity are related to an alleviation of the oxidative damage, at least partially, through the increase of some AOX metabolites and a reduction of Cu bioaccumulation, especially in the roots. Overall, the results suggest the potential of wrack to alleviate Cu-induced stress in barley plants.

## 560 - Airborne graphene-related materials as a possible new hazard for anemophilous plants

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Since the discovery of graphene in 2004, graphene-related materials (GRMs) led to the development of many innovative GRMs-enabled products, which might cause the release of GRMs into the environment during their whole life cycle. Thanks to their small size and weight and bi-dimensional shape, GRMs can easily be wind-dispersed, eventually landing on plants. So far, varying effects of GRMs have been reported, mostly concerning the vegetative body at different developmental stages. Nevertheless, little is known about the effects on sexual reproduction. Recently, it was shown that graphene oxide (GO) might alter pollen performance mainly because of its acidic properties. Nonetheless, also the less reactive few-layer graphene can affect pollen-stigma interaction if in high quantity. However, direct evidence is missing that anemophilous flowers do intercept airborne GRMs and that adherent GRMs interfere with pollen germinating on the stigmatic surface.

Stigmas of *Corylus avellana* L. (common hazel) were exposed to standardized GO dry depositions in a custom-built chamber. Then in a second chamber, the anemophilous pollination process was simulated using a fan to deposit known amounts of pollen over pristine and GO-treated stigmas. The adhesion of airborne GO onto stigmatic surfaces and its possible effects on pollen-stigma interaction were verified by environmental SEM and confocal microscopy. In a second experiment, pristine and GO-treated stigmas were gently immersed in H<sub>2</sub>O<sub>d</sub> before or after pollination, simulating rain washes, to verify if these can remove adherent GRMs from stigmatic surfaces or foster the potential toxic effect of GO.

Stigmas of *C. avellana* intercept and retain airborne GO even after H<sub>2</sub>O<sub>d</sub> washes. Nevertheless, GO presence does not significantly impair pollen germination. These laboratory-based results are the first direct evidence that anemophilous species are potentially exposed to airborne GRMs in a very delicate phase of their life cycle.

## **585 - TRH1 proton coupled HAK/KUP/KT potassium transporter confers root resistance to acidic conditions**

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Root evolution allowed plants to boost their transition out of aquatic habitats towards the conquest of land. In higher plants, roots show positive gravitropic response growing downwards along the gravity vector to acquire water and nutrients and to firmly anchor in the soil. Plant hormone auxin coordinates the plasticity of root system architecture providing developmental adaptation and allowing plants to cope with adverse soil conditions. The loss-of-function mutant of TRH1, a proton coupled potassium transporter of the HAK/KUP/KT family, results in impaired auxin homeostasis at the root tip causing defective root hair elongation and root agravitropism. Here, we show that in acidic conditions (pH 4.5), the developmental response of Arabidopsis primary root is reminiscent of the trh1 mutant grown at normal conditions (pH 5.7). Remarkably, low pH further disturbed auxin distribution within trh1 roots and enhanced root agravitropism. Exogenous application of a synthetic lipophilic auxin, 1-naphthaleneacetic acid (NAA), which is membrane-permeable independent of changes in pH, restored the root hair phenotype of wild type plants under acidic conditions. As TRH1 transporter is an important component of auxin transport in Arabidopsis roots, the NAA effect supports the notion that the acidification of the rhizosphere under low-pH conditions interferes with polar cell-to-cell auxin transport. To absorb essential nutrients, plants acidify the rhizosphere establishing a proton motive force. As anticipated, when wild type seedlings grew at pH 5.7 media without a buffering agent, the roots moderately acidified the rhizosphere. Conversely, trh1 roots significantly increased media acidification due to potentially an excess export of protons that resulted in proton rhizotoxicity severely inhibiting root elongation. These findings support a novel developmental role of HAK/KUP/KT potassium transporters to increase the fitness of Arabidopsis roots on overly acidic highly-toxic soil layers.

## **589 - Localization of D27-LIKE1 and its effect on salt tolerance and phyto-hormone metabolism in *Arabidopsis thaliana***

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D27-LIKE1 is an uncharacterized member of the D27 protein family in *Arabidopsis thaliana*. Previous studies have shown that its paralog, the AtD27 is a plastid localized protein that is necessary for strigolactone biosynthesis via the all-trans/9-cis- $\beta$  carotene isomerization similar to its homolog, the first described *Oryza sativa* DWARF27. The aim of the present work was to start to characterize AtD27-LIKE1, by identifying its location, the role in stress responses and phytohormone profile in mutant plants.

In this study, we first show that AtD27-LIKE1 has the same subcellular plastid localization as its paralog. In order to determine this, the cDNA encoding the AtD27-LIKE1 was fused to a GFP containing expression vector (35S:cD27LIKE1:GFP) followed by protoplast transfection. We furthermore examined the tissue specific expression where the GUS reporter gene was driven by the full length of the AtD27-LIKE1 promoter. To elucidate the biological functions of AtD27-LIKE1, the wildtype (Ler) mutant (d27-like1) and transgenic (pD27LIKE1:GUS) plants were tested under different stress conditions. Here we show that the lack of AtD27-LIKE1 induced salt stress tolerance in plants, when NaCl was added to the media in 75, 100, 125 and 150 mM concentrations. In addition, the AtD27-LIKE1 promoter under salt stress was not as active, as in control conditions. To ascertain it, AtD27-LIKE1 expression was measured in wildtype plants growing in NaCl containing media. An increased stress tolerance was observed which indicated possible differences in the phytohormone levels. A profiling of the phytohormones in the shoots and roots showed varying levels of salicylic acid in the shoots.

In order to understand the underlying mechanisms of how a member of a carotene isomerase protein family has effect on salicylic acid and can influence salt tolerance, further examinations are needed.

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## 592 - Detection of cold and salt stress responses in Capsicum by hyperspectral measurements

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Suboptimal cultivation conditions like low temperature and high soil or irrigation salinity are limiting factors in horticultural production. Also in chilli and bell pepper production, cold and salt stress can lead to a considerable reduction of the fruit yield. It has been shown that hyperspectral measurements allow an early detection of plant stress responses, already before onset of visible stress symptoms. Leaf reflectances of a bell pepper and a chilli cultivar were strongly affected by cold stress with wavelengths around 558.5 nm and 699.2 nm being the most sensitive ones. Salt stress, however, had a much weaker effect on the leaf reflectance in both cultivars. Furthermore, the chilli cultivar showed a higher leaf reflectance sensitivity to the applied stresses than the bell pepper cultivar. This coincides with the stress-related growth reduction, which occurred stronger and earlier in the chilli than in the bell pepper cultivar. Selected reflectance indices were monitored throughout 14 days of stress treatment regarding their potential for an early detection of cold or salt stress responses. A clear, significant distinction between unstressed and cold-stressed plants could be already achieved after 4 days for chilli and after 11 days for the bell pepper cultivar through the RGI (red green index). The NDRE (normalized difference red edge index) was significantly decreased in leaves of plants treated with single cold and cold plus salt after 11 and 14 days in both cultivars. However, the selected reflectance indices allowed no distinct separation between unstressed and salt-stressed plants in either of the cultivars. Early detection of plant stress responses by hyperspectral leaf analyses can help to adjust bell pepper and chilli cultivation. Moreover, stress response quantification is a prerequisite for breeding crops with higher stress tolerance levels.



**656 - Impact of gadolinium orthovanadates nanocrystals doped with Eu<sup>3+</sup> ions on germination, elongation, mass, cell viability and oxidative stress of wheat (*Triticum aestivum* L.)**

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Lanthanide-doped nanocrystals (LDNCs) are nanoparticles increasingly used in industry, as well as in medical and biological science, because of their spectroscopic properties. However, they may entail a risk of a harmful influence on the living organisms. For this reason, the impact of GdVO<sub>4</sub> doped with Eu<sup>3+</sup> nanocrystals on wheat (*Triticum aestivum* L.) seedlings, was investigated. After three days of incubation with colloids of LDNCs at the concentrations of: 0, 10, 50 and 100 µg/ml, several endpoints of plants were analysed. As a results, the nanoparticles did not affect number of roots, roots length, roots mass, hypocotyl length and hypocotyl mass, as well as germination rate of the seedlings. Similarly, oxidative stress determined on the basis of the amount of lipid peroxidation product (thiobarbituric acid reactive substances; TBARS) of the roots were not significantly changed after the exposure to GdVO<sub>4</sub>:Eu<sup>3+</sup> nanocrystals at all used concentrations. Moreover, TTC (tetrazolium chloride) assay did not show any differences in cells' viability. On the contrary, root cells of the treated seedlings contained less amount of Evans Blue (EB) when compared to the control.

**659 - The connection between glutathione transferases and redox state – a comparative analysis of two tomato cultivars**

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Glutathione transferases (GSTs) constitute an important enzyme family due to their broad spectrum of catalytic activities. They actively participate in the cellular detoxification processes, the metabolism of certain hormones, maintaining the cellular reduction-oxidation (redox) state, taking part of the regulation of plant development, also they are involved in stress responses. Through their glutathione-dependent specific activities, they are strongly connected to the glutathione system, which is a key metabolite of the cellular redox homeostasis. The ratio of the reduced and oxidized glutathione (GSH/GSSG) and its total amount can be an effective marker of cellular redox homeostasis, acting also in ROS perception in plants. Changes in the redox state are affected by environmental conditions and genetic properties.

In our research we aimed to compare stress responses of two tomato cultivars' (*Solanum lycopersicum* cv. Moneymaker and Ailsa Craig), especially targeting to investigate the connection between the redox status and certain SIGST genes. Four-week-old plants were treated by NaCl, mannitol and SA. The two tomato cultivars showed dissimilar stress sensitivities and several differences can be observed in their physiological parameters and in their redox state. 62 redox-related and SIGST genes were investigated by high-throughput quantitative PCR (HT-QPCR) which is capable to perform QPCR-based DNA/RNA analysis in an array-like format at high-speed, in a proprietary multiwell plate in 1536-format. The results showed both cultivar- and stress-specific changes in their expression patterns, which could be connected to the differences in the two cultivars' redox balance.

Financial support to the project was provided by the Hungarian National Research, Development and Innovation Fund (grant numbers are K 125265, PD 131909 and PD 131884).

**685 - Is the synthesis of strigolactones and ABA co-ordinately repressed under irrigated conditions? A case study on ORA47 in Arabidopsis and tomato**

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The ORA47 (Octadecanoid-Responsive AP2/ERF-domain transcription factor 47) transcription factor of Arabidopsis has been found to bind the promoter regions of ABA and strigolactone biosynthetic genes during normal, but not drought conditions. Since the transcription of those same genes is activated in leaves under drought, the possibility was raised that ORA47 acts as a repressor of strigolactone and ABA synthesis, to be removed under drought for the levels of these hormones to rise. Thus, being interested in the strigolactone-ABA cross-talk with respect to the plant water status, we set to elucidate the role of this transcription factor by contrasting plants with altered ORA47 expression and their respective control genotypes. Specifically, tomato ora47 TILLING lines in a M82 background with constitutively knocked ORA47 expression; and Arabidopsis lines overexpressing ORA47 under the control of an inducible promoter were obtained. Their molecular and physiological characterisation is still in progress; yet, preliminary results in tomato showed that the ora47 knockout line has lower stomata conductance under irrigated conditions, and possibly lower sensitivity to water deprivation than sister-line controls. The expression of the SINCE4 gene, biosynthetic of ABA, was indeed increased under irrigated conditions. Also, the stem of the ora47 knock-out line has the same functional hydraulic conductivity of its corresponding sister line, while the conductive xylem area is significantly lower. All of these observations are consistent with the initial hypothesis, though in need of further confirmation.

## TOPIC:

### Plant adaptation to climate change-related stress

#### **Keynote Lecture**

##### **Unveiling a new plant molecule involved in tolerance to abiotic stress**

**Rafael Catalá <sup>(1)</sup> - Rosa López-Cobollo <sup>(1)</sup> - Alvaro Berbís <sup>(1)</sup> - Jesús Jiménez-Barbero <sup>(1)</sup> - Julio Salinas <sup>(1)</sup>**

**CSIC, Centro de Investigaciones Biológicas Margarita Salas, Madrid, Spain <sup>(1)</sup>**

Abiotic stresses, such as low temperature, drought and high salt in soils, limit the growth and development of plants, and, in the case of crops, reduce yields causing important economic losses. To survive and reproduce under such adverse environments, plants have evolved sophisticated adaptive responses that range from rapid protective mechanisms, such as osmolyte accumulation, to developmental modifications, such as reduction in the shoot/root ratio, that ultimately promote tolerance and survival. We have identified a gene from *Arabidopsis*, RARE COLD INDUCIBLE 5 (RCI5), whose transcripts accumulate in response to low temperature and encodes an active flavin-containing monooxygenase (FMO) that belongs to a subclade of the *Arabidopsis* FMO phylogeny composed by seven proteins. Our results revealed that RCI5 and other FMOs of the subclade are involved in the biosynthesis of Trimethylamine N-Oxide (TMAO), a well-known naturally occurring osmolyte in animals, whose presence in plants has not yet been reported. Interestingly, TMAO is widely distributed among plants, including important crops, and accumulates when they are exposed to abiotic stress conditions. In addition, our data also indicate that TMAO operates as a protective osmolyte in plants, promoting appropriate protein folding, and as an activator of abiotic stress-induced gene expression. Consistent with these functions, TMAO enhances plant adaptation to low temperatures, drought and high salt. We have thus uncovered a new plant molecule that positively regulates abiotic stress tolerance.

## TOPIC:

### Plant adaptation to climate change-related stress

#### Oral Communications

##### 57 - Proteomics of developing cotton pollen reveals temporal patterns under heat stress

Farhad Masoomi-Aladizgeh <sup>(1)</sup> - Matthew McKay <sup>(2)</sup> - Mehdi Mirzaei <sup>(2)</sup> - Dana Pascovici <sup>(2)</sup> - Paul Haynes <sup>(3)</sup> - Brian Atwell <sup>(1)</sup>

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Heatwaves resulting from global warming are a leading threat to cotton crops because they specifically affect reproductive (gametophytic) development. We investigated pollen development using proteomics after exposure to heat (38/28 °C; day/night) in order to identify how gene products respond in tetrads, uninucleate and binucleate microspores, and mature pollen. A library consisting of 5257 *G. hirsutum* proteins was constructed using SWATH-MS, which led to quantification of 4501 proteins at the four distinct stages. Data analysis revealed that heat stress resulted in differential expression of 880, 360, 307 and 166 proteins at tetrad, uninucleate, binucleate and mature pollen stages, respectively. High numbers of differentially expressed proteins (DEPs) were identified in tetrads compared with the late developmental stages; these were associated with biological regulation, localization, response to stimulus and reproductive process. We conclude that proteins in tetrad cells responded more acutely to heat, for example by synthesizing cellular components that enable subsequent mitoses and structural changes to the haploid germ cells. On the contrary, fewer DEPs at the later stages might reflect a lower sensitivity to high temperatures. Identifying the stage-specific proteins will lead to the identity of key heat-responsive genes and new genetic tools for improved resilience of crops as climates keep warming.

**498 - Arabidopsis bZIP18 and bZIP52 accumulate in nuclei following heat stress where they regulate the expression of a similar set of genes.**

**Anna Johanna Wiese** <sup>(1)</sup> - **Lenka Steinbachová** <sup>(1)</sup> - **Ljudmilla Timofejeva** <sup>(1)</sup> - **Vojtěch Čermák** <sup>(2)</sup> - **Božena Klodová** <sup>(2)</sup> - **Ranjani Sri Ganji** <sup>(3)</sup> - **Mariana Limones-Mendez** <sup>(1)</sup> - **Pavel Bokvaj** <sup>(1)</sup> - **Said Hafidh** <sup>(1)</sup> - **David Honys** <sup>(1)</sup>

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Heat stress (HS) is a major abiotic stress that negatively impacts crop yields across the globe. Plants respond to elevated temperatures by changing gene expression, mediated by transcription factors (TFs) functioning to enhance HS tolerance. The involvement of Group I bZIP TFs in the heat stress response (HSR) is not known. In this study bZIP18 and bZIP52 were investigated for their possible role in the HSR. Localization experiments revealed their nuclear accumulation following heat stress, which was found to be triggered by dephosphorylation. Both TFs were found to possess two motifs containing serine residues that are candidates for phosphorylation. These motifs are recognized by 14-3-3 proteins, and bZIP18 and bZIP52 were found to bind 14-3-3  $\epsilon$ , the interaction of which sequesters them to the cytoplasm. Mutation of both residues abolished 14-3-3  $\epsilon$  interaction and led to a strict nuclear localization for both TFs. RNA-seq analysis revealed coordinated downregulation of several metabolic pathways including energy metabolism and translation, and upregulation of numerous lncRNAs in particular. These results support the idea that bZIP18 and bZIP52 are sequestered to the cytoplasm under control conditions, and that heat stress leads to their re-localization to nuclei, where they jointly regulate gene expression.

## TOPIC:

### Plant adaptation to climate change-related stress

#### ***Extended Elevator Pitches***

**23 - Acclimation reduces the vulnerability of wheat flag leaf photosynthesis to warming.**

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Global food security is expected to be threatened by climate change and global warming. Models predict that mean daily temperature could rise by 2–5°C by 2100. Such warming could limit photosynthetic activity and leaf-level metabolism and thus, reduce wheat crop performance, especially during the reproductive stages.

We assessed the vulnerability of wheat leaf photosynthesis at anthesis to future warming by using a novel high-throughput thermo-fluorometer system to measure  $T_{crit}$  – the temperature at which minimal chlorophyll a fluorescence rises rapidly, indicating disruption to photosystem II. The degree of acclimation of  $T_{crit}$  to sustained warming and thermal safety margins (difference between  $T_{crit}$  and the maximum growth temperature experienced in the field) were also determined for a diverse set of 24–50 wheat lines exposed to different thermal regimes in the field and in climate-controlled chambers.

Flag leaf  $T_{crit}$  ranged from 44.6–46.9°C in the field and from 42.1–44.6°C under climate-controlled conditions. Generally,  $T_{crit}$  acclimated to warming, increasing by 0.07–0.13 per 1°C increase in mean maximum air temperature at anthesis. Lines with low  $T_{crit}$  prior to sustained warming ('basal'  $T_{crit}$ ) increased more under warming than lines with high basal  $T_{crit}$ , suggesting an upper  $T_{crit}$  ceiling beyond which no lines exceeded. Without acclimation, thermal safety margins were exceeded for 60% of the wheat lines under a high-emission Representative Concentration Pathway (RCP) 8.5 IPCC scenario for 2090. This suggests most lines are highly vulnerable to future warming. However, acclimation reduced the number of vulnerable lines by half. While, the degree of acclimation of  $T_{crit}$  to warming in wheat is quantitatively small, it nonetheless helps minimize the effect of warming on wheat leaf energy metabolism.

**299 - High temperature patterns determine seed yield and quality in oilseed rape in relation to sulphur nutrition**

**Lethicia MAGNO MASSUIA DE ALMEIDA <sup>(1)</sup> - Jean-Christophe AVICE <sup>(1)</sup> - Annette MORVAN BERTRAND <sup>(1)</sup> - Marie Hélène WAGNER <sup>(2)</sup> - María REYES GONZALEZ-CENTENO <sup>(3)</sup> - Pierre-Louis TEISSEDER <sup>(3)</sup> - Jean Jacques BESSOULE <sup>(4)</sup> - Marina Le Guédard <sup>(5)</sup> - Tae Hwan KIM <sup>(6)</sup> - Alain MOLLIER <sup>(7)</sup> - Sophie BRUNEL-MUGUET <sup>(1)</sup>**

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High temperatures during the crop reproductive stage impact seed yield and quality. The changing climate will require consideration of the effects of high temperature events that differ from their intensity, their duration and their frequency over the seed quality-building stages. The impact of these features deserve to be investigated at the light of induced thermo-sensitization which can lead to alleviate expected negative impacts. In our work, maturing seeds of the Sulphur-demanding crop, oilseed rape, were exposed to several temperature sequences that varied in intensity, duration and frequency at the onset of seed maturation. Results-measured in seeds that were at the onset of maturation when the temperature stress occurred-indicated that (i) the longer the cumulated duration of the temperature stress, the more negatively impacted the quality criteria with decreased fatty acids (FAs) concentration, increased  $\omega 6$ :  $\omega 3$  ratio, lower seed membrane integrity and increased seed dormancy and (ii) a mild stress event prior to heat peaks had an alleviating effect on the negative impact of the later heat peaks (priming effect) on seed nitrogen, desiccation tolerance and the phytohormones involved in thermoinhibition. Sulphur restriction was positive on FAs, proteins concentrations and negative on breaking dormancy. In addition, Sulphur supply interfered with temperature modality, features such that positive impact of Sulphur limitation on boosting oxidative response were cancelled with intense late heat peaks. This work provides insights to define thermopriming protocols in relation to the timing of quality building processes, their respective optimal temperature and adequate Sulphur supply.

**Key words:** oilseed rape, seed quality, high temperature, sulphur, repeated stresses, priming, stress memory, thermotolerance.



## TOPIC:

### Plant adaptation to climate change-related stress

#### **Posters**

**580 - Recovery of photosynthetic activity and role of antioxidant defense during rehydration of *Haberlea rhodopensis* after freezing-induced desiccation**

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The resurrection plant *Haberlea rhodopensis* is unique with its ability to survive both desiccation to air dry state during summer and freezing temperatures during winter. Similar to drought, freezing stress induced desiccation of plants. In the present study we investigated the recovery of photosynthetic activity and antioxidant defense during the first hours of rehydration of plants after freezing-induced desiccation until their complete restoration after 7 days. The water uptake during the initial 15 hours was slow thus preventing cellular damages. The extent of electrolyte leakage gradually decreased in the course of rehydration of plants, reaching control values after 24 hours, indicating that the membrane integrity was preserved during recovery. A significant enhancement of the photochemical activity of PSII was observed after 9 h of rehydration. The photochemical activity of PSI recovered faster compared to PSII, most probably due to activation of alternative electron flow pathways. The antioxidant status of plants was estimated by native PAGE and subsequent in-gel staining and determination of the changes in isoenzyme profiles and relative total activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione-S-transferase (GST). Our results revealed 9 isoforms of SOD (5 Mn-, 2 CuZn- and 1 FeSOD), 2 of CAT, 5 of GR, and 10 of GST. The activity of all studied enzymes increased at the beginning of recovery. SOD and CAT activities declined after 7 h of recovery, while that of GR and GST remained higher up to 7d rehydration of dry leaves. The enhanced activity of antioxidant enzymes indicated their important role in overcoming oxidative stress during rehydration of *H. rhodopensis*.

This work was supported by the Bulgarian National Science Fund, Ministry of Education and Science (Project KP-06-H21/8).

## 586 - Genetic architecture of culm morphology involved in barley lodging resistance: multi-environment genome-wide association

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In cereals with hollow internodes, lodging resistance is influenced by morphological characteristics such as internode diameter and culm wall thickness. Despite the relevance of these traits reported in several studies in rice, their genetic control in barley is poorly understood. To fill this gap, we developed an image-analysis based protocol to accurately phenotype culm diameter and culm wall thickness across 261 barley accessions and integrated the resulting data with data for several agronomic traits and marker information from 50k SNP iSelect genotyping. Analysis of culm traits collected from field trials in 7 different environments revealed high heritability values, indicating a strong genetic control. However, the existence of genotype-by-environment interactions was also not negligible. The collection was structured mainly according to row-type, which had a confounding effect on culm traits as evidenced by phenotypic correlations. In addition, culm traits showed strong negative correlations with lodging but weak correlation with plant height across row-types, indicating the possibility of improving lodging resistance independent of plant height. We applied a multi-environment genome-wide association study (GWAS) using a mixed model approach and identified several QTLs with main and interaction effects for culm traits that were not associated with plant height. Furthermore, modeling heterogeneous variances within environments and unique pairwise genetic covariances among environments provided the best fitting in multi-environment GWAS. Analyses within row-type subpopulations identified subpopulation-specific QTLs. We found QTLs that either harbour known genes or are in close proximity to them. The possible role of candidate genes will be discussed. In summary, our study provides a basic platform for exploring natural genetic variation of culm morphology linked to barley lodging resistance and yield improvement.

Funding: this work was supported by grants ClimBar (FACCE on Climate Smart Agriculture) and BARISTA (FACCE-JPI SusCrop).

**602 - Stem photosynthesis affects the xylem recovery ability in the deciduous *Populus alba* but not in the evergreen *Laurus nobilis***

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Stem photosynthetic activity has been suggested to play relevant roles to cope with different biotic and abiotic stress, including drought, as demonstrated in different desert and semi-desert non-succulent species. The extra carbon gain (as derived by the stem re-assimilation of CO<sub>2</sub> released by respiration as well as by carbon assimilation at stem level) may play a relevant role also for maintaining the integrity of xylem hydraulic function during drought events and/or facilitate the recovery. In this light, stem photosynthesis can favour plant drought resilience and, then, limit tree die-back induced by ongoing climate change.

In the present study we performed measurements of hydraulic conductance and non-structural carbohydrate content in the evergreen *Laurus nobilis* L. and the deciduous *Populus alba* L. subjected to inhibition of stem photosynthesis and exposed to drought-recovery experiment. We aimed to test possible links between stem-level production of carbohydrates and xylem hydraulic functionality. Results highlight the relevance of stem photosynthesis to sustain xylem hydraulic recovery, especially in the deciduous species.

## **690 - ID-Chestnut: a mobile app for the tree chestnut management**

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The inventory and assessment of the phytosanitary status and risk situations of plants in chestnut areas are essential for management. In addition, contribute to the knowledge of phytosanitary tree conditions and to the choice of interventions to be performed. Insert here

These studies are usually time-consuming because they require careful analysis of field data and additional time for registration in a digital format. To reduce the time taken to register the field data a mobile application based on the AppSheet® platform ([www.appsheet.com](http://www.appsheet.com)) was developed. This application was used in the evaluation of trees carried out in different country sites, in Portugal. The IDTree application allows the digital registration of the dendrometric and phytosanitary tree variables, the photographic records of the overall state or particular conditions of trees, and their georeferentiation. Moreover, it is able to read information from QR-Code tags. The ID-Chestnut is a valuable resource in fieldwork, allowing to substantially reduce the time spent with the transcription of data from paper record to a digital medium. It also contributes to the best systematization of data, facilitating their editing process during fieldworks and its further analysis.

**Keywords:** Chestnut orchards, inventory, phytosanitary, digital registration, AppSheet

## **117 - Photosynthesis and respiration in a warming world: acclimation to hot nights does not offset wheat biomass reduction**

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Rising temperatures continue to challenge crop yields around the globe, with warmer growing seasons and increasingly frequent and intense heatwaves particularly problematic for wheat productivity. Yet, the understanding of how fundamental processes like respiration and photosynthesis may respond to such trends, and how these responses may influence crop growth, remains limited. We combined several novel, high-throughput methods to investigate the high temperature acclimation of respiration and photosynthesis in wheat in both field and controlled environment settings.

Up to a 40% reduction of leaf and root respiration rate was widely apparent in response to elevated growth temperature. We found that this response was driven predominantly by a 5-10°C increase in night-time growth temperature, whereas a 5°C day-time growth temperature increase had little effect. Leaf respiration was also more greatly affected by this rise in night growth temperature than it was by a multi-day, 38°C day-time heatwave.

The high temperature tolerance of photosystem II increased by several degrees with warming, indicating that photosynthetic electron transport also thermally acclimated to elevated growth temperature, although this was not contingent on whether warming occurred during day or night. Photosynthetic rate demonstrated some high temperature acclimation (a 25% increase in maximum assimilation rate, and a 1-2°C increase in optimum temperature of photosynthesis with rising growth temperature), although quantitatively less than that of respiration.

Despite all of these acclimation responses having the potential to improve wheat carbon use efficiency, we found that whole-plant biomass still decreased by 20% with a 5°C rise in night growth temperature. This raises questions as to whether photosynthetic and respiratory acclimation to high temperature are capable of sufficiently minimising the reduction in plant growth caused by supra-optimal temperatures.

## 215 - Warming climate modifies carbon fixation capacity of Betula species

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Northern forest ecosystems are exposed to rapid climate change, i.e. climate warming, extended growing seasons, changes in precipitation and drought, accompanied by increasing pressure of herbivores and pathogens. Betula species are fast-growing pioneer species that have key role in carbon capture of boreal forest ecosystems mediating responses of northern forest ecosystems to climate change. Silver birch (Betula pendula) is also attractive target for modern genomics-based biotechnology, phenotyping and research on resistance traits due to a small genome size and the availability of advanced breeding material. We have studied the impacts of climate warming on Betula species with two complementary approaches: (i) short-term artificial heating treatments (with infra-red heaters or growth chambers) causing direct acclimation responses, and (ii) long-term common garden experiments ('natural laboratory') for genetic adaptation of populations to different latitudes and climate. Our studies included four different Betula species (native in Finland), six provenances and several genotypes from each provenance. The short-term experiments with potted birch saplings indicated that artificial warming treatments clearly enhance photosynthesis and canopy biomass. However, common garden experiments revealed clear differences in several physiological parameters between the southern and northern provenances. In particular, northern provenances showed less height growth increment, higher stomatal conductance and lower intrinsic water-use efficiency (WUE) compared to southern provenances in similar experimental conditions. Higher gas exchange rates were measured for the northern provenances compared to the southern, but this did not realize into better height growth increment. We also demonstrate differences in physiological traits between the short shoot and long shoot leaves. Genetic differences in carbon capture and water efficiency were accompanied by differences in leaf chemistry, metabolism, leaf spectral reflectance and herbivory resistance traits.

**242 - Physiological and anatomical phenotypes sustained by weak alleles of Arabidopsis stomatal development genes at optimal and supraoptimal temperature**

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Stomata number, size and distribution determine the maximum potential for gas exchange. These morphological traits influence transpiration and photosynthesis -and thence plant survival and performance- under different environments. Stomatal abundance, measured as stomatal density and stomata proportion, is a complex trait set gradually during leaf development and growth. In Arabidopsis, a network of key regulatory genes directs the process, influenced by gene activity and environmental growth conditions. Loss of function alleles of key positive regulators render stomataless plants, but their hypomorphic alleles can generate viable plants with informative stomatal phenotypes. We analysed morphological and physiological phenotypes of a set of Arabidopsis mutants carrying hypomorphic alleles for stomatal development genes, which display distinct SA phenotypes. We assessed their physiological performance through non-invasive imaging techniques at optimal (22°C) and supra-optimal (30°C) growth temperatures. This includes their photosynthetic activity, and their leaf temperature, as an estimation of transpiration. We recorded anatomical traits such as stomatal density, stomatal size and leaf thickness, and examined the impact of temperature regimes on plant growth by measuring leaf numbers, projected rosette area, dry weight and Feret's diameter of the rosette in the various genotypes grown under the different conditions. We made comparisons among growth conditions and genotypes, to uncover possible correlations between morphological and physiological characters at optimal and under supraoptimal temperatures, and will present the results of our analysis. This work was supported by the national grant AGL2015-65053-R) and the Castilla-la Mancha Government (SBPLY/18/180501/000009).

### **330 - The components of desiccation and other stress tolerance mechanisms in Bryophytes: revisit the old story**

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Bryophytes are not primitive precursors of vascular plants, but the diverse and highly evolved representatives of an alternative adaptation strategy, some fascinating physiology of their own. With their successful strategy they are prominent in oceanic temperate forests, tropical cloud forests, bogs and fens, polar and alpine fellfields and tundras. Bryophytes and vascular plants operate at different scales in relation to gravity, surface tension, laminar boundary layer, transport processes in the ambient air. Scale therefore has major physiological consequences: in many ways bryophytes function differently from vascular plants. They use water when it is available, and suspend metabolism when it is not. In the course of drying out and rehydrating they must pass through the levels of water stress experienced by DT vascular plants. They only transiently face the problem of metabolizing under water stress. It is like a 'drought avoidance' strategy in vascular plants. To understand the various physiological processes and stress responses of bryophytes comparing with higher plants' reactions it is essential to know the actual water status of the bryophyte tissue. Substantial external capillary water is generally present, and its amount can vary widely without affecting cell water status, which can result in difficulties in expressing precise actual water content (WC). The knowledge of full-turgor WC is principal. Desiccation tolerance is partly constitutive, allowing survival of rapid drying, and employs an active rehydration-induced repair and recovery. Bryophytes are capable of effective light absorption during their desiccation, rehydration, freezing and melting, with the help of coexisting alga and vascular plant energy dissipation mechanisms. Authors summarize the physiological mechanisms, morphological features and alternative strategies that make bryophytes successful in a constantly changing terrestrial (stressful) environment.

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**350 - Changes of ScBx gene expression and benzoxazinoid synthesis level in rye (*Secale cereale* L.) due to low temperature.**

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Benzoxazinoids (BXs) are secondary metabolites synthesized by many species of the Poaceae family, including rye. These compounds are primarily important in plant defence strategies against biotic and abiotic stresses. The majority of previous studies were focused on the influence of BX on pests, pathogenic fungi and weeds. The knowledge about the opposite relationship: how different factors affect Bx gene expression is still fragmentary and limited. An impact of low temperature on BX synthesis and related gene expression is, on the one hand, poorly understood, and on the other - very important for winter cultivars such as most cultivars of *Secale cereale* L.

The aim of this study was to determine the effect of a low temperature of 4°C, effective in the vernalization, on the biosynthesis of BXs (HBOA, DIBOA, GDIBOA, DIMBOA, GDIMBOA, MBOA) and expression of related genes (ScBx1-ScBx5, Scgl) in three inbred rye lines at control and low temperature - 21, 70 and 77 days after germination (dag). After seven weeks of cultivation, the expression level of all analysed genes and BXs content decreased compared to the first time point (21 dag), both in cold treated and control plants. At this time, we observed higher gene expression level and BX content in cold treated plants. Oppositely, on 77<sup>th</sup> dpi, the expression level of genes and the synthesis level of BXs in untreated plants usually increased. Therefore we can conclude that the treatment of rye seedlings with low temperature for a period of seven weeks decreases the level of reduction of BX synthesis and gene expression compared to untreated plants.

#### 451 - Cold tolerance in maize: new insights in 2 near-isogenic lines

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Maize (*Zea mays* L.) is a crop affected by many abiotic stresses such as cold, well known to cause extensive damages. With climate change require maize to be sowed earlier in order to reduce the impact of summer's water deficiency, an improved understanding of cold tolerance has become increasingly important. It has thus become necessary to determine the key actors of cold tolerance to better respond to various climatic challenges. The AMAIZING Biotechnology and Bioressources investment project combines genetic, genomic and ecophysiological analyses with high throughput phenotyping and genotyping to carry out genomic predictions and association studies, the global aim being the identification of polymorphisms responsible for characteristics of agronomic interest such as yield, quality and tolerance to soil abiotic stress, including cold stress.

We subjected a pair of maize near-isogenic lines targeting a major quantitative trait locus for cold tolerance to contrasted temperature treatments of several weeks and further analyzed them with a trio of -omics assays consisting in the analysis of the transcriptome (Illumina NexSeq500), the metabolome via primary and specialized metabolites (GC-MS and LC-MS), and the proteome by focusing on cell wall proteins or soluble proteins (GC-MS). These analyses enabled the identification of biomarkers responding either to the treatment, the genotype or the interaction (genotype x treatment). A large part of the genes responding to the genotypic effect, specifically during the cold condition, were located in the divergence zone of the NILs. Most of these differentially expressed genes, but also some differentially abundant proteins, between genotypes in the cold condition were validated by RT-qPCR. The complementary results between the trio of -omics have been interconnected, which allowed us to highlight and better understand the mechanisms of cold tolerance in maize for the obtention of potential new targets for breeding.

**608 - Unraveling the impact of combined salt and heat stresses on tomato plants (*Solanum lycopersicum* L.)**

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In a scenario of unprecedented climatic instability, where plants are simultaneously exposed to different abiotic stresses, compromising their productivity, it is imperative to understand how they respond to these environmental challenges. In this sense, this study focuses on characterizing the response of tomato plants (*Solanum lycopersicum* L.) to the co-exposure to salt (100 mM NaCl, via irrigation) and heat (42 °C – 4 h/day) stress for 21 days. Although salt and heat, individually, led to a decrease in both length and biomass of roots and shoots, the impact on biometric parameters was much more pronounced with the combined treatment, demonstrating a synergistic effect. This pattern was also present when analysing the content of photosynthetic pigments, with plants subjected to salt and heat co-exposure showing much lower chlorophylls and carotenoids levels than those of the single stressors. Curiously, and although the single stresses negatively affected the relative electron transport rate and the effective quantum yield of the photosystem II (PSII), their combination restored the photochemical efficiency of PSII to control levels.

In what concerns the redox status of the plants, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in shoots decreased in all treatments, but roots of plants under heat-stress (single and combined with salt) presented increased levels of this reactive oxygen species. Nonetheless, the over-production of H<sub>2</sub>O<sub>2</sub> did not appear to induce severe oxidative damage, since lipid peroxidation did not increase in any treatment. This finding can be related to the astonishingly higher content in proline found in roots and shoots of both sets of plants under salt stress (single and combined with heat).

Overall, the results show that, although growth reduction is expected from plants exposed to a combination of salt and high temperatures, *S. lycopersicum* can employ different mechanisms to ensure adequate photosynthetic performance and redox status under these conditions.

## 610 - The impact and importance of high quality field data for crop model calibration

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Process-based crop simulation models (CSM) are valuable tools for assessing GxExM interactions and quantifying climate change impacts on crops. Ex-ante evaluations of adaptation options to water stress require well-validated CSMs which are continuously improved and evaluated. This requires high quality data from model-driven field experiments. Therefore, detailed data for weather, soil, and crop growth and development was collected in one season of barley (cv. RGT planet) field experiments in Denmark. This dataset meets the highest standards for crop model improvement as defined by the modelling community. To evaluate the importance and impact of data quality on model calibration results, the CSM APSIM was calibrated for one location, first with a low (Q1), then with a medium (Q2), and finally with the high (Q3) quality dataset. Q1 represents a typical scenario of limited data availability for CSM calibration (e.g. limited soil description, few in-season phenology and biomass measurements). In a Q2 scenario usually better soil descriptions and phenology and biomass measurements at different crop stages are available. Phenology was predicted accurately with all quality levels, but the highest accuracy was achieved using the Q3 dataset (normalized root mean square error NRMSE (%) Q1: 11.5, Q2:11.6, Q3: 4.4). LAI was overestimated with all quality levels; however, the Q3 based calibration results were closest to the observations (NRMSE (%) Q1: 86.9, Q2:83.10, Q3: 69.30). Final grain yield was underestimated with Q1 and Q2 and slightly overestimated with Q3. The most accurate yield prediction was achieved with Q3 (NRMSE (%) Q1: -6%, Q2: -3.13 %, Q3: 1.38%). Findings from this study support our primary hypothesis that calibrating a CSM with high quality data increases prediction accuracy, however the impact of data quality varies depending on the specific process. Here, calibrating LAI and grain yield (complex traits) required more comprehensive datasets than calibrating phenology.

## 612 - Characterization of bark and wood photosynthesis in *Fraxinus ornus*

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Leaves are generally the most important photosynthetic organs in woody plants, but chloroplasts can also be found in organs optimized for other functions. So far, little attention has been paid to the photosynthetic efficiency of stem chloroplasts. In this study, the photosynthetic efficiency of stems, both at the bark and wood compartments, was investigated in *Fraxinus ornus* L. potted saplings. To this aim, optical methods such as spectral reflectance, fluorescence microscopy, chlorophyll fluorescence imaging and in vivo spectroscopy were applied in cut twig sections and in the corresponding leaves, considered as reference controls. Experimental data revealed a light gradient in the stem along the radial direction, with blue radiation being mainly absorbed by the outer bark, whereas a far-red enriched radiation reaches the underlying xylem and pith. Although a decreasing chlorophyll concentration gradient from the outer bark to the pith was observed, chlorophyll autofluorescence was detected also along the xylem rays and in the pith. Despite a lower concentration of chlorophylls, the bark showed a photosynthetic efficiency comparable to that of the leaf (e.g. similar maximum quantum yield of PSII). By providing a comprehensive scenario of the characteristics of bark and wood photosynthetic systems in *F. ornus*, future studies may better focus on understanding the functional role of stem photosynthesis. More research is needed to clarify the species-specific distribution of chloroplasts and their photosynthetic performance within the different stem compartments.

## 624 - Identification and characterization of genetic loci for culm diameter in barley

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Barley (*Hordeum vulgare* L.) is one of the most important cereal crops and it has been extensively used as a model for plant genetic analyses and breeding. Stem lodging in barley can cause a reduction in grain yield and quality. Beside reducing plant height, other strategies could be adopted to reduce the risk of lodging, such as by manipulating traits associated with the morphology and the composition of culm.

Multi-Environment GWAS analysis on a panel of European spring two-row barleys led to the identification of three QTLs for culm morphology with stable effects across environments. For the selected QTLs, lines carrying contrasting alleles and contrasting phenotypes for culm diameter were subsequently crossed for estimating QTL effects. Within each cross, the F1 plants were backcrossed to the respective small culm parents to develop BC1F1 segregating populations. The resulting BC1F1 plants are being used to develop Double Haploids (DH) lines carrying different combinations of alleles for the target QTL(s). Further evaluation of selected parents confirmed their contrasting phenotypes for the culm diameter. In parallel, the QTL regions are scanned for candidate genes to be targeted for identification of allelic variants by screening of the HorTILLUS mutagenized population developed by University of Silesia.

An additional goal of our research is the identification of mutants for culm morphology traits in the six-row barley mutagenized population TILLMore, developed by University of Bologna. A total of 57 lines selected from a preliminary field screening were grown in the greenhouse in completely randomized blocks with 10 replicates. The analysis of phenotypic data allowed us to identify mutants with increased/decreased culm diameter compared to the wild type Morex background.

This work provides the basis for the development of lines carrying favourable alleles for culm diameter and identifying the underlying genes controlling this trait.

Funding: this work was supported by the BARISTA grant (FACCE-JPI SusCrop).

## **632 - Drought stress memory in an epiphytic bromeliad potentially involves pigment and nitro-oxidative metabolisms**

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Epiphytic bromeliads are under frequent exposition to drought periods in the forest canopies since they are detached from the soil. Thus, it is likely these bromeliads present drought stress memory, which is the capacity of plants to show increased tolerance to a current stress event due to a previous exposition and recovery. Hence, we aimed to evaluate if the epiphytic bromeliad *Acanthostachys strobilacea* show improved biochemical responses to a second drought and recovery cycle, as indication of stress memory mechanisms. In a controlled environment chamber, 90-day-old plants were exposed to one (D1) or two (D2) drought-recovery cycles of 14 days of irrigation withholding and 5 days of rewatering each. Control plants (CT) were irrigated throughout the experiment. Most antioxidant parameters, pigments (chlorophylls and carotenoids) and osmoprotectants (proline and total amino acids) were increased after both drought cycles in D1 and D2, with full or partial recovery to CT levels after rewatering. These adjustments possibly prevented membrane and photosynthetic damage in D1 and D2 plants because no alterations in lipid peroxidation or PSII efficiency were detected. Particularly, the second drought in D2 plants resulted in significantly higher levels of osmoprotectants, pigments, S-nitrosothiols (SNOs), glutathione reductase (GR) and S-nitrosogluthione reductase (GSNOR) activities than the first drought. Hence, these results strongly suggest a stress memory response in this bromeliad, potentially involving pigment and nitro-oxidative metabolisms. The increased pigments might have enhanced the capacity of light-harvesting and prevented reduction in photosynthesis during the second drought in comparison to the first. Also, the increased osmoprotectants and GR activities possibly aided in preventing excess ROS, potentially generated from the enhanced electron transport rate caused by increased pigments in the second drought. Finally, the SNOs and GSNOR response clearly suggest the involvement of nitric oxide regulation in the second drought exposure and stress memory in this species.

**642 - Stem blackout: short-term effects of stem shading on hydraulics and non-structural carbohydrate dynamics in *Fraxinus ornus* L. during drought and rehydration**

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Leaves are the main photosynthetic organs in woody plants, but in some species stem photosynthesis can significantly contribute to the tree carbon budget through assimilation of locally-respired CO<sub>2</sub>. This extra carbon source in the form of non-structural carbohydrates (NSCs) might be crucial especially when drought stress induces leaf stomatal closure and phloem transport is impaired, and in post-drought recovery processes. However, studies investigating the role of stem photosynthesis on the plant NSC metabolism under water availability fluctuations are under-represented in the literature.

Stems of potted *Fraxinus ornus* L. saplings were covered with aluminium foil to inhibit stem photosynthesis and irrigation was immediately withheld. Plants reached the target xylem water potentials of -3.5 MPa (corresponding to 50% loss of xylem hydraulic conductivity, PLC) in ca. one week and then were re-irrigated to field capacity. In addition, to test the hypothesis that sugars locally produced by stem photosynthesis would favour stem water uptake and hydraulic recovery, branches from adult trees were dehydrated to induce embolism formation and then soaked in water under light or dark conditions. In well-watered, drought-stressed and re-irrigated plants, as well as in soaked branches, NSC (soluble sugars and starch) concentration and osmotic potentials were measured in wood and bark separately, together with PLC.

Shaded stems of potted plants did not differ in xylem hydraulics with respect to light exposed ones, but showed short-term changes in the NSC concentration and partitioning under drought and drought relief, as well as less negative osmotic potentials in the wood. Our results indicate stem photosynthesis as an important source of local, readily available NSCs, which could be crucial to endure drought and to enhance the hydraulic recovery capability.



## **653 - Physiological response of *Arabidopsis thaliana* with modified BPMs expression to moderate heat stress**

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In the *Arabidopsis thaliana* (L.) Heynh. genome, six BPM genes encode proteins containing BTB/POZ (Bric-a-Brac, Tramtrack, Broad Complex) and MATH (Meprin and TRAF Homology) domains. Recent studies have shown that BPM proteins play an important role in heat stress through the negative regulation of DREB2A, a transcription factor controlling the expression of many genes in response to drought and heat stress. Moreover, BPM1 protein was shown to be stabilized and accumulated under moderate heat stress. Physiological response of *Arabidopsis* seedlings with modified BPMs expression to moderate heat stress was investigated in wild type (wt), the line overexpressing BPM1 gene (oeBPM1) and the line with downregulation of BPM1, 4, 5 and 6 genes (amiR-bpm). Seedlings were exposed to 37 °C for six hours. Plant material was collected at two time points – immediately after exposure to elevated temperature and after a recovery period of 24 h at the cultivating temperature (24 °C). The status of photosynthetic apparatus was analyzed by chlorophyll a fluorescence transient (OJIP test). The content of pigments (carotenoids, chlorophyll a and b), proline, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and level of lipid peroxidation, as well as the activity of the antioxidant enzymes catalase, superoxide dismutase, guaiacol and ascorbate peroxidases were measured spectrophotometrically. Immediately after exposure to 37 °C, seedlings of all three lines showed reduced proline content compared to the non-stressed control groups. The photosynthetic performance index and pigment content were decreased in the wt and oeBPM1 immediately after stress. Seedlings with altered BPMs expression showed different dynamics of H<sub>2</sub>O<sub>2</sub>, lipid peroxidation and antioxidant enzymes. Different physiological response of wt, oeBPM1 and amiR-bpm to moderate heat stress could be related to altered expression of BPMs in examined lines.

**670 - Future high temperature impacts and plastic responses are challenged by higher CO<sub>2</sub> on common bean's best sources of heat-tolerant germplasm.**

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Common bean is a global food security staple of particular significance as a source of affordable protein and micronutrients. It is essential to identify strategies to develop climate-smart beans to mitigate the impact of steadily increasing growing season temperatures and elevated CO<sub>2</sub>. We characterised the response of the best available sources of heat tolerant germplasm to a range of chronic (season-long) higher temperatures with either ambient or elevated CO<sub>2</sub>. We observed different genotypic breaking points for chronic high temperatures, beyond which yield was compromised. Under stress below the breaking point, two developmentally plastic response strategies emerged. Mesoamerican interspecific crosses maintained yield until a higher temperature, switching to a more indeterminate habit to stay green and flowering for longer to achieve enough sinks to partition into seed. On the other hand, determinate Andean genotypes presented more losses—maintaining fewer but bigger seeds—as temperature increased. These plastic responses were not always successful under future CO<sub>2</sub> levels. Higher CO<sub>2</sub> generally increased intrinsic water use and vegetative biomass. Yet combined with higher temperature, it did not consistently cause re-partitioning into either more or larger pods or seeds. Only Mesoamerican interspecific crosses with heat tolerance derived from *P. acutifolius* successfully partitioned and increased yield under enriched CO<sub>2</sub>. When combined with future temperatures, they also maintained yield by presenting bigger seeds and/or more pods. Not all plastic strategies will rise to the challenge of combined higher temperature and CO<sub>2</sub>. We need to identify and exploit both functional and developmental plastic phenotypes from diverse germplasm to develop climate-smart and nutritive crops.

# 701 - Recovery of photosynthetic activity and role of antioxidant defense during rehydration of *Haberlea rhodopensis* after freezing-induced desiccation

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## Recovery of photosynthetic activity and role of antioxidant defense during rehydration of *Haberlea rhodopensis* after freezing-induced desiccation

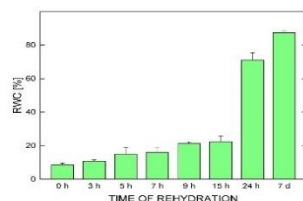
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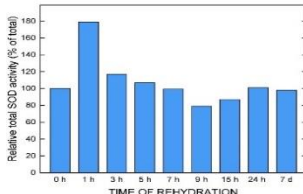
In the present study we investigated the recovery of photosynthetic activity and antioxidant defense during the first hours of rehydration of the resurrection plant *Haberlea rhodopensis* after freezing-induced desiccation until its complete restoration after 7 days. *Haberlea rhodopensis* Friv. is a perennial herbaceous rock polichydric plant, a preglacial relict, whose "age" is probably over two million years. Nowadays it can be found only in the Balkans, mainly in the Rhodope mountains in Bulgaria. It is considered as a homoiochlorophyllous desiccation-tolerant plant, since it preserves its chlorophyll content during dehydration. *H. rhodopensis* has the ability to survive both desiccation to air dry state during summer and freezing temperatures during winter. Similar to drought, freezing stress induced desiccation of plants. During rehydration, the plants should activate protective mechanisms to eliminate the damages caused by desiccation and subsequent rehydration.

### Plant Material and Methods

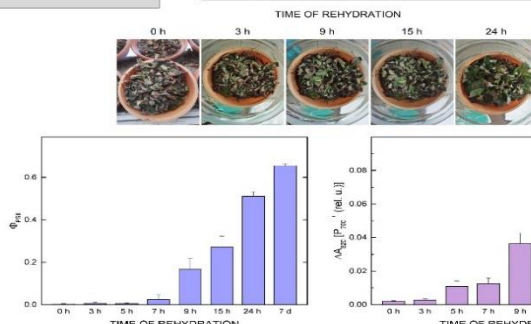
- Haberlea rhodopensis* plants were exposed to cold and freezing temperatures in natural conditions during autumn and winter, and after reaching air-dry state due to freezing-induced desiccation they were rehydrated in laboratory conditions in spring time. The measurements were conducted on dry leaves (0 h) and after 1, 3, 5, 7, 9, 15, 24 h, and 7 d of rehydration.
- Membrane integrity was assessed by measuring the extent of electrolyte leakage (EC 215, Hanna Instruments, USA).
- The changes in the activity of PSII were studied by measuring the chlorophyll fluorescence emission (PAM-2500, Walz, Effeltrich, Germany).
- The redox state of P700 was monitored by measuring the absorption changes at 810/860 nm (Walz ED 7000W-E emitter/detector unit connected to a PAM 101E main control unit).
- Plant leaf proteins were extracted according to Mladenov et al. (2015) with some modifications. Native PAGE were performed according to Laemmli (1970), emitting SDS, under nonreducing and nonreducing conditions.
- In-gel staining for activity of antioxidant enzymes: Superoxide dismutase (SOD) - Azevedo et al. (1998); Catalase (CAT) - Chandrasekhar and Scandalios (1983); Glutathione reductase (GR) - Anderson et al. (1995); Glutathione S-transferase (GST) - Ricci et al. (1984).



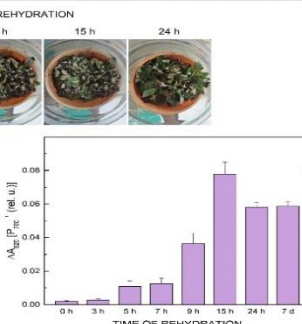
**Fig. 1.** The kinetics of recovery of the relative water content (RWC) of the air-dried plants consisted of 2 phases - the water uptake during the initial 15 hours was slow, thus preventing cellular damages and thereafter the rate of rehydration increased.



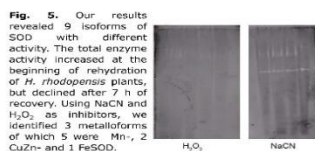
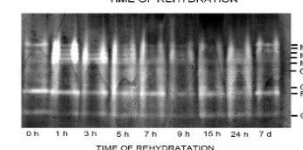
**Fig. 2.** Upon rehydration of plants the extent of electrolyte leakage gradually decreased, reaching control values after 24 hours, indicating that the membrane integrity was preserved during recovery.



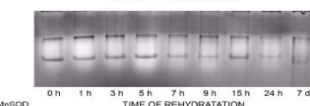
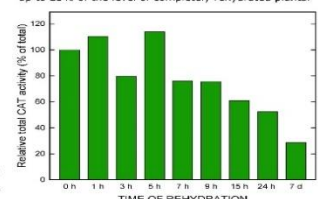
**Fig. 3.** The quantum yield of PSII photochemistry in the light adapted state (Fv/Fm) significantly increased after 9 h of rehydration, when the values of Fv/Fm increased up to 25% of the level of completely rehydrated plants.



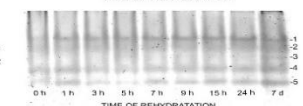
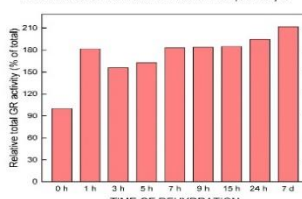
**Fig. 4.** Photochemical activity of PSI was observed after 5 h of rehydration of plants. PSI activity recovered faster compared to PSII, most probably due to activation of alternative electron flow pathways.



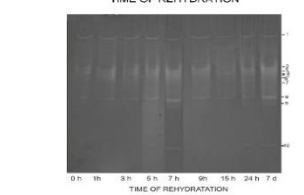
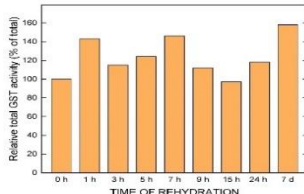
**Fig. 5.** Our results revealed 9 isoforms of SOD with different activity. The total enzyme activity increased at the beginning of rehydration of *H. rhodopensis* plants, but declined after 7 h of recovery. Using NaCN and H<sub>2</sub>O<sub>2</sub> as inhibitors, we identified 3 metalloforms of which 5 were Mn-, 2 CuZn- and 1 FeSOD.



**Fig. 6.** Two isoforms of CAT with different activity could be found in *H. rhodopensis* leaves. The total enzyme activity increased at the beginning of rehydration of plants, but declined after 7 h of recovery.



**Fig. 7.** The isoenzyme profile of GR showed the presence of 5 isoforms with distinct activities. A strong increase in the activity of GR was observed during rehydration of dry leaves until their full recovery after 7 days, when it is 2 times higher than that measured in dried plants.



**Fig. 8.** In the leaves of *H. rhodopensis*, GST is represented by 10 isoforms. The activity of GST increased in the first hours of rehydration and remained high up to 7 days when plants were completely recovered.

### Acknowledgments

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**TOPIC:**

**Plant development and flowering**

**Keynote Lecture**

**Mechanisms underlying cellular differentiation in flowers**

**Kerstin Kaufmann**

**Humboldt-Universität zu Berlin, Germany**

Plant cells are enigmatic because the 'identity' of each individual cell results from combination of positional and genetic factors. Kerstin Kaufmann will present progress on mechanisms underlying cellular differentiation focusing on functions of transcription factors and on single cell omics.

## TOPIC:

### Plant development and flowering

#### **Oral Communications**

#### **80 - Light-dependent H<sub>2</sub>O<sub>2</sub> scavenging as a critical process in the photocontrol of axillary bud outgrowth?**

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In rosebush, axillary bud outgrowth strictly depends upon light since it does not occur in darkness, in contrast to many plant models such as arabidopsis or tomato. A minute amount of light is sufficient to induce bud outgrowth and it has been shown that the bud is the site of light perception. Aside from well-known actors regulating bud outgrowth (phytohormones, nitrogen, sugar...), cytokinins have been notably shown to be the major actor of early bud outgrowth photocontrol events. Recently, we demonstrated that H<sub>2</sub>O<sub>2</sub> and redox status are critical parameters to engage bud outgrowth in light. Thus, one may question their involvement in the photocontrol of bud outgrowth.

We show that H<sub>2</sub>O<sub>2</sub> remains, in darkness, at a high level that is comparable to that observed in the dormant bud, while it decreases in light, enabling the bud to outgrow. The high level of H<sub>2</sub>O<sub>2</sub> appears to be the consequence of a repressed scavenging activity rather than an increase in its production. Indeed, the expression of ascorbate glutathione cycle genes are strongly downregulated in darkness while they are highly upregulated in light. Moreover, both the synthesis pathway and the quantity of glutathione were decreased in darkness, while the opposite occurred in light. Surprisingly, the expression of RBOHs genes encoding NADPH oxidases, the main contributors of H<sub>2</sub>O<sub>2</sub> production, decreased both in light and darkness and thus appears not to be involved in the photocontrol of bud outgrowth.

In conclusion, H<sub>2</sub>O<sub>2</sub> appears to be a new contributor to the photocontrol of bud outgrowth. We are currently investigating the crosstalk between H<sub>2</sub>O<sub>2</sub> and cytokinins to improve our understanding of the photocontrol mechanism of bud outgrowth.

**Keywords:** Ascorbate-glutathione cycle, Bud outgrowth; H<sub>2</sub>O<sub>2</sub>; Light; Photocontrol; RBOHs; Rosebush

## 204 - The evening complex protein OsLUX is necessary for rice flowering

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**ITQB, NOVA University, Oeiras, Portugal**<sup>(1)</sup> - **Leibniz-Institut für Gemüse- und Zierpflanzenbau, Leibniz-Institut für Gemüse- und Zierpflanzenbau, Grossbeeren, Germany**<sup>(2)</sup>

Rice (*Oryza sativa* L.) is the staple food for more than 3.5 billion people. Ensuring the continuous production and quality of this cereal is of utmost importance for human nutrition. Flowering time, particularly the time when plants shift from vegetative to reproductive stage (heading-date), dictates the quality and quantity of the grains. Early flowering and high temperature will impact on pollen fertility; late flowering and cold will impair flower development. Studies in the model plant *Arabidopsis thaliana* allowed the identification of three circadian clock members, ELF3, ELF4, and LUX that form a protein complex at dusk (evening complex (EC)) and regulate plant growth and flowering. The rice genome has two ELF3 homologs (OsELF3.1 and OsELF3.2) and it was shown that mutating either of them results in late flowering. Yet, studies of other members of the EC in rice or other cereals remains elusive. Here, we sought to study the role of the EC in rice flowering-time.

In order to better understand the function of the EC regulating flowering time in rice, we have generated mutant lines of OsLUX, OsELF3.1 or OsELF3.2 and double mutants for OsELF3.1 and OsELF3.2 using the CRISPR/Cas9 system. The late-flowering phenotype of OsELF3 mutants was evident and concordant with previous reports. Though we expected that OsLUX mutants displayed a late flowering phenotype as well, most OsLUX mutant lines failed to transit from the vegetative to the reproductive stage entirely. In order to identify the molecular mechanisms underlying this phenotype, we have analysed the expression of flowering time genes in these mutants. Known flowering activators were down-regulated whereas the flowering repressor OsPRR37 was up-regulated in the evening. In this talk, I am going to present our current hypothesis for the involvement of the EC in the control of rice flowering time.

**429 - The anti-florigen TERMINAL FLOWER 1 orchestrate plant architecture coordinating floral transition and vascular bundle development .**

**Alice Pajoro**<sup>(1)</sup> - **Edouard Severing**<sup>(2)</sup> - **He Gao**<sup>(2)</sup> - **Maida Romero-Branchat**<sup>(2)</sup> - **George Coupland**<sup>(2)</sup>

**National Research Council, Institute of Molecular Biology and Pathology, Rome, Italy**<sup>(1)</sup> - **Max Planck Institute for Plant Breeding Research, Department of Plant Developmental Biology, Cologne, Germany**<sup>(2)</sup>

During floral transition the identity of the shoot apical meristem (SAM) switches causing the organs formed on its flanks to change from leaves to flowers. In parallel, the stem elongates and thickens providing nutritional and physical support to the growing inflorescence. Gene regulatory networks (GRNs) controlling the switch in SAM identity have been established, but the extent to which these influence stem development remains unclear. We used transcriptomics, confocal microscopy and transgenesis to show that proteins that regulate floral transition in the SAM also exhibit precise spatial patterns of expression in the inflorescence stem, and that mutation of these floral regulators affects stem morphology. The florigen FLOWERING LOCUS T is expressed specifically in phloem companion cells of the inflorescence stem, whereas the anti-florigen TERMINAL FLOWER 1 (TFL1) is expressed in the phloem parenchyma. The *tfl1* mutant has reduced cell numbers in the cambium, xylem and phloem of inflorescence stem vasculature bundles, and transgenic expression of TFL1 only in the SAM of *tfl1* mutants restores its meristematic functions but weakly rescues the vasculature defect. Thus, the inflorescence protein TFL1 has additional roles in contributing to radial growth of the inflorescence stem, and our data provide the basis for further analyses of flowering GRNs in inflorescence stem development.

## TOPIC:

### Plant development and flowering

#### ***Extended Elevator Pitches***

##### **108 - A triple florigen system is essential for flowering and panicle architecture in rice**

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**University of Milan, Department of Biosciences, Milan, Italy**<sup>(1)</sup> - **University of Milan, Department of Agricultural and Environmental Sciences, Milan, Italy**<sup>(2)</sup> - **Max Planck Institute, Department of Plant Developmental Biology, Cologne, Germany**<sup>(3)</sup>

Rice (*Oryza sativa*) flowering is accelerated upon exposure of plants to short day lengths (SD). Under such conditions, two florigenic proteins encoded by *HEADING DATE 3a* (Hd3a) and *RICE FLOWERING LOCUS T 1* (RFT1), are expressed in leaves and move through the phloem to reach the shoot apical meristem (SAM). At the SAM, they assemble into higher-order complexes that include 14-3-3 proteins and bZIP transcription factors, to reprogram gene expression, start panicle morphogenesis and promote internode elongation. In plants exposed to SD, both Hd3a and RFT1 expression is induced, whereas exposure to LD induces RFT1 expression only. This pattern of expression could account for redundant as well as independent roles as flowering triggers. Here, we describe genes that are common targets of both photoperiodic and florigenic signalling pathways. To this end, we quantified and compared the SAM transcriptomes of wild type plants induced to flower by SD, and of transgenic plants harbouring inducible versions of either Hd3a or RFT1, whose signalling was activated artificially under LD.

The screen identified a small cluster of genes whose expression depends upon SD and the florigens. The group included all known genes promoting panicle development or internode elongation, as well as novel regulators of the floral transition. Among them, we unexpectedly identified a florigen-like gene, *FLOWERING LOCUS T-LIKE 1* (FT-L1), whose expression is strongly induced at the apex during floral commitment, and dependent upon Hd3a and RFT1. Expression and protein-protein interaction data indicate that FT-L1 does not show molecular features typical of other florigens. However, EMS and CRISPR mutants showed that FT-L1 promotes flowering and controls panicle architecture and seed production by inhibiting secondary branching.

These data support a novel model for transition to flowering in rice, whereby a triple florigen system integrates environmental signals to initiate panicle morphogenesis and drive its subsequent development.



### 134 - SEEDSTICK controls Arabidopsis fruit size by regulating cytokinin levels and FRUITFULL

**Maurizio Di Marzo** <sup>(1)</sup> - **Humberto Herrera-Ubaldo** <sup>(2)</sup> - **Elisabetta Caporali** <sup>(1)</sup> - **Ondřej Novák** <sup>(3)</sup> - **Miroslav Strnad** <sup>(3)</sup> - **Vicente Balanzà** <sup>(4)</sup> - **Ignacio Ezquer** <sup>(1)</sup> - **Marta Adelina Mendes** <sup>(1)</sup> - **Stefan de Folter** <sup>(2)</sup> - **Lucia Colombo** <sup>(1)</sup>

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Upon fertilization, the ovary increases in size and undergoes a complex developmental process to become a fruit. We show that cytokinins (CKs), which are required to determine ovary size before fertilization, have to be degraded to facilitate fruit growth. The expression of CKX7, which encodes a cytosolic CK-degrading enzyme, is directly regulated post-fertilization by the MADS-box transcription factor STK. Similar to stk, two ckx7 mutants possess shorter fruits than wild type. Quantification of CKs revealed that stk and ckx7 mutants have high CK levels, which negatively control cell expansion during fruit development, compromising fruit growth. Overexpression of CKX7 partially complements the stk fruit phenotype, confirming a role for CK degradation in fruit development. Finally, we show that STK is also required for the expression of FUL, which is essential for valve elongation. Overall, we provide novel insights into the link between CKs and the molecular pathways that control fruit growth.

## 158 - Transcriptome reprogramming in the Arabidopsis male germline during pollen tube growth

**Chandra Shekhar Misra** <sup>(1)</sup> - **Daniel Sobral** <sup>(2)</sup> - **Jörg Becker** <sup>(1)</sup> - **Pedro Barros** <sup>(3)</sup>

**Instituto Gulbenkian de Ciência, Plant Genomics Group, Instituto Gulbenkian de Ciência, Lisbon, Portugal**  
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**Faculdade de Ciências e Tecnologia - Universidade Nova de Lisboa, Lisbon, Portugal** <sup>(2)</sup> - **Instituto de**  
**Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Lisbon, Portugal** <sup>(3)</sup>

A pollen tube's journey through the transmitting tissues of the pistil leads to changes in its gene expression profile. However, to what extent this reflects changes in the transcriptome of the sperm cells (SC) and vegetative nuclei (VN) it transports has been ignored so far. This question is of particular importance since sperm cells are believed to acquire competence for fertilization during pollen tube growth. However, such a study was challenging so far because of the difficulty in collecting enough material to study the transcriptomic changes of SC and VN during pollen tube growth. Now we have compared the transcriptomes of SC and VN isolated from mature pollen grains and from pollen tubes grown semi in-vivo (SIV). We used an optimised SIV system and an available fluorescent marker line to isolate GFP labelled SC and RFP labelled VN by FACS, followed by a low input RNA-seq protocol. We compared the transcriptomes of SC and VN isolated from mature pollen grains and from pollen tubes grown semi in-vivo. Moreover, to understand how many genes are elicited in SC and VN only in presence of female cues, we also analyzed the SC and VN from pollen tubes grown in vitro. Our data suggest that SC and VN undergo extensive transcriptomic changes, some of which are elicited only when they have travelled through the pistil, revealing hitherto unidentified transcripts that may be important for fertilization and pollen tube growth. Our transcriptome data also sheds light on the role of genome-wide alternative splicing during pollen tube growth, an important developmental aspect that remains unknown. More detailed analyses of gene families and pathways will be presented. Our study provides the most comprehensive overview of transcriptome dynamics during Arabidopsis pollen tube growth to date.

### **322 - Understanding meristem differentiation at single cell level during flower development**

**Jose M Muino**<sup>(1)</sup> - Xiaocai Xu<sup>(1)</sup> - Manuel Neumann<sup>(1)</sup> - Cezary Smaczniak<sup>(1)</sup> - Kerstin Kaufmann<sup>(1)</sup>

**Humboldt University, Philippstr. 13, Berlin, Germany**<sup>(1)</sup>

Plant meristems are small populations of undifferentiated cells that proliferate and differentiate to form plant organs. However, the precise regulatory mechanisms underlying the formation of distinct mature cell types in an organ-specific manner are still not well understood.

Here, we use single-cell RNA-seq experiments at different time points of flower development to understand the transcriptomic trajectories during cellular differentiation. The inducible AP1-GR system is used to focus the analysis on the early stages of floral meristem development. The analysis of the data shows the importance the association of cell cycle with cell differentiation, and predicts direct regulatory links between flower developmental and cell cycle regulators.

In addition, we map our single cell transcriptomic data to a 3D reconstructed inflorescence meristem based on confocal microscopy of ~25 reporter genes. Based on this methodology, we can predict the expression of ~1,000 genes in any cell population of interest. We show the potential of this methodology by predicting the expression of ~1,000 genes in the AP3 versus AG expression domains, and showing its good agreement with bulk RNA-seq data of FACS-purified cells from these domains.

In summary, we use single-cell methodologies to characterize the dynamics of the transcriptome during flower development, and we validate a method to map the single-cell data into the 3D reconstructed flower meristem.

#### **435 - Conserved regulatory networks acting during phellem development in Arabidopsis and cork oak roots**

**Pedro Barros <sup>(1)</sup> - Ana Rita Leal <sup>(1)</sup> - Helena Sapeta <sup>(1)</sup> - Boris Parizot <sup>(2)</sup> - Nick Vangheluwe <sup>(2)</sup> - Joana Belo <sup>(1)</sup> - Tom Beeckman <sup>(2)</sup> - M. Margarida Oliveira <sup>(1)</sup>**

**ITQB NOVA, GPlantS Unit, Oeiras, Portugal <sup>(1)</sup> - Ghent University & VIB-UGent, Ghent University, Department of Plant Biotechnology and Bioinformatics, Gent, Belgium <sup>(2)</sup>**

The longevity and high activity of the cork cambium (or phellogen) from *Quercus suber* (cork oak) are the cornerstones for the sustainable exploitation of a unique raw material. Cork (or phellem) is mainly composed of dead cells with a high deposition of suberin in the cell walls, providing the first defense against abiotic/biotic stresses in organs undergoing secondary growth. To identify novel regulators of cork development/differentiation, we performed histological and transcriptomic studies following phellem ontogeny in *Arabidopsis* and cork oak roots. We found that root phellogen activity initiates 1-week after sowing in both species. We further sequenced the translome of newly developed phellem cells in *Arabidopsis* through Translating Ribosome Affinity Purification followed by mRNA sequencing (TRAP-SEQ). We found that the phellem translational landscape is organized in three main domains related to synthesis of cell wall components, energy production, and response to stimulus. Novel players related to suberin monomer transport and assembly, as well as novel transcription regulators were identified.

In cork oak, we further performed a comparative transcriptomic analysis using roots grown under control, osmotic and heat stress conditions. We selected root segments undergoing secondary (SD) and primary development, to assess the differential impact of the tested conditions. Independently of the stress, the transcriptomic landscape of SD-region showed enrichment in the expression of genes related to cell-wall modifications, mainly lignification and suberization. A total of 215 *Arabidopsis* genes identified by TRAP-SEQ showed homology to cork oak genes enriched in the SD zone, including multiple transcription factors and enzymes related to suberization, phenylpropanoid and lipid metabolism. Osmotic and heat stress had contrasting effects on phellem suberization, partially related to differential regulation of ABA signaling genes. Our work provided an unprecedented resolution of the regulatory networks acting during phellem development, which might further contribute to design new strategies to improve cork production.

#### **464 - Functional analysis of AtDRIF genes during the development of Arabidopsis thaliana seedlings**

**Ana Marques**<sup>(1)</sup> - **Rómulo Sobral**<sup>(1)</sup> - **Ana Cunha**<sup>(1)</sup> - **Sweta Singh**<sup>(1)</sup> - **Jõao Raimundo**<sup>(1)</sup> - **Sara Laranjeira**<sup>(1)</sup> - **Manuela Costa**<sup>(1)</sup>

**BioISI, University of Minho, Braga, Portugal**<sup>(1)</sup>

Plants are highly dependent on environmental signals to coordinate and synchronize their development with the most favourable conditions. Light is a major cue controlling early stages of plant development when individuals are more vulnerable to the environment. The development of plant seedlings buried in the soil (no light input, skotomorphogenesis) is characterized by the growth of the embryonic stem (hypocotyl) and the formation of an apical hook, a curvature of the stem that protects the apical meristem from soil abrasion. When the seedlings emerge from the soil, the perception of light initiates a transition characterized by a decrease in the growth rate of the hypocotyl and by the opening of the apical hook (photomorphogenesis). Many families of genetic factors are known to be involved in the transition from skoto to photomorphogenesis, however a link between them is still illusive. Our lab has been studying the previously unknown role of a unique MYB-like gene family during the dark to light transition. In Arabidopsis, this family is composed by five homologs, three of which are very similar, suggesting they may be functionally redundant. The triple knockout mutant shows a shorter hypocotyl than wild type and the loss of the apical hook when grown in the dark. An RNAseq approach was used to identify genetic factors that are affected in the triple mutant during early stages of development. The results suggest that members of this unique MYB-like family control key genes on the regulatory network that regulates the formation of the apical hook and hypocotyl elongation during the transition from skoto to photomorphogenesis. Identifying the yet unknown molecular mechanisms associated to this MYB-like family is of the utmost importance as proper development of seedlings is critical for plant fitness and crop yield, particularly in a rapidly changing environment.

## **475 - Genetic control of juvenile phase-specific cuticle deposition and cuticle-mediated plant response to drought in maize**

**Giulia Castorina**<sup>(1)</sup> - **Matteo Chiara**<sup>(2)</sup> - **David Horner**<sup>(2)</sup> - **Frédéric Domergue**<sup>(3)</sup> - **Gabriella Consonni**<sup>(1)</sup>

**Università degli Studi di Milano, Department of Agricultural and Environmental Sciences, Milan, Italy**<sup>(1)</sup> - **Università degli Studi di Milano, Bioscience Department, Milan, Italy**<sup>(2)</sup> - **University of Bordeaux - INRA Bordeaux, Laboratoire de Biogenèse Membranaire, CNRS, Bordeaux, France**<sup>(3)</sup>

In all higher plants, the outer surface of the aerial parts is covered by the cuticle, a complex lipid layer that constitutes a barrier against environmental stresses. Only few genes involved in cuticle deposition have been characterized in maize and its hormone-mediated regulation is still poorly understood.

We show in this work that the MYB transcription factor ZmMYB94/fused leaves1 (fdl1) is a key regulator of cuticle deposition during maize juvenile vegetative phase. Biochemical analysis showed that wax and cutin were significantly altered in fdl1-1 mutant plants, suggesting that FDL1 is required for the deposition of both cuticular components. These data corroborated previous results obtained from mutant phenotypic analysis showing that fdl1 is involved in the correct epicuticular waxes distribution as well as in the formation of the whole cuticle layer during post-germinative organ separation.

To further investigate the role of FDL1 in the control of cuticle biosynthesis we performed an Illumina based RNA-sequencing experiment. About 1600 differentially expressed genes were detected between fdl1-1 and wild type. Their analysis led to the identification of a set of candidate genes implied in lipid metabolism. Variation in their expression nicely correlated with cuticular alterations observed at the biochemical level.

We explored the effect of drought on cuticle properties and showed that in drought condition maize seedlings are able to decrease cuticle-mediated leaf permeability, thus reducing water loss and enhancing plant tolerance. Similar results were obtained in ABA treated seedlings. Moreover, drought and ABA influenced fdl1 and other cuticle related genes at the transcriptional level.

Our data provide novel tools for the selection of genetic variants able to improve cereal crop tolerance against environmental conditions that limit yield.

#### **488 - The evening complex is necessary for rice flowering activation**

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**ITQB, UNL, Oeiras, Portugal**<sup>(1)</sup> - **IGZ, Leibniz-Institut für Gemüse- und Zierpflanzenbau, Grossbeeren, Germany**<sup>(2)</sup>

Flowering time dictates the reproductive success of flowering plant species and is intrinsically linked to quality and quantity of crop grain production. Early flowering and high temperature will impact on pollen fertility; late flowering and cold will impair flower development. Studies in the model plant *Arabidopsis thaliana* allowed the identification of three circadian clock members, ELF3, ELF4, and LUX that form a protein complex at dusk named evening complex (EC) that regulate plant growth and flowering. Rice (*Oryza sativa* L.) is the staple food for more than 3.5 billion people and ensuring the continuous production and quality of this cereal is of utmost importance for human nutrition. The rice genome has two ELF3 homologs (OsELF3.1 and OsELF3.2) and it was shown that mutating either of them results in late flowering. Yet, studies of other members of the EC in rice or other cereals remains elusive. Here, we sought to study the role of the EC in the control of rice flowering-time.

We have generated mutant lines of OsLUX, OsELF3.1 or OsELF3.2 and double mutants for OsELF3.1 and OsELF3.2 using CRISPR/Cas9. The late-flowering phenotype of OsELF3 mutants was evident and concordant with previous reports. However, both OsLUX and OsELF3.1OsELF3.2 mutants failed to transit from the vegetative to the reproductive stage entirely suggesting the presence of an active EC also in cereals. In order to identify the molecular mechanisms underlying this phenotype, we have analysed the expression of flowering time genes in these mutants by RNA-seq. Known flowering activators were down-regulated whereas the flowering repressor OsPRR37 was up regulated in the evening indicating a possible pathway for the EC to control flowering-time.

**522 - FSD1: a plastidial, nuclear and cytoplasmic enzyme with antioxidant, osmoprotective and developmental functions**

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Superoxide dismutases (SODs) are enzymes detoxifying superoxide to hydrogen peroxide. It is known that SODs determine plant abiotic stress tolerance, but the knowledge about their in vivo developmental expression and subcellular localisation is still elusive. Therefore, we aimed here to reveal in vivo developmental expression, subcellular, tissue- and organ-specific localisation of iron superoxide dismutase 1 (FSD1) in Arabidopsis using advanced confocal microscopy. FSD1-GFP temporarily accumulated at the site of endosperm rupture during seed germination. In emerged roots, it showed the highest abundance in cells of the lateral root cap, columella, and endodermis/cortex initials. The largest subcellular pool of FSD1-GFP was localised in the plastid stroma, while it was also located in the nuclei and cytosol. We found that *fsd1* knockout mutants exhibit reduced lateral root number and this phenotype was reverted by genetic complementation. Mutant analysis also revealed a requirement for FSD1 in seed germination during salt stress. Salt stress tolerance was coupled with the accumulation of FSD1-GFP in Hechtian strands and superoxide removal. It is likely that the plastidic pool is required for acquiring oxidative stress tolerance in Arabidopsis. This study suggests new developmental and osmoprotective functions of SODs in plants.

This work was supported by the Czech Science Foundation GACR, grant Nr. 19-00598S.



### **37 - Plant growth development and B-Box transcription factors**

**Javier Botto**<sup>(1)</sup> - **Carlos Crocco**<sup>(1)</sup> - **Gabriel Gomez-Ocampo**<sup>(1)</sup>

**IFEVA-CONICET, Agronomy Faculty, Univerity of Buenos Aires, CABA, Argentina**<sup>(1)</sup>

The B-box (BBX) proteins are zinc-finger transcription factors containing one or two B-box motifs. BBX proteins control growth and developmental processes including seedling photomorphogenesis, photoperiodic regulation of flowering, shade avoidance, and responses to biotic and abiotic stresses. The phytohormones and the BBX proteins have key roles in regulating plant growth and development; however, their interplay and underlying mechanisms are not fully understood. In this talk, I will show novel results on the functional characterization of BBX members as a key transcription factors in signaling regulatory networks of different phythormones. I will also discuss the use of BBX engineering crops to reduce phytohormone sensitivity and increase yield productivity in abiotic stress conditions.

## TOPIC:

### Plant development and flowering

#### Posters

##### 550 - Expansin gene transformation promotes transgenic cotton fiber elongation

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Cotton fiber quality defines its spinnability in the form of yarn and its textile performance. A candidate expansin gene, EXPA1, expressed under two different promoters namely GhSCFP and CaMV35S, spatially and temporally, in a local cotton variety CEMB-66 for its potential in fiber quality improvement. Expansin is a unique, cell wall loosening gene, and a non-enzymatic protein that is prominent for its significant role in plant cell wall expansion, and cellulose deposition. A significantly higher expression of EXPA1 under GhSCFP in Cotton boll was observed as compared to other plant parts. While its expression under CaMV35S promoter was found to be consistent in all tissues including cotton boll. The temporal expression profile was quite interesting with a gradual increasing pattern of EXPA1 under both promoters from 1DPA (days post anthesis) to 18DPA and decreased expression from 24 to 30 DPA. The transgenic cotton plants were found to have 22-40% more cellulose than non-transgenic wild type control cotton plants. Fiber lengths were improved by 10% and 11% in FSEXPA1 and 35SEXPA1 transgenic cotton plants respectively. Closed observation of fiber twist and topology was made through a Scanning electron microscope (SEM) and fluorescent microscope. Agronomic traits of cotton plants such as yield, cotton bolls, and average boll weights calculated for both transgenic and control plants. The total yield (seed and lint) was found to be improved by 16-17% in both transgenic groups as compared to control ones. The study significantly compared the two most commonly used promoters, GhSCFP, and CaMV35S, in the context of a fiber elongation-promoting gene EXPA1 expression in transgenic cotton. The results revealed that GhSCFP promoter could be used more efficiently in fiber related gene transformation when compared with CaMV35S which being constitutive in nature preferred for expression in all parts of the plant. The developed material during this study can be interesting for the breeders to use for cotton fiber quality improvement.

**Key words:** Expansin, Cotton, Fiber, Transformation, Cell wall, Promoters

## 563 - Structure and development of leaf glandular trichomes in *Cleome amblyocarpa* (Cleomaceae)

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The distribution, structure and development of glandular trichomes (GTs) on the leaves of *Cleome amblyocarpa* was studied using light as well as scanning and transmission electron microscopy. Histochemical tests were performed to identify the sites of the secondary metabolites accumulation. It was revealed that GTs of 4-6-8 series of cells with spherical secretory heads are uniformly distributed over the leaf surface. The positive reactions with NADI stain, Sudan B, Fluorol Yellow and Naturstoff Reagent indicated the content of terpenoids, lipids and lipophilic compounds and flavonoids correspondingly in secretion of GTs.

The head cells of mature GT have relatively thin cell walls and large nucleus, small vacuoles dense cytoplasm rich of organelles. Smooth endoplasmic reticulum (SER) and plastids are highly developed. Sections of plastids are long, anastomosing. Within 3D-reconstructed fragment of the secretory cell the plastid presented the continuous network.

The development of GT starts as the enlargement of one epidermal cell and continued with a series of anticlinal and subsequent periclinal divisions that result in the mature GT. In growing GTs cytoplasm of head cells contained a lot of free ribosomes, endoplasmic reticulum (ER) was mostly in the form of long granular cisterns. The sections of plastid were small, rounded. During GT maturation the cisterns of granular reticulum were replaced by tubules of SER, free ribosomes disappeared, plastids became long, cell polarity was established. Numerous myeloid bodies associated with plastids and vacuoles were found in head cells during maturation. We propose that partial plastid degradation gives place to development of other type of plastids and this replacement of plastid populations is the essential stage of secretory cell development.

### 593 - Do strigolactones regulate the translation?

**Magdalena Korek** <sup>(1)</sup> - **Agata Daszkowska-Golec** <sup>(1)</sup> - **Marek Marzec** <sup>(1)</sup>

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**(1)**

Strigolactones are a group of plant hormones that inhibit the growth of axillary buds, reducing the shoot branching. However, new roles of strigolactones in plant growth, development, and responses to biotic and abiotic stresses were described based on the strigolactone mutants in various species analysis. Up to now, only one strigolactone mutant was identified in barley (*Hordeum vulgare*) in our lab - the hvd14.d line. Mutant carrying change in gene encoding strigolactone receptor HvD14 is insensitive to strigolactone treatment. Our analyses demonstrated that hvd14.d line produces almost twice as many tillers as wild-type plants, and mutant had shorter but more branched roots when compared to parent variety.

To understand the molecular basis of the hvd14.d phenotype, the deep sequencing of the 4-week-old plant transcriptomes was performed under optimal growth conditions. Gene expression was analyzed in shoot and roots tissue of both mutant and parent variety. We found 1 420 and 6 664 differentially expressed genes (DEGs) between genotypes in leaf and root tissue, respectively. Moreover, we focused also on genes expressed in common up- (97) and down-regulated (165) in mutant shoot and roots.

Gene ontology (GO) enrichment analysis revealed the most overrepresented GO terms (from all three categories) of genes that were upregulated in both mutant tissues, which includes: 1) peptide/ amide biosynthetic process; 2) translation; 3) structural constituent of ribosome; 4) RNA binding; and 5) ribosome. Whereas in the set of genes that were downregulated in hvd14.d, the overrepresented GO terms were mainly related to photosynthesis. Strikingly, the obtained data suggest the role of strigolactones in the regulation of translation what was not previously reported.

### 639 - The NtMAX3 gene regulates leaf angles and shoot branching in *Nicotiana tabacum*

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Strigolactones (SLs) are a new class of hormones involved in the regulation of diverse physiological processes, including shoot branching and root development. The SLs biosynthesis gene MAX3/CCD7, encodes a carotenoid cleavage dioxygenase required for the synthesis of SLs, have been cloned and characterized in different plants. However, the gene encoding MAX3/CCD7 has not been identified in tobacco (*Nicotiana tabacum*) thus far. Here, we identified and cloned the NtMAX3 gene in tobacco genome. Sequence comparison indicated that MAX3 had high homology with plant MAX3 proteins. The expression patterns of NtMAX3 in different tissues were analyzed by determined by quantitative PCR, the results showed that the NtMAX3 gene was strongly expressed in roots. Tissue-specific expression of NtMAX3, as examined with a promoter-glucuronidase (GUS) gene fusion, was observed predominantly expressed in roots, as well as stems. Furthermore, we demonstrated that NtMAX3 expression was induced by 1-naphthalene acetic acid, but was down-regulated by the application of salicylic acid and sucrose. Tobacco plants that constitutively overexpress NtMAX3 showed smaller leaf angles. Further expression analysis reveals that the NtTAC1, a key leaf angle regulated gene, was significantly decreased. We also used CRISPR-Cas9 technology to generate mutants for NtMAX3, and there was an increased leaf angle and shoot branching phenotype in NtMAX3 mutant plants. These data suggest that NtMAX3 play key roles in regulating the axillary branch and leaf angle processes by mediating the synthesis of SLs in tobacco.

## 662 - The ALOG family members OsG1L1 and OsG1L2 regulate inflorescence branching in rice

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Human population growth makes of primary importance to find new ways to improve agricultural crop production and meet the increasing food demand. In this context, inflorescence architecture is one of the key agronomical traits which determines grain yield; thus, it has been a major target for crop domestication and improvement.

In *Oryza sativa*, inflorescence architecture is established at early stages of reproductive development. During vegetative phase, the Shoot Apical Meristem (SAM) produces leaves. When a rice plant undergoes floral transition, the SAM differentiates into Inflorescence Meristem (IM), which in turn gives rise to primary branch meristems (pBMs). The pBMs can produce Spikelet Meristems (SMs) or form a more complex architecture with Secondary Branches Meristems (sBMs), that will in turn produce SMs. When the SM differentiation occurs, the meristem loses its indeterminate state to become determinate, stopping all sorts of branching potential. This leads to the development of Floral Meristems (FM), that differentiate into floral organs.

The ALOG gene TAWAWA1 (TAW1) has been shown to be a regulator of meristem activity: it promotes IM activity and the suppression of the phase change to SM identity. Combining laser microdissection of rice inflorescence meristems with RNA-seq, we observed that other two members of the ALOG gene family, OsG1-like 1 (OsG1L1) and OsG1L2, present an expression profile similar to TAW1. Furthermore, the loss-of-function CRISPR mutants *osg1l1* and *osg1l2* present a phenotype similar to the *taw1* mutant, suggesting that these three genes may act in related pathways controlling inflorescence development.

A transcriptome analysis was performed on *osg1l2* mutant, suggesting the interaction of OsG1L2 with other genes known to control inflorescence architecture. The obtained dataset was also used to generate a gene regulatory network (GRN).

The analysis of the loss-of-function CRISPR mutant of the homeodomain-leucine zipper transcription factor gene *OsHOX14* suggests that the proposed GRN can be useful to identify players involved in inflorescence development in rice.

## **663 - Characterization of REM genes involved in the reproductive development of *Arabidopsis thaliana***

**Francesca Caselli <sup>(1)</sup> - Veronica Maria Beretta <sup>(1)</sup> - Otho Mantegazza <sup>(1)</sup> - Rosanna Petrella <sup>(1)</sup> - Humberto Herrera-Ubaldo <sup>(2)</sup> - Stefan de Folter <sup>(2)</sup> - Martin M Kater <sup>(1)</sup> - Veronica Gregis <sup>(1)</sup>**

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The REproductive Meristem (REM) gene family encodes for transcription factors belonging to the B3 DNA binding domain superfamily. In *Arabidopsis thaliana*, this family is composed of 45 members, preferentially expressed during flower, ovule, and seed developments. Three members of this family, REM34, REM35 and REM36 were shown to be expressed from the earliest stages of the reproductive development of *Arabidopsis*, in the inflorescence meristem and in the flower meristems; however, their role in these tissues is still unclear. To functionally characterize these genes, REM\_RNAi lines, in which the three genes of interest were downregulated, as well as 35S:REM34-EAR dominant repressor lines were employed. At the same time, single and multiple mutant combinations were generated using CRISPR-Cas9 genome editing system.

While wild type plants are characterized by a spiral phyllotaxy, in which successive siliques arise at an angle of 137.5°, the single and multiple knock-out mutants, as well as the REM\_RNAi and 35S:REM34-EAR lines, displayed an altered phyllotactic pattern, with the divergence angles distributed around three main values: 90°, 140° and 180°.

The analysis of several marker lines and the effect of exogenous auxin applications clearly showed that auxin was correctly produced and distributed in the inflorescence meristem, but the perception of this hormone was perturbed in the rem mutants.

We also discovered that REM35 was able to interact with REM34 as well as with a subset of Auxin Responsive Factors, which loss-of-function mutant is also characterized by a defective phyllotactic pattern.

Taken together, these findings suggest that REM34, REM35 and REM36 are involved in the control of auxin response in the inflorescence, in complex with ARFs.

## 682 - What is behind the mechanism of anther opening regulation in response to weather?

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**(1)**

Anther dehiscence is a process of anther opening and releasing of pollen grains. Before the dehiscence starts, developing anther primordium must undergo cell differentiation which produces various cell types like septum or stomium. These cell types then degrade. This creates a slit in between pollen sacs. But this slit is not big enough to allow pollen grains to participate in pollination and fertilization. At the end of dehiscence, anther must dehydrate which causes the anther walls, formed by epidermis and endothecium, to bend outward and makes pollen grains fully exposed. Precise timing of this process is necessary especially in context of weather. This last phase of dehiscence can be postponed due to rain or dew, as we showed in both *A. arenosa* and *A. thaliana* anthers. We are now trying to investigate various fluorescent marker lines and also relevant mutants to understand what is the physiological state of epidermal and endothelial cells in the moment of dehydration. Our main objective is to elucidate what the trigger of the last part of dehiscence is.



#### 14 - Rice small protein OsSTR1 controls cell stability and stress responses

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Small peptides play important roles in plant development and responses to abiotic and biotic stresses. We have identified a novel OsSTR1 gene encoding a small peptide comprised of 76 amino acids. OsSTR1 is mainly expressed in the leaf, stem, and root, and its expression is induced by drought treatment. Fluorescence analysis shows that OsSTR1 localizes in inner nuclear membrane. Transgenic Arabidopsis plants overexpressing OsSTR1 exhibited enhanced drought stress tolerance as compared to the wild type. In addition, OsSTR1-overexpressing plants displayed enhanced expression of pathogen-related genes including PR1 and PDF1.2 and abiotic stress-related genes including RDA29A and CPK6, indicating that OsSTR1 is involved in the control of biotic and abiotic stresses. OsSTR1 directly interacts with rice E3 SUMO ligase and modified with SUMO (Small Ubiquitin-related Modifier), strongly indicating that the function and stability of OsSTR1 are modulated by sumoylation. OsSTR1 also directly interacts with an inner nuclear membrane protein SUN (SAD1/UNC-84 DOMAIN PROTEIN), indicating that OsSTR1 can function as a linker of nucleoskeleton and cytoskeleton complex. OsSTR1 forms homodimer itself and also heterodimer complex with a basic helix-loop-helix transcription factor, suggesting that OsSTR1 can regulate gene expression as a transcription factor. Our data indicate that OsSTR1 plays crucial functions in various stress responses by the establishment of cell polarity, cell migration, cell mechanosensing, and gene expression. This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center no. PJ01327601), Rural Development Administration, Republic of Korea. This work was also supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center No. PJ01330802), Rural Development Administration, Republic of Korea.

## 16 - Arabinogalactan proteins: deciphering their function while looking at the sugars

**Jessy Silva** <sup>(1)</sup> - **Sílvia Coimbra** <sup>(1)</sup> - **Matthew Tucker** <sup>(2)</sup>

**LAQV Requimte, Sustainable Chemistry, University of Porto, Porto, Portugal** <sup>(1)</sup> - **School of Agriculture, Food, and Wine, University of Adelaide, Adelaide, Australia** <sup>(2)</sup>

By 2050, the human population is estimated to reach nine billion. To achieve the needs to sustain this growing population, we will have to double agriculture yields without increasing arable land. Seeds are the final product of sexual reproduction process in spermatophytes and are essential units for plant propagation. As agricultural activities rely on this event, in order to improve productivity, we must first understand the mechanisms and the factors regulating the complex process of seed formation.

Arabinogalactan proteins (AGPs) are highly glycosylated proteins deeply involved in plant reproduction. 90% of the molecules are constituted by arabinogalactan polysaccharides, composed of arabinose, galactose and minor sugars such as glucuronic acid (GlcA), fucose and rhamnose. AGP glycosylation is catalyzed by various glycosyltransferases (GTs) in the secretory pathway. Over the past few years, several efforts have been made to characterize these enzymes but many of them remain to be identified. Recently, it was proposed that AGPs bind and store  $\text{Ca}^{2+}$  through GlcA residues at the plasma membrane.

As the sugar moiety of AGPs seems crucial for their function, we took a step towards the disclosure of the role of a specific group of GTs: the glucuronosyltransferases (GlcATs). GlcATs appear to have an important mission on ensuring the presence of GlcA on AGPs for posterior binding to  $\text{Ca}^{2+}$ . Promoter analysis using multiple YFP fusions were obtained to determine the GlcATs expression patterns.

Mutants of GlcATs were grown in calcium-deficient and non-deficient mediums. Plants with reduced size, smaller siliques, aborted ovules, non-viable pollen and non-fertilized ovules were found leading to the hypothesis that GlcATs are involved in two processes signalized by  $\text{Ca}^{2+}$ : pollen development and pollen tube attraction to the ovule.

Finally, this work highlights the importance of the sugar portion for AGPs' function, providing a deeper understanding of AGPs during reproduction.

## **21 - Molecular and functional characterization of S1/P1 nucleases involved in plant programmed cell death.**

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In animals and plants, degradation of cellular DNA and RNA is an inherent step of PCD, role and mechanism of this process it's different. Previous studies have demonstrated that S1/P1 nucleases are mainly responsible of nucleic acids degradation in plants. As a result of their high homology, to well described fungus nucleases S1/P1 with catalytic activity in low pH and in presence of zinc-ions, it was assumed that the same conditions occurs during hydrolysis of the plant's DNA.

Our research exhibits that plant S1/P1 nucleases have a high diversity of the catalytic requirements. Some of them demonstrate activity in neutral pH and in presence of calcium or manganese ions, while low pH and zinc ions inhibit their activity. These information suggest exact step of PCD when degradation of nucleic acids occurs. To better understand structures features responsible for enzymatic properties of plants S1 nucleases, we performed a directed mutagenesis of regions close to activity center of ENDONUCLEASE 4 and examined influence of this mutations synergistically or independently on activity properties of enzyme.

We determined cell localization of S1/P1 proteins during different cell death processes. We observed transport of three nucleases during starvation and culture senescing from endoplasmic reticulum to vacuoles. While during salicylic acid-induced PCD we observed formation of protein bodies near nucleus. Its suggest various S1/P1 nucleases mode of action in different type of programmed cell death in plants.

We examined expression patterns of S1 nucleases family members using GUS reporter gene system. Our results suggest high diversity of expression, both in different time and tissues. Promoters of members of S1-like family show distinctive but in some cases overlapping expression pattern.

Diversity of expression patterns, cell localization and catalytic properties of S1/P1 nucleases suggesting that role of degrading nucleases in plants is more complex that it was previous assumed.

## 25 - Role of CRK5 protein kinase in embryogenesis of *Arabidopsis thaliana*.

**Abu Imran Baba** <sup>(1)</sup> - Ildikó Valkai <sup>(1)</sup> - Nitin Labhane <sup>(2)</sup> - Koczka Lilla <sup>(3)</sup> - Norbert András <sup>(4)</sup> - Éva Klement <sup>(5)</sup> - Zsuzsanna Darula <sup>(5)</sup> - Katalin Medzihradszky <sup>(5)</sup> - László Szabados <sup>(1)</sup> - Attila Fehér <sup>(1)</sup> - Gábor Rigó <sup>(1)</sup> - Ágnes Cséplő <sup>(1)</sup>

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During the development of embryos in plants, a fine hormonal crosstalk involving (ethylene, gibberellin, brassinosteroid) are important in addition to the important role of auxin. The transport of auxin performed mainly by the PINs efflux and the AUX1/LAX1 auxin influx transporters. It was earlier reported that both efflux and influx auxin transporters are pivotal for determination of embryo polarity. In this study we present some novel insights for the auxin-GA crosstalk in auxin signaling and the regulatory role of the *Arabidopsis thaliana* CRK5 protein kinase during embryo development. We report that *Atcrk5-1* mutant has a delayed embryogenesis accompanied with diminished gibberellic acid (GA) levels. In addition, the *Atcrk5-1* mutant was also reported to be incapable of maintaining the local auxin concentration during embryo formation as observed by decreased expression of the auxin sensor DR5::GFP. The abundance of the polar auxin transport (PAT) proteins PIN1, PIN4 and PIN7 in the *Atcrk5-1* mutant embryos was also decreased. Therefore, we propose that AtCRK5 protein kinase additionally to its regulatory role in root gravitropic responses and hypocotyl development can also contribute to the embryo development in *Arabidopsis thaliana* through fine tuning of auxin-GA level by phosphorylation of polar auxin transport (PAT) proteins.

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## 28 - Modifications of cell wall and storage materials during the culture of isolated endosperm of kiwiberry

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Plant endosperm cells are rich in storage substances. Our work aimed to study the cytological events, which occur in isolated endosperm of kiwiberry, *Actinidia arguta* cv. Bingo cultured on media based on Murashige and Skoog (1962) macro- and microelements and vitamins. Callus induction medium (CIM) and *Actinidia* endosperm medium (AEM) were supplemented with 0.5 mg/l of thidiazuron and 2 mg/l of 2,4-D with 5 mg/l of kinetin, respectively. Callus proliferation was observed on both of media, but shoot buds induction occurred only on CIM, after c.a. 6 weeks of the culture. For histochemical analysis callus was collected after 0, 8, 15, 35 and 42 days of the culture and stained with Toluidine blue O, Periodic acid - Schiff reagents, Aniline blue black, sudan black B and auramine O. In order to detect epitopes of pectins, extensins, hemicelluloses and arabinogalactan proteins (AGPs), 42 day old callus was designed to immunohistochemical analysis, where selected antibodies were used. The fresh isolated endosperm was composed of thick-walled cells, abundant in proteins and lipids. During the culture on CIM, starch grains were detected in dedifferentiated cells and on the surface of explants the cutin presence was stated. Phenolic deposits in callus cells were also visible. Cells of endosperm-derived callus on AEM showed lower contents of starch and proteins. The presence of some extensin (JIM11, JIM12, JIM20) and pectic (LM19, LM20) epitopes was noticed in calli from CIM and AEM. Additionally, pectic (LM5, LM6), hemicellulose (LM25) and AGPs (LM2) epitopes were detected only in callus cultured on AEM. Floccular or net-like structures composed of, among others, pectins were observed in cells from CIM and AEM, respectively. Observed differences in wall composition and storage materials accumulation between explants cultured on CIM and AEM can be a result of different culture conditions and cell competence.

#### **46 - Auxin homeostasis and signalling are key factors that control nitrogen- and dark-mediated adventitious root formation in petunia**

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Adventitious root (AR) formation in the stem base of shoot tip cuttings is a key developmental process underlying clonal propagation of several ornamental crop species. AR formation in *Petunia hybrida* is inhibited by nitrogen limitation in stock plants but promoted by dark incubation of cuttings before planting. We investigated whether the homeostasis and signalling of auxin is involved in nitrogen- and dark-mediated AR formation. Temporal distribution of levels of indole-3-acetic acid (IAA) and of transcripts of genes that control auxin homeostasis and function was analysed in the stem base in response to high versus low nitrogen supply to stock plants and to dark versus light exposure of cuttings. In addition to the use of GC-MS/MS, a petunia specific microarray and quantitative RT-PCR, auxin source capacity in cuttings and auxin concentration in the rooting zone were manipulated. IAA concentration was only marginally affected by nitrogen content of cuttings but was during AR induction enhanced by dark incubation. Early IAA accumulation in the dark depended on the upper shoot as auxin source and was enhanced after apical IAA supply. Dark exposure stimulated RNA accumulation of auxin-related genes. In particular, expression of Ph-PIN1 and of genes controlling auxin signalling including Ph-IAA14, Ph-ARF8, Ph-ARF10 and Ph-SAUR14 was enhanced, while the latter four were repressed in nitrogen-limited cuttings, particularly in the dark. Basal auxin application partially substituted the effect of dark exposure on rooting, whereas the auxin response of AR formation was strongly depressed by nitrogen limitation. Increased auxin delivery from the upper shoot and enhanced auxin signalling in the stem base contribute to dark-stimulated AR formation, while nitrogen limitation inhibits AR formation down-stream of the auxin signal. Targeted mutagenesis of *P. hybrida* by *Agrobacterium*-mediated transformation with RNA-guided Cas endonucleases is currently being established to analyse the functions of candidate genes putatively controlling these processes.

#### 47 - TRH1, an auxin-mediated organizer of Arabidopsis root system architecture

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A network of the transmembrane polarly localized PIN-FORMED (PIN) proteins contributes to auxin gradient formation in plant organs. PIN1/2/3 are major auxin efflux components, shaping auxin homeostasis and hence cell patterning and polarity in the root. While a member of Arabidopsis KT/HAK/KUP K<sup>+</sup> transporter family, TRH1 also controls auxin transport. Auxin distribution in trh1 roots is distorted resulting in agravitropism and defective root-hair elongation. To obtain a systems-level view of these auxin-mediated developmental processes, TRH1 expression was driven to specific root cell layers by PIN promoters pinpointing the acropetal (pPIN1) or the basipetal (pPIN2) auxin routes and the lateral auxin transport in statocytes (pPIN3). TRH1 expression in the acropetal route by pPIN1 complemented root agravitropism, but failed to restore root-hair morphogenesis. In contrast, TRH1 expression in the basipetal route by pPIN2 resulted in normal root-hair morphogenesis, while root gravitropism remained impaired. TRH1 expression mainly in root statocytes driven by pPIN3 failed to restore both gravitropism and root-hair elongation. Substantial transcriptional changes were identified between the pPIN1 and pPIN2 expression systems, demonstrating a powerful model to uncouple the molecular basis of root gravitropism and root-hair development. Variations were observed in gene networks that likely control root response to gravity or cell differentiation highlighting the role of unknown transcription factors (TFs). While in total 53 TFs were differentially expressed, 45 and 8 were identified in the pPIN1 and pPIN2 expression systems, respectively. In line with single-cell RNA-sequencing data of Arabidopsis root, the expression profile of most of these TFs was mapped either at stele cells upon pPIN1 gene expression or root epidermis and cortex upon TRH1 expression by pPIN2. Overall our findings demonstrate an auxiliary but nevertheless fundamental role of TRH1 in auxin canalization and maintenance of auxin homeostasis in the Arabidopsis root apex, shaping distinct traits of root system architecture.

**65 - The Solanaceous cystine-knot miniproteins are metallocarboxypeptidase inhibitors acting in stress defence and development.**

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The cystine-knot proteins identified in Solanaceous species are a group of small proteins ( $\leq 50$  aa long in the mature form) characterized by a peculiar three dimensional structure called cystine-knot, which possess inhibitory activity against metallocarboxypeptidase. Their unique structural scaffold is due to the presence of three intertwined disulfide bonds conferring resistance to high T, extreme pH and proteolysis. Another characteristic of the cystine-knot miniproteins is the presence at the N terminus of a signal peptide for secretion. Studies on potato, tomato and tobacco cystine-knot metallocarboxypeptidase inhibitors (MCPI) demonstrated their responsiveness to various type of abiotic stresses. Moreover, the over-expression of a potato MCPI conferred resistance to the herbivore *Chilo suppressalis* and the fungus *Magnaporthe oryzae* in transgenic rice, suggesting a function in the plant response to biotic stress. More recently, it was shown that altering the expression pattern of two tomato cystine-knot metallocarboxypeptidase inhibitor (TCMPs) during flower and fruit development can result in early fruit production. To shed light on the role of these proteins in reproductive development, we have undertaken a yeast two hybrid analysis to detect interactive partners. One of the identified interactors belongs to the B-box zinc finger protein family which comprises Zn finger transcription factors with regulatory roles in different developmental processes and B-box proteins lacking the transcriptional regulation domain. TCMPs interact with a BBX protein of the second type whose principal function in *A.thaliana* is to interfere with the formation of protein complexes that regulate flowering. Our results indicate that the overexpression of TCMPs favours the sympodial termination in tomato and its ectopic overexpression in *A. thaliana* induces early flowering and increases FT expression, thus suggesting that TCMP might modulate the activity of flowering regulatory multiprotein complexes.



## 116 - Multiple Auxin-Response Regulators Enable Stability and Variability in Leaf Development

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Plant hormones such as auxin and gibberellin (GA) play a central role in many developmental processes throughout the plant's life. Auxin signal transduction is mediated by the antagonistic activity of transcriptional activators (Auxin Response Factors/ARFs) and repressors (Aux/IAAs). Both activators and repressors belong to gene families. However, how plants balances between stability and flexibility in organ development and how the expanded gene families contribute to this is largely unknown. We addressed these questions using tomato leaf development as a model by generating multiple mutants in auxin-response components and analysing genetic interactions among them. Our results suggest that the existence of multiple Aux/IAAs and class A-ARFs stabilizes the developmental output of auxin, and that tuning their activity enables shape variability. Further genetic interactions between A, B and C-ARFs suggest partially overlapping function of A and C-ARFs in the regulation of growth during leaf development. Global transcriptomic analysis, combined with genetic analysis of genotypes with different activities of A and C-ARFs, suggest a prominent role for GA as a mediator of auxin and these ARFs in the regulation of initiation and growth in tomato leaf development. In addition, our results suggest that auxin affect leaf shape and size by balancing between growth and differentiation. In general, we propose a model by which organ development is coordinated by the interaction of two hormonal cues: Auxin, a local growth regulator that activates GA, which have a broader effect on tissue-wide differentiation.

## 152 - SYNSTIGMA TURNS THE FIG INTO A LARGE FLOWER

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Synstigma is a structure formed by clusters of two to several stigmas, whether in the same or between different flowers. Although rare in Angiosperms, synstigmas are found in approximately 500 out of the roughly 750 *Ficus* species (Moraceae). This floral structure is associated with fig-fig wasp pollinating mutualism. The synstigma structure and the pollen tube pathways were studied in six *Ficus* species from section *Americanae* to check the hypothesis that the synstigma allows a cluster of pollen grains deposited on a stigma to emit pollen tubes that can grow laterally and fertilize surrounding flowers. Syconia containing recently pollinated stigmas were collected and dissected and the stigmas processed for analyses in light and scanning transmission electron microscopies. The arrangement of the synstigmas across species can be spaced or congested, with the number of stigmas per synstigma ranging from two to 20. Contact between the stigmas in a synstigma occurs by the intertwining of the stigmatic branches and papillae, which can be classified as firm or loose. The pollen tube grows through the stigmatic exudate and the intercellular spaces of the transmitting tissue until reaching the ovule micropyle. Curvy pollen tubes growing from one stigma to another were observed in five out of the six species studied. The curvilinear morphology of pollen tubes in the synstigma likely results from competition between chemical cues produced by different female gametophytes. Chemical signals emitted at the same time by the egg cells of the flowers near a pollinated/oviposited flower may cause interruptions in the pollen tube growth, as evidenced by the numerous curves in its structure. Synstigma seems to be a key adaptation ensuring that seed production by flowers is not exploited by fig wasps in actively pollinated *Ficus* species (Fapesp, CNPq, Capes).

## 156 - Maternally expressed transcription factors mediate genome dosage response in Arabidopsis

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Seeds are fundamental units for plant propagation and adaptation. Their development is an intricate process that requires the coordinated development of the zygotic tissues (embryo and endosperm) and the seed coat (a maternal sporophytic tissue). Polyploidization plays a major role in plant evolution and diversification. The triploid block is a post-zygotic reproductive barrier in plants which results in the inability of polyploid genotypes to generate viable seeds when crossed with non-polyploid genotypes. Triploid block is associated with the defective development of the seed endosperm due to an altered proliferation-cellularization signalling, particularly when paternal genome-dosage excess occurs. Recently, paternal regulators underpinning the sensitivity to the increased paternal genome dosage have been proposed as active players establishing the triploid block; however, the role of maternal regulators in this phenomenon remains unknown and unexplored. Here, we demonstrate that disruption of genes controlling the flavonoid biosynthetic pathway (FBP) in **maternally derived tissues (like the seed coat)** leads to rescue of the triploid block. We also demonstrate that maternal-specific loss of function of two transcription factors controlling the FBP suppresses the increase in seed size effect typically observed in paternal-excess interploidy crosses. Our results indicate a relevant role for these transcription factors in the bidirectional crosstalk between zygotic and maternal tissues following fertilization.

**KEY WORDS:** Seed development, interploidy crosses, triploid block, maternal effects, seed coat, sporophyte, genome dosage, hybridization barriers.

## 161 - Regulatory effect of polyamines on pollen germination and pollen tube elongation of tobacco plants

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Several signalling pathways have been shown to be involved in the regulation of pollen germination and pollen tube elongation. Among others, exogenously applied polyamines were found to strongly affect pollen maturation, pollen tube emergence and elongation. Our aim was to demonstrate the relation among exogenous polyamines, reactive oxygen species and nitric oxide in the regulation of pollen germination and pollen tube elongation in tobacco plants (*Nicotiana tabacum*, SR1). We have found that putrescine had a somewhat positive effect on pollen tube emergence, but negatively regulated its further elongation; spermidine enhanced both processes, while spermine had negative effect on pollen germination but did not influenced pollen tube growth. Furthermore, our data indicated that PAs regulate pollen germination primarily via regulating the ROS level, while tube elongation primarily influencing the NO level. Taken together, our results further supported the involvement of PAs in the regulation of pollen germination and elongation affecting ROS and/or NO levels in a polyamine- and cellular-region-specific way.

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## **170 - Role of auxin and polyamines in the regeneration processes of *Arabidopsis thaliana***

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Over recent decades, various culture conditions have been established for plant regeneration. A common mode of plant regeneration is de novo organogenesis, the other way is somatic embryogenesis (SE). Plant hormones, mainly auxins are important in the regulation of plant regeneration. Furthermore, auxin may interact with polyamines in several physiological processes. Our aim was to examine the effect of auxin and polyamines on the induction of SE or organogenesis. To this end, we used a root culture based regeneration system which was previously established in our laboratory. Root explants of freshly germinated seedlings and whole seedlings of *Arabidopsis thaliana* were used in this system. To induce regeneration processes low auxin concentration followed by a strong temporal cytokinin treatment was used. If the root explants were kept on medium containing cytokinin, organogenesis occurred. However, if the root explants were transferred onto a hormone-free medium at a right time, the primordia were converted to somatic embryos. Interestingly, the morphogenic response of the root was blocked, if the shoot was not removed before cytokinin treatment. Using auxin transport inhibitor it was shown that shoot-derived auxin is responsible for this blockage. Furthermore, among polyamines, spermine and spermidine, also induced the regeneration of the roots of whole seedlings. Based on this result we suggest that polyamines may be involved in the regulation of regeneration processes through the inhibition of auxin transport.

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## **179 - Genetic control of juvenile phase-specific cuticle deposition and cuticle-mediated plant response to drought in maize**

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In all higher plants, the outer surface of the aerial parts is covered by the cuticle, a complex lipid layer that constitutes a barrier against environmental stresses. Only few genes involved in cuticle deposition have been characterized in maize and its hormone-mediated regulation is still poorly understood.

We show in this work that the MYB transcription factor ZmMYB94/fused leaves1 (fdl1) is a key regulator of cuticle deposition during maize juvenile vegetative phase. Biochemical analysis showed that wax and cutin were significantly altered in fdl1-1 mutant plants, suggesting that FDL1 is required for the deposition of both cuticular components. These data corroborated previous results obtained from mutant phenotypic analysis showing that fdl1 is involved in the correct epicuticular waxes distribution as well as in the formation of the whole cuticle layer during post-germinative organ separation.

To further investigate the role of FDL1 in the control of cuticle biosynthesis we performed an Illumina based RNA-sequencing experiment. About 1600 differentially expressed genes were detected between fdl1-1 and wild type. Their analysis led to the identification of a set of candidate genes implied in lipid metabolism. Variation in their expression nicely correlated with cuticular alterations observed at the biochemical level.

We explored the effect of drought on cuticle properties and showed that in drought condition maize seedlings are able to decrease cuticle-mediated leaf permeability, thus reducing water loss and enhancing plant tolerance. Similar results were obtained in ABA treated seedlings. Moreover, drought and ABA influenced fdl1 and other cuticle related genes at the transcriptional level.

Our data provide novel tools for the selection of genetic variants able to improve cereal crop tolerance against environmental conditions that limit yield.

## **202 - Effect of phytochromes activation in vivo and in vitro on physiological and biochemical processes in plants with different types of development**

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Activation of phytochromes stimulates considerable changes in physiological and biochemical processes in plants. But these effects of phytochrome activation in vivo and in vitro on plants with different photoperiodic sensitivity, spring and winter crops, and early-maturing and late-maturing plants have not been studied. In these conditions we investigated the effect of phytochrome activation by red light (660 nm) on morphogenesis, enzyme and phytohormones activity, carbohydrates and protein metabolism in wheat lines (*Triticum aestivum* L.) isogenic by VRN and PPD genes, and in soybean E lines (*Glycine max* (L.) Merr.), spring and winter rapes (*Brassica napus* var. *Oleifera*), early and late ripening tomato varieties (*Lycopersicon esculentum* Mill.). In conditions in vitro during the irradiation of the callus cultures of the VRN and PPD genes isolines of wheat with red light (RL), morphogenesis processes got intensified. These processes were more intense in the lines with the dominant genes VRN-A1a and VRN-D1a, as well as in the lines PPD-D1a and PPD-A1a, than in the lines with the dominant genes VRN-B1a and PPD-B1a, which in vivo developed more slowly. Red light irradiation of callus cultures of soybean isogenic lines E genes in vitro enhanced morphogenetic processes in photoperiodically neutral (PPN) lines with recessive e1e2 genes more significant than in short-day lines (SD) with dominant E1E2 genes. Under in vitro conditions in the callus culture of spring rape and early-ripe tomato varieties, RL exposure enhanced morphogenetic processes more significantly than in winter rape and late-ripe tomato varieties. The RL irradiation of plants of soybean lines isogenic by the E genes under the conditions of the vegetative experiment led to increase both in growth processes and in activity of GA and IAA, but at the same time it resulted in a decrease in ABA activity, as well as a decrease in the accumulation of carbohydrates in the leaves of the SD line; also it didn't change the intensity of these processes in the PPN line. Irradiation of RL of tomato seedlings caused an acceleration of the transition to the late-ripening plants flowering, without changing the timing of transition to flowering in early-ripening plants planted in open field. After irradiation with RL, the activity of sucrose phosphate synthase, sucrose synthase, invertase and amylase, as well as the accumulation of carbohydrates in leaves of tomato seedlings plants changed. So, the effects of phytochrome activation can be realized through genetic control of plants development.

## **208 - Effect of HSP90 in stem cell homeostasis in *Arabidopsis thaliana*.**

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Heat shock protein 90 (HSP90), an evolutionary conserved molecular chaperone, is considered as a well-connected hub in molecular networks. It is involved in the regulation of a huge repertoire of substrate proteins that are collectively referred as clients. HSP90 play a crucial role in areas as diverse as development and homeostasis, whereas its role in the transcriptional control of environmentally responsive genes and their interaction with epigenetic factors have recently begun to be studied. In plants, reproductive development is controlled by complex gene-regulatory networks of transcription factors. These networks integrate the information from endogenous, hormonal and environmental regulatory pathways and converge at the Shoot Apical Meristem (SAM), a well-defined structure at the top of the shoot apex in *Arabidopsis*. SAM serves as the source of cells for all initiating lateral organs, while stem cell population is maintained constant in the SAM. The molecular effector of the stem cell maintenance is the WUSCHEL/CLAVATA3 feedback loop. Here we show that plants with reduced HSP90 levels show abnormal shoot organization such as fasciation and loss of apical dominance. This effects are due to altered meristem organization. On the molecular level the marker gene WUSCHEL and other meristematic genes show altered expression levels, while the CLAVATA3 gene shows ectopic expression as well. Furthermore, we provide evidence that HSP90 bind on the promoter of WUSCHEL, exerting a role in the transcriptional control of this key meristematic gene.



**223 - A PHABULOSA dependent control of Gibberellin homeostasis regulates Middle Cortex formation in Arabidopsis.**

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In both animal and plants development involves histological and anatomical changes. Arabidopsis Ground Tissue (GT) development is a paradigmatic example of those changes as its post-embryonic maturation coincides with the generation of an additional tissue layer, the Middle Cortex (MC). The plant hormone gibberellin (GA) is a key regulator of MC formation as alterations in its signalling and homeostasis result in variations in MC development. Nonetheless, little is known about how GA signalling and homeostasis are regulated during GT maturation. We demonstrate that the HOMEODOMAIN LEUCINE ZIPPER III (HD-ZIPIII) transcription factor PHABULOSA (PHB) is a master regulator of MC formation by controlling both GA signalling and catabolism. We show that PHB regulates GA homeostasis in the vascular tissue, promoting the stability of the DELLA protein GAI (GIBERELLIN INSENSITIVE). Furthermore, we show that the PHB/GA module is fundamental for the correct formation of the MC. Our results represent a step forward in understanding the molecular mechanisms controlling root development.

## **237 - Phenological Assessment of Maize Hybrids Under High Plant Population Density**

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A field experiment was conducted during 2014 and 2016 rainy season at Tudun Wada, Kano and Samaru, Zaria in the Northern Guinea Savanna of Nigeria in order to assess the phenological components of maize-hybrids at high plant population. The two locations fall under the northern Guinea Savanna zone of Nigeria. Tudun Wada, usually receives less rainfall than Samaru Zaria because it lies at the fringes of the Guinea savanna zone. Specific soil characteristics in each location were determined according to the IITA analytical procedures. The experiment consisted of two plant populations of 53,333 plants $\text{ha}^{-1}$  and 88,888 plants $\text{ha}^{-1}$  as main plot and 8 drought-tolerant maize-hybrids and 2 controls as sub-plot laid out in a randomized split-plot design and replicated three times. Data were collected on the following parameters: Number of days to 50% tasseling: number of days to 50% silking: days to silking (silking date), anthesis-silking interval (ASI): and number of days to 95% maturity: The study indicated that the recently developed maize-hybrids were tolerant to high plant population of 88,888 plants  $\text{ha}^{-1}$ . Phenological parameters of the hybrids were generally shorter in Zaria than in Tudun Wada. High plant population showed decrease in phenological responses. Interaction studies also showed that maize-hybrids responded differently to the high plant population of 88,888 plants $\text{ha}^{-1}$ . Therefore, recently developed maize-hybrids showed better adaptation to biotic stress.

**Key words:** plant population, maize-hybrids, phenology.

## 255 - Analysis of sex determining regions of Y chromosome in a dioecious plant *Silene latifolia*

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*Silene latifolia* is a dioecious plant species established as a model for studying sex determination and evolution of sex chromosomes. As in the mammalian XY system, the Y chromosome contains the key genes localized in the sex determining regions.

These regions are Gynoecium Suppressing Factor (GSF), Stamen Promoting Factor (SPF) and Male Fertility Factor (MFF). Our project is aimed at analysing the GSF region with the main goal to identify specific genes involved in the gynoecium formation suppression.

Based on differential expression data we selected a number of candidate genes/sequences with expression differing between males, females and hermaphrodites. To verify the localization of the selected sequences in the GSF region we are using two parallel approaches.

The first of them is based on the low-copy FISH method which we have optimized for use in plants. We also have probes marking the edges of the GSF region.

The second approach is deletion mutant mapping via PCR. Using mutant plant DNAs with deletions in the GSF region, we are testing these for the absence of our candidate sequences as well as published markers in the vicinity of the GSF region. This way we are refining the physical map of the Y chromosome, which will enable us to lower the number of sequences for the FISH experiments.

Knowledge of chromosomal localization of sex-linked genes and their function will help understand sex determination in plants. Moreover, it will lead to better understanding of evolution of sex chromosomes in general, e.g. chromosomal aberrations and rearrangements.

This work is financially supported by the Czech Science Foundation (grant no. 19-02476Y).

## 271 - Functions of class XI myosins in Arabidopsis

Eve-Ly Ojangu <sup>(1)</sup> - Birger Ilau <sup>(1)</sup> - Kristiina Talts <sup>(1)</sup> - Heiti Paves <sup>(1)</sup> - Erkki Truve <sup>(1)</sup>

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The actomyosin cytoskeleton coordinates numerous processes in plants: determining cell architecture, movement of organelles and vesicles, development, fertility, senescence. Although the general function of myosins is established decades ago, it is not well understood why plants need two classes of myosins and what are the individual functions of each myosin. In this presentation we concentrate on class XI myosins, encoded by 13 genes in Arabidopsis. Studies using T-DNA mutants, dominant negative or RNAi inhibition of genes by several groups, including us, have demonstrated that many myosins have largely redundant functions whereas myosin XI-K is often functioning as a “master regulator” of actomyosin-dependent processes.

The reduced cell size was first described in these mutants. Both the tip growth and diffuse expansion of cells (predominantly epidermal cells) were disturbed. Soon it became evident that class XI myosins contribute to the cytoplasmic streaming, which may be one of the reasons why the mobility of various organelles was changed. Reduced cell size leads to size reduction of shoot organs and roots. This affects the fertility as the elongation of stigmatic papillae in mutants is delayed and the pollen tube growth is slowed down. Fertility is affected in mutants also because the polar auxin transport is irregular. These defects in turn lead to the reduced seed-set. In addition, class XI myosins contribute to actin remodelling by stimulating turnover and shape changes in actin filaments or bundles. Many studies carried out by different groups, including us, complement each other and will be described in the presentation. Nonetheless, the functions of class XI myosins are not yet fully understood. For instance, whether large organelles or small vesicles constitute the main myosin cargoes? Is it correct that class XI myosins mediate cell division, although we and others have not observed any changes of cell numbers in mutant plants?

## **280 - Characterization and genetic analysis of vegetative phase change in natural populations of *Arabidopsis thaliana*.**

**Erin Doody**<sup>(1)</sup> - **Scott Poethig**<sup>(1)</sup>

**Department of Biology, University of Pennsylvania, Philadelphia, United States**<sup>(1)</sup>

Variation in the timing of developmental transitions, or heterochrony, is a mechanism by which many organisms adapt to changing environments. This variation occurs both between and within a species and is regulated by interplay between genetic and environmental cues. A better understanding of the genetic basis of natural variation in developmental timing will therefore provide important insights into the mechanisms by which organisms become adapted to their environments. In plants, shoot development is characterized by two distinct transitions: vegetative and reproductive phase changes. Vegetative phase change is the transition from juvenile to adult vegetative growth and reproductive phase change the transition from vegetative to reproductive growth. While natural variation in reproductive phase change has been extensively studied in many plant species, including *Arabidopsis thaliana*, natural variation in vegetative phase change remains poorly characterized. To better understand how the juvenile to adult transition is regulated in natural populations of *A. thaliana*, a group of over 100 ecotypes originating from a wide range of geographical locations were phenotypically characterized for timing of vegetative and reproductive phase changes. These experiments revealed that timing of these two developmental transitions was decoupled in the majority of natural accessions, indicating that the observed variation is both genetically regulated and heterochronic in nature. To reveal potential genetic loci involved in regulating the variation in the timing of the vegetative phase transition, a genome-wide association study (GWAS) was done using phenotypic data from 87 natural accessions. This analysis revealed a significant candidate locus in a highly polymorphic intergenic region on chromosome three. Several candidate genes in this region are currently being characterized to validate the role of this locus in the regulation of vegetative phase change.

### **283 - Night-Length Information drives photoperiodism in poplar**

**Mariano Perales** <sup>(1)</sup> - **Paolo M Triozzi** <sup>(1)</sup> - **Daniel Alique** <sup>(1)</sup> - **Daniela Gomez-Soto** <sup>(1)</sup> - **Isabel Allona** <sup>(1)</sup>

**Centre for Plant Biotechnology and Genomics UPM – INIA, Universidad Politécnica de Madrid, Madrid, Spain** <sup>(1)</sup>

Day length is a key indicator of seasonal information that determines major patterns of behavior in plants and animals. Day length measurement requires a photoperiodic time measurement mechanism (PTM) formed by 3 main components (1) a photosensory system, which includes photoreceptors, (2) the circadian clock system, and (3) A mobile photoperiodic signal that translates photoperiod information from the photosensory system to the target organs. Plant components of the PTM have been largely studied in Arabidopsis flowering time regulation however photoperiod perception in perennial models show conserved and particular features. In poplar trees, photoperiodism plays a major role in growth cessation during the autumn as well as activating the resumption of shoot growth in the spring, both processes controlled by FLOWERING LOCUS T expression levels and critical for the survival of perennial plants over winter. It has been shown that the conserved role of poplar orthologs to Arabidopsis CONSTANS (CO) directly activates FT expression. Overexpression of poplar CO is, however, not sufficient to sustain FT expression under short days, pointing to the presence of an additional short-day-dependent FT repression pathway in poplar. We find that night length information is transmitted via the expression level of a poplar clock gene, LATE ELONGATED HYPOCOTYL (LHY), which controls FT expression. Repression of FT is a function of the night extension and LHY expression levels. Functional and computational analysis support a night length derived signaling operating in poplar PTM.

**293 - Mutations at the miR172 target site of orthologous PETALOSA TOE-type genes cause dominant double-flower phenotype in peach, rose, carnation and petunia.**

**Stefano Gattolin**<sup>(1)</sup> - **Marco Cirilli**<sup>(2)</sup> - **Stefania Chessa**<sup>(1)</sup> - **Alessandra Stella**<sup>(1)</sup> - **Daniele Bassi**<sup>(2)</sup> - **Laura Rossini**<sup>(2)</sup>

**CNR, Istituto di Biologia e Biotecnologia Agraria (IBBA), Milan, Italy**<sup>(1)</sup> - **Università degli Studi di Milano, Dipartimento di Scienze agrarie e ambientali (DISAA), Milan, Italy**<sup>(2)</sup>

Flowers with supernumerary petals have been selected by humans throughout history, and in several ornamental plants the so called ‘double flower’ varieties have added commercial value. In many cases a single dominant locus was identified as responsible for this sought-after trait. High-resolution linkage mapping in segregating *Prunus persica* (peach) progenies allowed us to identify a TOE-type euAP2 transcription factor as the candidate gene linked to the phenotype in this species. Members of the euAP2 family have been shown to play a critical role in the ABCDE model proposed to explain flower organ development in model species. Genomic resequencing data revealed a deletion of the miR172 binding site in this gene, likely impairing its regulation and resulting in double-flower formation. Using allele mining approaches, we then identified an array of analogous mutations in orthologous TOE-type genes of important ornamentals: *Rosa hybrida* (rose), *Rosa rugosa* (Japanese rose), *Petunia hybrida* (petunia) and *Dianthus caryophyllus* (carnation). In all cases insertions or deletion variants cause the disruption of the miR172 target sequence and of the C-terminal portion of the encoded protein. Despite the phylogenetic distance between these eudicots, which diverged in the early Cretaceous, the identified TOE-type genes all belonged to a single subgroup, which we named PETALOSA (PET). Similarity searches revealed the presence of PET sequences in various other plant species. As a proof of concept of the conserved function of these genes, CrispR-Cas9-induced lesions within the miR172 target site of *Nicotiana tabacum* (tobacco) PET genes resulted in the development of supernumerary petaloid structures. The identification of pet alleles and the possibility of identifying and engineering PET genes to obtain the desirable double-flower trait in different plants provide valuable tools to develop new varieties for the ornamental market.

**295 - Physiological and biochemical characterization of bud dormancy: Evolution of sugar metabolism, hormonal profile and antioxidant systems in a high chill peach variety**

**Jose Antonio Hernández** <sup>(1)</sup> - **Pedro Díaz-Vivancos** <sup>(2)</sup> - **Nuria Alburquerque** <sup>(2)</sup> - **Domingo Martínez** <sup>(3)</sup> - **Gregorio Barba-Espín** <sup>(2)</sup> - **José Ramón Acosta-Motos** <sup>(2)</sup> - **Esther Carrera** <sup>(4)</sup> - **Jesús García-Bruntón** <sup>(5)</sup>

**CBAS-CSIC, Group of Fruit Trees Biotechnology, Murcia, Spain** <sup>(1)</sup> - **CEBAS-CSIC, Group of Fruit Trees Biotechnology, Murcia, Spain** <sup>(2)</sup> - **University Miguel Hernandez, Department of Food Technology, Alicante, Spain** <sup>(3)</sup> - **IBMCP-CSIC, Group of Hormonal metabolism and plant development regulation, Valencia, Spain** <sup>(4)</sup> - **IMIDA, Group of Fructiculture, Murcia, Spain** <sup>(5)</sup>

The levels of antioxidant enzymes, sugar and starch contents and the evolution of ABA and GAs during bud dormancy were studied in a peach variety, grown in two geographical areas. In both locations, starch contents progressively increased, reaching a peak in ecodormancy (EC), and then decreased at dormancy release (DR). Sorbitol and sucrose were the more abundant soluble sugars in peach buds. Sucrose and sorbitol declined at DR, mainly in the cold area. Glucose and fructose progressively rose during the dormancy process, reaching the highest values at DR.

APX peaked at the onset of endodormancy (ED) and declined at DR in both locations. In the temperate area, MDHAR peaked at the beginning of ED, progressively decreased in ED and EC and increased at DR. DHAR peaked at the end of EC and decreased at DR. In general, GR activity declined during ED and progressively increased until DR. SOD peaked at the end of ED in both zones. Then, SOD activity decreased, returning to initial values. In both locations, POX peaked at the end of PD, showing the lowest values at DR. CAT activity displayed a progressive decrease during the dormancy period in both locations, reaching a minimum at DR, mainly in the temperate zone.

GA7 was the major bioactive GA in both zones. In the temperate zone, GA7 levels were unchanged, whereas in the cold zone, GA7 progressively decreased during the dormancy period, especially at DR. In both locations, ABA decreased during the dormancy period, especially in the cold zone. As a consequence, a reduction in the ABA/total GAs ratio was observed, being more evident in the cold zone.

The possible interaction between the antioxidant and carbohydrate metabolism and the evolution in ABA and GAs in breaking bud dormancy is discussed.

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## 298 - Improvement of indirect leaf area measurements

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Leaf or canopy area is a key parameter for both farmers and plant scientists to monitor and/or model the growth and the well-being of plants. Hydrological processes, such as transpiration, evaporation and rainfall interception are also related to it.

Although direct LAI measurement techniques are accurate, those are relatively laborious and time-consuming. To overcome these disadvantages a couple of indirect optical methods have been developed. Ceptometry is one of the most widely used since the early 1990s. The LP-80 ceptometer uses a linear array of PAR (photosynthetically active radiation) sensors for non-destructive LAI measurements.

Optimal illumination conditions for the measurement is one of the most important aspects that are not uniformly handled by experts.

The sensitivity of ceptometer-based LAI values to PAR was investigated by readings under uniformly overcast and clear sky conditions and also by setting the device into logger mode on a day with cloud drifts when light conditions changed rapidly.

$PAR < 1700 \mu\text{mol m}^{-2} \text{s}^{-1}$  was found to be inadequate light condition in our study regarding leaf area measurement with LP-80 ceptometer. We found that measurements under inadequate light conditions could cause an underestimation of LAI. A simple method was devised for wheat and maize to correct raw ceptometer data collected under non-ideal light conditions.

Using the corrected LAI values, the ceptometer data showed a significantly better fit (higher  $R^2$ , smaller mean average error and closer to zero mean signed error values) to the destructive LAI data for both wheat and maize. With the help of the correction equations, the use of the LP-80 ceptometer could be extended to days when light conditions are not ideal. The correction method also helps overcoming practical limitations of the device in monitoring leaf area variation in time and space, when illumination conditions can considerably change during the measurement.

### **314 - Floral development of Cecropieae species (Urticaceae) with emphasis on the tubular calyx and united androecium**

**Giseli Donizete Pedersoli<sup>(1)</sup> - André Luiz Gaglioti<sup>(2)</sup> - Simone Pádua Teixeira<sup>(1)</sup>**

**Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil<sup>(1)</sup> - Institute of Botany, Herbarium SP, São Paulo, Brazil<sup>(2)</sup>**

Small flowers, with tubular calyces and united androecium added to small numbers of organs per whorl are found in species of Cecropieae (Urticaceae). In order to elucidate the processes that lead to the formation of such floral conditions, this study compared the morphology of the developing flowers of *Cecropia pachystachya* Trécul, *Coussapoa microcarpa* (Schott) Rizzini and *Pourouma cecropiifolia* Mart.. Buds and flowers were collected and examined under scanning electron and light microscopes. The tubular calyx originates from the activity of a peripheral annular meristem that elongates and forms a tube with two (*C. pachystachya*) or three free lobes (*C. microcarpa*, *P. cecropiifolia*). In the staminate floral meristem the androecium primordium arises as a central bulge that elongates and gives rise to two stamens that remain with the filaments basally united (*C. pachystachya*) or filaments totally united with the anthers free (*C. microcarpa*) or the anthers basally united (*P. cecropiifolia*). In the pistillate floral meristem the gynoecium primordium also arises as a central bulge that elongates and gives rise to two carpel primordia: one expands and forms a carpel with a cleft and the other does not differentiate and remains rudimentary at the base of the developing bud. Pistillate and staminate flowers result of the stamen or carpel absence, respectively, from inception. Petals are also absent from inception. The formation of the tubular calyx and united androecium occurs very early in development characterizing a congenital union. The union of anthers by the connectives in *C. microcarpa* (union between cell walls at the base and through a weak cohesion of the epidermal cells at the median region) is an uncommon condition in Urticaceae. The floral development of Cecropieae is quite similar and less labile than in the other Urticaceae species, indicating that this tribe should be considered a well-defined taxonomic group.

### 338 - A molecular framework for understanding root growth angle contro

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Scientific Research Unit, Italian Space Agency, Rome, Italy <sup>(1)</sup> - FBS, University of Leeds, Leeds, United Kingdom <sup>(2)</sup> - MPIMP, Max Plank Institute, Berlin, Germany <sup>(3)</sup> - Dpt. Experimental Plant Biology, Charles University, Prague, Czech Republic <sup>(4)</sup> - IST, Institute of Science and Technology, Vienna, Austria <sup>(5)</sup>

Growth angle is a crucial determinant of root system architecture and function. The trajectories of roots growing through the soil are most often determined in reference to gravity, in which case the growth angle is known as gravitropic setpoints angles (GSAs). A defining characteristic of organs growing at GSA is that when orientation is shifted in the gravity field, gravitropic response returns the root to its GSA. In the case of roots with vertical GSAs, such as the primary roots of many species, that graviresponse is always a downward one. In contrast, lateral roots growing at non-vertical GSAs must have the capacity to bend up as well as down. In this project, we have investigated the similarities and differences in GSA control between primary and lateral roots. Firstly, we show that in both primary roots and lateral roots graviresponse is angle-dependent, i.e. the bend rate is proportional to the angle of displacement from the GSA. Further, we show that in both primary and lateral roots, these angle-dependent gravitropic responses are associated with changes in auxin distribution that are entirely in accordance with the Cholodny-Went model of tropic growth. Finally, we show that differences in sensitivity to auxin between primary and lateral roots are an important component of the capacity to maintain vertical and non-vertical GSAs respectively. Together, these data provide a coherent mechanistic framework for understanding, and possibly manipulate, growth angle control in the root.

### **362 - Functional studies of hot pepper CYP707A family genes: Identification of their catalytic reaction and analysis of their roles in plant**

**Young Hee Joung <sup>(1)</sup> - Hyun Min Kim <sup>(1)</sup> - Se Hee Park <sup>(1)</sup> - Seo Young Park <sup>(1)</sup> - Thanh Dat Mai <sup>(1)</sup> - Ju Hui Do <sup>(1)</sup>  
- Tuan Viet Do <sup>(1)</sup> - Won Choi <sup>(1)</sup> - Hye Min Jang <sup>(1)</sup> - In yeong Na <sup>(1)</sup> - Hyeon Bae Hwang <sup>(1)</sup> - Sang Hoon Ma <sup>(1)</sup>  
- Jae Sung Shim <sup>(1)</sup> - Chul-Ho Yun <sup>(1)</sup>**

**Plant Molecular biology Lab, Chonnam National University/School of Biological Sciences and Biotechnology, Gwang-ju, Korea, Republic Of <sup>(1)</sup>**

Change of abscisic acid (ABA) homeostasis affect to various ABA mediated stress and developmental responses. Homeostasis of ABA is regulated by balance of biosynthesis and catabolism processes. The ABA catabolism pathway involves the CYP707A family, encoding ABA 8'-hydroxylase. Here we have identified that four *Capsicum annuum* cytochrome P450 genes (CaCYP707A1, 2, 3, and 4) have ABA hydroxylation activity. Expressions of CaCYP707As have been validated to increase under drought stress condition. To identify the catalytic function of the CaCYP707As, these proteins were cloned into *E. coli* expression vector and heterologously produced in *E. coli* system. These recombinant proteins were incubated with ABA in NADPH condition and catalyzed ABA to 8'-hydroxy ABA, phaseic acid. ABA hydroxylation activity was also demonstrated in CaCYP707As-overexpressing tobacco plants, which ABA level was lower than non-transgenic plant and exhibited drought sensitive phenotype. More interestingly, these transgenic tobacco plants showed that down regulated seed formation. The phenotypical analysis of pollen showed that the development of pollens were not complete. In addition, the pollen viability of transgenic tobacco plants were significantly lower than non-transgenic plant. Our results indicate that CaCYP707As regulate the seed formation and pollen viability through their ABA hydroxylation activity.

**375 - Expression analysis of relevant genes involved in de novo shoot organogenesis and somatic embryogenesis of *Jatropha curcas* L.**

**Juan José Torres-Ruíz<sup>(1)</sup> - Teresa Ponce-Noyola<sup>(1)</sup> - Ana Carmela Ramos-Valdivia<sup>(1)</sup> - Adriana Garay-Arroyo<sup>(2)</sup> - Estela Sandoval-Zapotitla<sup>(3)</sup>**

**Centro de Investigación y de Estudios Avanzados IPN, Departamento de Biotecnología y Bioingeniería, CDMX, Mexico<sup>(1)</sup> - Instituto de Ecología, Universidad Nacional Autónoma de México, CDMX, Mexico<sup>(2)</sup> - Jardín Botánico del Instituto de Biología, Universidad Nacional Autónoma de México, CDMX, Mexico<sup>(3)</sup>**

*Jatropha* (*Jatropha curcas* L., Euphorbiaceae), a potential biodiesel plant, has created remarkable interest all over the world for the use of its seed oil as a commercial source of biodiesel. Due to the unreliability of oil content in its seeds and low economic returns, planting of *Jatropha* in agriculture has been restricted. Investigation of molecular basis of plant regeneration through De novo Shoot Organogenesis (DNSO) or Somatic Embryogenesis (SE) is an immediate need to apply biotechnological breeding strategies that allow to exploit its great potential. In this study, we analyze morphologically and histologically regenerated structures through DNSO and SE pathway obtained from cotyledons cultured in MS medium supplemented with high concentration of 6-benzyladenine in relation to indole acetic acid; and 2,4-dichloro-phenoxyacetic acid, respectively. Analysis of longitudinal serial sections showed the formation of either meristemoids which differentiated into shoots with vascular connection to the initial explant; or pro-embryogenic masses with cells containing amyloplasts, that will give rise to somatic embryos. On the other hand, we examined during development of these structures the expression levels of 5 transcription factors previously studied in *Arabidopsis thaliana* as ESR2 and PLT3 involved in DNSO, AGL15 and RKD4 relevant for SE, and WUS implicated in both processes using real-time quantitative PCR. All 5 genes increased their expression levels compared to non-regenerating calluses, showing different expression patterns and maximum relative ratios from 1.23 to 18.63 times during the development of shoots and embryos. This work provided a first glimpse of expression patterns of genes associated to in vitro morphogenesis of *Jatropha curcas*, a non-model plant with great economic potential.

**378 - Differential transcriptomic profile between early- and late-maturing sweet cherry varieties in response to GA<sub>3</sub> reveals a role of gibberellins during fruit ripening**

**Nathalie Kuhn** <sup>(1)</sup> - **Jonathan Maldonado** <sup>(2)</sup> - **Claudio Ponce** <sup>(3)</sup> - **Macarena Arellano** <sup>(3)</sup> - **Alson Time** <sup>(3)</sup> - **Lee Meisel** <sup>(3)</sup> - **Salvatore Multari** <sup>(4)</sup> - **Stefan Martens** <sup>(4)</sup> - **José Manuel Donoso** <sup>(5)</sup> - **ESTHER CARRERA** <sup>(6)</sup>

Pontificia Universidad Católica de Valparaíso, Pontificia Universidad Católica de Valparaíso/ Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Quillota, Chile <sup>(1)</sup> - Universidad de Chile, Universidad de Chile, Facultad de Ciencias Agronómicas, Departamento de Producción Agrícola, Santiago, Chile <sup>(2)</sup> - Universidad de Chile, Universidad de Chile, Instituto de Tecnología de los Alimentos, Santiago, Chile <sup>(3)</sup> - Fondazione Edmund Mach, Department of Food Quality and Nutrition, Centro Ricerca e Innovazione, Fondazione Edmund Mach, Trentino, Italy <sup>(4)</sup> - INIA, INIA Rayentué, Rengo, Chile <sup>(5)</sup> - Universidad Politécnica de Valencia, Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-Consejo Superior de Investigaciones Científicas, Valencia, Spain <sup>(6)</sup>

The ripening process in non-climacteric fruits is controlled mainly by abscisic acid, though other hormones may also participate. GA<sub>3</sub> treatment is known to affect ripening, causing a delay in color development, anthocyanin accumulation and fruit softening. However, there is few information about the role of gibberellins (GAs) at the physiological and molecular level during ripening.

In sweet cherry fruits GA<sub>4</sub> and GA<sub>3</sub> accumulate prior to ripening initiation, but then reduce their content as the fruit colors. GA<sub>4</sub> is negatively correlated with anthocyanin and sugar content. Additionally, differences in the GA<sub>3</sub> effect in contrasting maturity-time sweet cherry varieties have been found, but the molecular basis of this difference has not been further explored.

Here we found that anthocyanin content and several ripening related parameters were affected by GA<sub>3</sub> when applied prior to ripening initiation in early as well as in late sweet cherry varieties. GA<sub>4</sub> was present in both varieties, however only in the late variety it tended to increase its content. Additionally, a differential transcriptomic profile in response to GA<sub>3</sub> in late- compared with early-maturing variety was found, which was accompanied with differences in the maturity index IAD (Index of Absorbance Difference), which reveals the presence of phenolic compounds, including anthocyanins. We found that in the late-maturing variety the IAD curve was delayed and the expression changes of several genes did not occur after the GA<sub>3</sub> treatment. In contrast, in the early variety the IAD curve was not delayed but reduced its magnitude at maturity and an important number of genes changed after the treatment. The results suggest that GAs possibly delay but not affect ripening in late varieties, by slowing several ripening related pathways, whereas GAs negatively affect ripening in early varieties possibly by controlling pathways related to the ripening process.

**391 - Expression analysis of relevant genes involved in de novo shoot organogenesis and somatic embryogenesis of *Jatropha curcas* L.**

**Juan José Torres-Ruíz <sup>(1)</sup> - Teresa Ponce-Noyola <sup>(1)</sup> - Ana Carmela Ramos-Valdivia <sup>(1)</sup> - Adriana Garay-Arroyo <sup>(2)</sup> - Estela Sandoval-Zapotitla <sup>(3)</sup>**

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*Jatropha* (*Jatropha curcas* L., Euphorbiaceae), a potential biodiesel plant, has created remarkable interest all over the world for the use of its seed oil as a commercial source of biodiesel. Due to the unreliability of oil content in its seeds and low economic returns, planting of *Jatropha* in agriculture has been restricted. Investigation of molecular basis of plant regeneration through De novo Shoot Organogenesis (DNSO) or Somatic Embryogenesis (SE) is an immediate need to apply biotechnological breeding strategies that allow to exploit its great potential. In this study, we analyze morphologically and histologically regenerated structures through DNSO and SE pathway obtained from cotyledons cultured in MS medium supplemented with high concentration of 6-benzyladenine in relation to indole acetic acid; and 2,4-dichloro-phenoxyacetic acid, respectively. Analysis of longitudinal serial sections showed the formation of either meristemoids which differentiated into shoots with vascular connection to the initial explant; or pro-embryogenic masses with cells containing amyloplasts, that will give rise to somatic embryos. On the other hand, we examined during development of these structures the expression levels of 5 transcription factors previously studied in *Arabidopsis thaliana* as ESR2 and PLT3 involved in DNSO, AGL15 and RKD4 relevant for SE, and WUS implicated in both processes using real-time quantitative PCR. All 5 genes increased their expression levels compared to non-regenerating calluses, showing different expression patterns and maximum relative ratios from 1.23 to 18.63 times during the development of shoots and embryos. This work provided a first glimpse of expression patterns of genes associated to in vitro morphogenesis of *Jatropha curcas*, a non-model plant with great economic potential.

#### **401 - Cell wall and storage materials modifications during the culture of isolated endosperm of kiwiberry**

**MOHIB ABDULLAH <sup>(1)</sup> - KATARZYNA SALA <sup>(2)</sup> - EWA KURCZYNSKA <sup>(2)</sup> - MONIKA TULEJA <sup>(1)</sup> - MARZENA POPIELARSKA-KONIECZNA <sup>(1)</sup>**

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Cell wall and storage materials modifications during the culture of isolated endosperm of kiwiberry

Plant endosperm is composed of cells full of storage substances. Our present work aimed to study the cytological events, which occur in isolated endosperm of kiwiberry, *Actinidia arguta* 'Bingo' cultured on media based on Murashige and Skoog (1962) macro- and microelements, and vitamins. Callus induction medium (CIM) and *Actinidia* endosperm medium (AEM) were supplemented with 0.5 mg/l of thidiazuron and 2 mg/l of 2,4-D with 5 mg/l of kinetin, respectively. Callus proliferation was observed on both of media, but shoot buds induction on CIM only, after c.a. 6 weeks of the culture. For histochemical analysis fresh isolated endosperm and callus after one, two, four and six weeks of the culture were collected and stained with toluidine blue O, periodic acid Schiff reaction, aniline blue black, sudan B black and auramine O. For immunolabelling procedure six weeks old callus was analysed using of selected primery monoclonal antibodies to detect epitopes of pectins, extensins, hemicelluloses and arabinogalactans (AGP) proteins. The fresh isolated endosperm was composed of thick walled cells, containing abundance of proteins and lipids. During the culture on CIM in dedifferentiated cells were detected starch grains and on the surface of explants the cutin formation was stated. Phenolic deposits in callus cells were also visible. Cells of endosperm-derived callus on AEM showed smaller contents of starch and proteins. Regarding immunohistology, the presence of some extensins (JIM11, JIM12, JIM20) and pectins epitopes (LM19, LM20) was noticed in calli proliferated on CIM and AEM. Additional pectins (LM5, LM6), hemicelluloses (LM25) and AGPs epitopes (LM2) were detected only in callus cultured on AEM. Floccular and net-like structures of pectins were observed in cells from CIM and AEM, respectively. Differences in cell wall composition and storage materials accumulation observed in explants cultured on CIM and AEM can be resulted from different culture conditions and cell competence.



**421 - Rootstock effects on objectively assessed morphological traits of *Vitis vinifera* berries (cv Cabernet Franc) during ripening.**

**Dimitrios Taskos <sup>(1)</sup> - Miltiadis Christopoulos <sup>(2)</sup> - Georgia Ouzounidou <sup>(2)</sup>**

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Rootstocks affect scion physiology, vine vegetative and reproductive growth, and berry development. In this context, to define appropriate harvest time for a given wine style, winemakers use subjective methods to empirically assess berry color and texture in the field. The aim of the present work was the study how rootstocks affect berry morphological traits assessed using objective methods. The study was conducted in a 7-years old commercial vineyard in North Greece, planted with cv. Cabernet Franc (*Vitis vinifera* L.) and grafted onto four widely used rootstocks (110R, 41B, GR, SO4). Determination of berry peel color was carried out with a digital chromameter and expressed as L\*, a, b, h°, C and Color Index [CI=(180- h°)/(L\*+C)] parameters. Berry firmness (BF), corresponding to the peak penetration force (g), and berry compression force (g) was recorded at 1mm intervals by a digital texture analyzer. Three samplings were conducted from veraison to technological maturity. At veraison, berries of GR had the more intense red-violet coloration (low h° and b, high a and CI) but showed the lower color evolution towards harvest. At technological maturity, berry color differences among rootstocks decreased. Berries from GR and 41B vines had higher BF values at veraison and 110R showed the lowest softening rate during ripening. When all samplings are considered, GR had the highest BF values whereas 110R the lowest. SO4 berries at maturity had relatively lower firmness than the rest rootstocks.

#### **422 - Rootstock effects on objectively assessed morphological traits of *Vitis vinifera* berries (cv Cabernet Franc) during ripening**

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#### 449 - Cell wall modifications by $\alpha$ -XYLOSIDASE1 are required for seed and fruit size

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Cell wall modifications are of pivotal importance during plant development. Among cell wall components, xyloglucans are the most abundant hemicellulose in primary cell walls, and can connect the cellulose microfibril surface to affect cell wall mechanical properties. Changes in xyloglucan structure are known as the major factor regulating cell growth. Therefore, the degradation of xyloglucan is an important modification that alters the cell wall. The  $\alpha$ -XYLOSIDASE1 (XYL1) gene encodes the only  $\alpha$ -xylosidase acting on xyloglucans in *Arabidopsis thaliana*. Here, we show that mutation of  $\alpha$ -xyl1 strongly influences seed size, seed germination, and fruit elongation. We found that the expression of XYL1 is directly regulated in developing seeds and fruit by the MADS-box transcription factor SEEDSTICK (STK). We demonstrate that XYL1 complements the stk smaller seed phenotype. Finally, by Atomic Force Microscopy (AFM), we investigate the role of XYL1 activity in maintaining tissue stiffness and growth, confirming the importance of cell wall modulation in shaping organs.

#### 454 - STRUCTURE AND FUNCTIONS OF HYDROPOTES IN NYMPHAEACEAE DURING LEAF GROWTH

**Elizabeth Kordyum** <sup>(1)</sup> - **Sergey Mosyakin** <sup>(2)</sup> - **Vasyl Brykov** <sup>(1)</sup>

**Institute of Botany, National Academy of Science of Ukraine, Cell Biology and Anatomy, Kyiv, Ukraine** <sup>(1)</sup> -  
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Specialized trichomes on the leaf abaxial surface of Nymphaeaceae plants have been called “hydropotes” (drinking water). Hydropotes are assumed to play an important role in the transport of ions and water into and out the plant as well as in the chelation, sequestration, and detoxification of metals, that may be one of ways to provide plant adaptation to the aquatic environment and their tolerance to metal toxicity. Hydropotes of floating green leaves of *Nuphar lutea* and *Nymphaea alba* differ from the surrounding epidermal cells in a rounded form and consist of three cells growing in a stack: the base cell, the lens-shaped cell, and the bowl-shaped cell. We showed that leaf rudiments appear in the middle of the rhizome apex and young leaves etiolated in the absence of light are very tightly enclosed by the extended bases of petioles of mature leaves. The edges of the leaf blade on both sides of the petiole are twisted in 3–5 scrolls. Each etiolated leaf in *N. lutea* is densely covered with thin long glandular hairs, which are also formed inside the scrolls. On the basis of the similarity in initiation and first development of glandular hairs and hydropotes, which are typical for floating leaves, we consider glandular hairs as hydropotes and define two types of hydropotes: type I – the apical cell of three-celled structure continues to divide, and type II – an apical cell does not divide. Functional role of type I is assumed to be in the protection and facilitation growth of young leaves inside rosette, type II – in the accumulation and removal of some substances of endogenic and exogenic origin from a plant. The obtained data could be interpreted as indirect evidence in favor of the concept of sister relationships of *Nuphar* and Cabombaceae.

## **465 - Correlation Between Rock Type and Growth Behavior of Vegetation in Terms of Transport Infrastructure**

**Elisabeth Wiegele** <sup>(1)</sup> - Martin Schneider

**Carinthia University of Applied Sciences, Civil Engineering & Architecture, Villach, Austria** <sup>(1)</sup>

In the area of transport infrastructures, spontaneous vegetation is exposed to a considerable safety risk and combated by chemical or mechanical means. Glyphosate is one of the most widely used agents worldwide. Glyphosate is harmful to the environment and to health; As a result, alternative and environmentally friendly solutions are being researched in many places. As part of the research project Green Logix of the Carinthia University of Applied Sciences, an analysis of the plant growth with different rock materials and recycling products was carried out. For this purpose, test boxes with different rock materials - different types of track ballast and recycled products - were filled and observed. The germination and growth behavior of the emerging plants on the different substrates was systematically documented. The analysis of plant growth in connection with different rock materials and recycling products with different test arrangements (exposures, contaminations with organic components) is the main objective of this work. The hypothesis of this research paper is: To what extent does the rock material influence the plant growth? Is there a correlation between petrography and vegetation density?

#### **504 - Beta subunits of the nascent polypeptide associated complex play their role in male gametophyte development and protein translation in *Arabidopsis thaliana***

**Bozena Klodova** <sup>(1)</sup> - Jan Fila <sup>(1)</sup> - David Potesil <sup>(2)</sup> - Zbynek Zdrahal <sup>(2)</sup> - David Honys <sup>(1)</sup>

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Basic transcription factor 3 (BTF3) was described as the  $\beta$  subunit of Nascent polypeptide-associated complex (Also referred to as NAC $\beta$ ) which, forming a heterodimer together with the NAC $\alpha$  subunit, binds to ribosome and assists in protein folding in yeast *Saccharomyces cerevisiae*. NAC $\beta$  is crucial for development of higher eucaryotes, as its silencing proved to be embryo lethal<sup>3</sup>. Our study aims to uncover NAC $\beta$ 's function in *Arabidopsis thaliana*. In plants, several publications propose its involvement in stress tolerance as positive transcription regulator<sup>4-6</sup>. Interactions between both NAC $\beta$  paralogues and five NAC $\alpha$  paralogues in *Arabidopsis thaliana* were previously studied in our lab and heterodimerisation between subunits was confirmed together with NAC $\beta$ 's potential to bind to the ribosome, suggesting conserved functional mechanism in protein folding. We also discovered the family-specific conserved NAC domain to be essential for the dimer formation. Furthermore, the *Arabidopsis thaliana* null mutant (*nac $\beta$ 1nac $\beta$ 2*) in NAC $\beta$  subunits was created in our lab, which showed lower chlorophyll content and defects in pollen germination and pollen tube growth. To deepen the understanding about molecular mechanisms behind the *nac $\beta$ 1nac $\beta$ 2* phenotype, flower bud transcriptome and proteome of the *nac $\beta$ 1nac $\beta$ 2* double homozygous mutants were analysed resulting in 1965 differential expressed genes (DEG) and 407 differentially expressed proteins (DEPs) compared to Columbia-0 wild type. These data imply NAC $\beta$ 's involvement in pollen development, stress tolerance, ribosome assembly and photosynthesis together with starch metabolism. Also, upregulation of other chaperones such as HSP70 was observed in both studies. Altogether, NAC has a significant role in *Arabidopsis* male germ-line development, fertilisation and photosynthesis, probably by influencing the translation efficiency. It is also possible that NAC's function and regulation is interconnected with other plant chaperone pathways.

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## 505 - Unisexual flower development in two Fagaceae species: a molecular approach

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**Biosystems and Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Braga, Portugal** <sup>(1)</sup> - **Centro de Investigação e de Tecnologias Agro-Ambientais e Biológicas (CITAB), Universidade of Trás-os-Montes and Alto Douro, Vila Real, Portugal** <sup>(2)</sup>

In several plant species the initiation of male and female organs occurs at different timeframes, leading to a temporal separation in the emergence of male and female flowers, known as dichogamy. This is a highly adaptive trait that enhances cross-pollination as a means to avoid inbreeding. This characteristic has been observed in various species of the Fagaceae family, such as *Castanea sativa* and *Quercus suber*. Both are monoecious species, presenting male flowering before female flowering. Despite the overall importance of these species, the mechanisms that control flowering induction and unisexual flower development are still elusive.

In the present work, a characterization of members of the ABCDE MADS-box gene family, recruited in the flower meristem to specify flower organ identity, was performed, and the sex-biased gene expression and identification of novel protein-protein interactions suggest that unisexuality in these species might be related to a redeployment in the dynamics of the ABCDE model. Phylogenetic profiling led to the identification of homologs of genes known to be involved in flowering induction and repression, and their role in flower development was assessed with functional studies in *Arabidopsis*. Expression analysis of these homologs throughout several seasons in *Q. suber* suggests that male and female flowering may be triggered in separate induction events, a pattern that may be conserved in *C. sativa*. Transcriptomic analysis has shown that the flower induction events and flower development might be intrinsically dependent on specific climatic conditions, and the current climate change scenario poses a serious threat for fruit productivity in these species, as flowering becomes more inconsistent.

Overall, the results here presented constitute a step forward in the elucidation of the mechanisms responsible for unisexual flower development in monoecious species of agronomic interest. This knowledge may be applied in the maximization of agricultural practices, and consequent increase in production yield.

**518 - The beta-subunit of nascent polypeptide associated complex plays a role in flowers and siliques development of *Arabidopsis thaliana***

**Jan Fíla** <sup>(1)</sup> - **Božena Klodová** <sup>(1)</sup> - **David Potěšil** <sup>(2)</sup> - **Miloslav Juříček** <sup>(3)</sup> - **Petr Šesták** <sup>(1)</sup> - **Zbyněk Zdráhal** <sup>(2)</sup> - **David Honys** <sup>(1)</sup>

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Angiosperm flower development together with male gametophyte development represent important processes of plant reproduction, which are controlled by a common activity of plethora genes. The function of nascent polypeptide associated complex (NAC) was widely described in yeast and several information are also available about its role in various plant species. The knock down of individual NAC subunit(s) led usually to a higher stress sensitivity.

In *Arabidopsis thaliana* genome, there are five genes encoding the NAC $\alpha$ -subunit, and two genes encoding the NAC $\beta$ -subunit. The double homozygous mutants of both NAC $\beta$  genes (i.e. they did not carry any functional allele of any candidate gene paralogues), were acquired by a conventional cross of two publicly available T-DNA insertion lines. These double homozygous mutants showed several phenotypic traits different from the Columbia-0 wild type plants, such as delayed development, lower chlorophyll content in leaves, abnormal number of flower organs, and abnormally short siliques that carried a lower number of seeds per silique.

Both NAC $\beta$  genes were characterized in more detail – the phenotype of the double homozygous mutant was complemented by a functional NAC $\beta$  copy. Then, both NAC $\beta$  genes were localized to nuclei and cytoplasm and their promoters were active in many organs (leaves, cauline leaves, flowers, pollen grains, and siliques together with seeds). Since flowers were the most affected organs by nac $\beta$  mutation, the flower buds' transcriptome was identified by RNA sequencing, and their proteome by gel-free approach. The differential expression analyses of transcriptomic and proteomic datasets suggest the involvement of NAC $\beta$  subunits in stress responses, male gametophyte development, and photosynthesis.

The research was financially supported from the Czech Ministry of Education, Youth and Sports (LTC20050), the Czech Science Foundation (19-01723S), and the European Regional Development Fund-Project "Centre for Experimental Plant Biology" (No. CZ.02.1.01/0.0/0.0/16\_019/0000738).



## 520 - Deciphering the gametophytic role of *Arabidopsis thaliana* translation initiation factor (eIF3e)

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In higher plants, male and female gametophyte development is a crucial step in sexual reproduction. To guide plant developmental plasticity comprising male and female development, translation initiation factors perceive various developmental and environmental cues to influence cell as well as mRNA specific translatability and hence cellular function. Among all eukaryotic translation initiation factors known in plants, eIF3 complex is considered to be the largest involved in both sporophytic and gametophytic development. Here, we report that eIF3e subunit in *Arabidopsis thaliana* is required for male and female gametogenesis. Our current results suggest that functional loss of eIF3e not only affects pollen development post pollen mitosis I (PMI) and pollen germination, but also impacts on embryo-sac cell fate specification resulting in defect in fertilization. Mutation in eIF3e allele causes reduced transmission through male and female gametophyte. We also show that eIF3e protein localizes throughout pollen development and is expressed in female gametophyte especially in embryo sac and developing embryos. Concomitantly, regulators of mRNA translation, PAPB3 and PABP5 co-localize with eIF3e in pollen and pollen tubes, suggesting a possible function of eIF3e association with RNA and translational control. Moreover, our preliminary work have identified interacting partner of eIF3e in pollen and pollen tube by LC MS/MS including translation initiation factor G1 (eIF3G1), family of actin bundling protein, GERMIN-LIKE PROTEIN, cell wall invertase protein and TGF-BETA receptor interacting protein (TRIP1). Our findings revealed that although eIF3e is not a core subunit of eIF3 complex, plays a critical role on pre and post fertilization events in combination with other translation factors and mRNA binding protein.

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## **574 - Characterizing Monolignol Oxidoreductases from the Berberine Bridge Enzyme-like Protein Family in *Arabidopsis thaliana***

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Among the flavoprotein superfamily, the members of the berberine bridge enzyme-like (BBE-like) subfamily (pfam 08031) are present in bacteria, fungi and plants. Based on sequence similarities, BBE-like enzymes have been identified throughout the plant kingdom, ranging from two in the moss *Physcomitrella patens* to 57 in western poplar (*Populus trichocarpa*). *Arabidopsis thaliana* harbors 27 genes encoding BBE-like proteins (AtBBE-like). Microarray expression analyses suggest that AtBBE-like proteins are involved in various stress-induced plant responses as well as developmental processes. In silico characterization of AtBBE-like subgroup six, which consists of five genes (AtBBE-like 13, 15, 24, 25 and 26), showed high similarities in sequence and structure. AtBBE-like 13 and 15 have been found to oxidize monolignols to their corresponding aldehydes [1]. However, the biological functions of the individual AtBBE-like genes in subgroup six are yet unknown and appear to be highly diverse, very specific, and non-systemic.

In order to investigate the in planta functions of AtBBE-like subgroup six, we have now generated GUS reporter lines for all genes of AtBBE-like subgroup 6 as well as loss-of-function mutants for AtBBE-like 13 and 15. In this work, we document expression patterns and find the genes to be expressed mainly in roots, where the individual genes show different expression patterns on the tissue level. Additionally, AtBBE-like 15, 24 and 26 reporter lines show expression at distinct stages of flower development, and may, thus, play a role in flower development.

Further phenotypic description of loss-of-function mutants, detailed descriptions of reporter lines and physiological experiments comparing mutant to wild type plants will widen our understanding of subgroup six of the AtBBE-like protein family.

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[1] Daniel et al. (2015) The Journal of Biological Chemistry 290: 18770–1878

## **588 - Effect of different light spectrum on growth and development of Silver birch (*Betula pendula* Roth) cultures in vitro**

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Light emitting diodes (LED) offer many advantages over conventional fluorescent lighting, as fluorescent tubes are widely used as light source for in vitro cultures, they are energy-consuming, produce excess heat that must be dissipated and produce a broad spectrum with spectral regions unnecessary for plant growth. LEDs are energy efficient, produce less heat and the spectral composition can be adjusted to specific requirements. Currently indoor farming systems utilise adapted LED lighting spectrums for various crop species, whereas there are limited solutions for micropropagation of woody tree species. Our aim was to develop an innovative LED lighting system specifically adapted for in vitro propagation of silver birch (*Betula pendula* Roth) clones. Cultures were grown under LED lighting with three different spectral compositions: 1) Red+Blue (RB) (peak intensities at 660 and 442nm, respectively), 2) Red+Green+Blue (RGB) (peak intensities at 660, 530 and 442nm, respectively), 3) Red +Orange +Yellow +Green +Blue (RGBYO) (peak intensities at 660, 610, 530 and 442nm) and fluorescent tubes (FL) as control. Photon flux density was constant ( $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). To evaluate the effect of different light spectrum we compared plant growth parameters and propagation ability. We did not observe significant differences of growth parameters (stem length, number of internodes, length of third internode) nor multiplication index between LED and control lighting (FL). Plants under RGBYO grew slightly better than control plants, but RB plants underperformed – stem length and multiplication index was lower than in control plants (no statistically significant differences detected). The use of a narrower spectral composition (RB) resulted in a reduction of plant growth, compared with the control lighting (FL), whereas a broader spectrum (RGBYO) showed increased plant growth performance. The energy efficiency of LED lighting and the possibilities to arrange specific light spectrum combinations promotes the replacement of traditional fluorescent tubes.

Keywords: LED, spectral composition, micropropagation.

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**597 - Microscopic and Transcriptomic Analysis of Pollination Processes in Self-incompatible Taraxacum koksaghyz**

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The transition of the Russian dandelion *Taraxacum koksaghyz* (Asteraceae) to a profitable, alternative crop producing natural rubber and inulin requires the optimization of several agronomic traits, cultivation conditions and harvesting procedures to improve the yield. However, efficient breeding is hindered by the obligatory sexual outcrossing of this species. Several other asters have been investigated to determine the mechanism of self-incompatibility, but the underlying molecular basis remains unclear. We therefore investigated the self-pollination and cross-pollination of two compatible *T. koksaghyz* varieties (TkMS2 and TkMS3) by microscopy and transcriptomic analysis to shed light on the pollination process. Self-pollination showed typical sporophytic self-incompatibility characteristics, with the rare pollen swelling at the pollen tube apex. In contrast, cross-pollination was characterized by pollen germination and penetration of the stigma by the growing pollen tubes. RNA-Seq was used to profile gene expression in the floret tissue during self-pollination and cross-pollination, and the differentially expressed genes were identified. This revealed three candidates for the early regulation of pollination in *T. koksaghyz*, which can be used to examine self-incompatibility mechanisms in more detail and to facilitate breeding programs.

## 607 - Role of microRNAs in the photo-control of bud burst in Rosa 'Radrazz'

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Shoot branching is a major determinant of plant architecture, and as such impacts yield and visual quality, in order to meet consumer expectations. Architecture is an essential element in visual quality of the plant which is important in ornamental plants. Shoot branching is highly dependent of environmental factors, especially light. To study the environmental control of branching, we focus on the rosebush (Rosa 'Radrazz'), an important plant in ornamental horticulture that has an absolute requirement of light to trigger bud burst. The main objective of our team is to tackle the complexity of the endogenous factors like hormones (SL, IAA, GA, CK, ABA), nutrient (sugar, nitrogen) and ROS regulatory network behind the branching process in response to the environment. While great progress has been made in terms of transcriptional regulation of light-regulated rose bud outgrowth, little is known about the contribution of post-transcriptional regulations to this process. To provide new insights in post-transcriptional mechanisms underlying the control of bud outgrowth, we started elucidating the role of microRNAs. In this purpose, a combined high throughput sequencing of small RNAs and mRNAs transcriptome of axillary buds under different light conditions was used. Thus, a total of 160 conserved microRNAs belonging to 47 microRNAs families and 110 putative novel microRNAs were identified and analysed based on their differential expression in dormant (dark condition) versus outgrowing buds (light condition). Their putative target genes were predicted by bioinformatics analysis. Expression patterns of selected pairs of candidates (microRNAs and target genes) were validated by qRT-PCR. In order to validate microRNAs-target gene interactions, transient co-agroinfiltration assay of *Nicotiana benthamiana* leaves were carried out with quantitative GFP fluorescence analysis. Finally, the biological role of selected microRNAs was tested by heterologous overexpression in *Arabidopsis thaliana*.

## **614 - Involvement of B-box MicroProteins in the reproductive development of tomato**

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**(1)**

Genes coding for B-box proteins are widespread in all eukaryotic genomes analyzed so far. In Arabidopsis and tomato, B-box Zn finger proteins contain one or two B-box domains which represent a sequence motif for protein-protein interaction. Many members of this family also possess a CCT-domain, which is associated with a role in transcriptional regulation and nuclear transport.

The diverse functions of B-box proteins in plant growth and development range from the involvement in seedling photomorphogenesis, shade avoidance, seed germination and photoperiodic regulation of flowering. In Arabidopsis, several B-box proteins have been well-characterized. For instance, AtBBX30 and AtBBX31 MicroProteins were proved to actively participate in the flowering process mediating the recruitment of CONSTANS (CO), a positive regulator of flowering time in a repressor complex with TOPLESS (TPL) with downstream inhibition of FLOWERING LOCUS T (FT) expression.

The aim of this study is to functionally characterize two B-box proteins of tomato, BBX16 and BBX17, highly homologous to AtBBX30 and AtBBX31 also in light of our recent observation that BBX16 interacts with TCMP-2, a tomato cystine-knot miniprotein which is involved in flower and fruit production. First, we have demonstrated that TCMP-2 physically interacts also with AtBBX31 and when overexpressed in Arabidopsis causes anticipated flowering as well as an increased level of FT. In parallel, we have overexpressed SIBBX16 and SIBBX17 in Arabidopsis to find out whether they might have a regulatory function in flowering similar to AtBBX30 and AtBBX31. The study of the expression patterns of SIBBX16 and SIBBX17 in different phases of flower and fruit development is ongoing in tomato by using Agrobacterium tumefaciens-mediated transient expression of reporter gene constructs.

## 617 - Cytokinin and its role in flowering time control

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Plants undergo a major physiological change as they transition from vegetative to reproductive development. The precise timing of this process is responsible for their reproductive success. The hormone cytokinin is involved in regulating flowering time but the relevant cytokinin genes and its link to known flowering pathways are not well known. We compared the flowering time of selected cytokinin metabolism and signaling mutants with a higher or lower cytokinin content or signaling activity. This analysis revealed that cytokinin promotes the induction of flowering independent of photoperiod and independent of leaf number. Upon a shift from short to long day conditions, the hormone was required for full induction of FT (FLOWERING LOCUS T) and TSF (TWIN SISTER OF FT). Interestingly, our gene expression studies and genetic analyses identified a functional link between the cytokinin and the age pathway, which is defined by the antagonistic expression of the microRNAs miR156 and miR172 targeting several SQUAMOSA PROMOTER BINDING LIKE (SPL) and APETALA2 (AP2)-like transcription factor genes. The underlying molecular mechanisms of this crosstalk will be discussed.

## 626 - CLIMBER - Confronting CLIMate change impacts in BarLEy and Rice

Laura Rossini <sup>(1)</sup> - Carlotta Ferrario <sup>(1)</sup> - Roberta Rossi <sup>(1)</sup> - Letizia Cornaro <sup>(2)</sup> - VM Beretta <sup>(2)</sup> - Francesca Caselli <sup>(2)</sup> - Vittorio Zanzani <sup>(2)</sup> - Marta Mendes <sup>(2)</sup> - Salar Shaaf <sup>(1)</sup> - Abhisek Biswas <sup>(1)</sup> - Alessandro Vajani <sup>(1)</sup> - Agnieszka Janiak <sup>(3)</sup> - Beata Chmielewska <sup>(3)</sup> - Raffaella Battaglia <sup>(4)</sup> - Maria von Korff <sup>(5)</sup> - David Stephen Horner <sup>(2)</sup> - Veronica Gregis <sup>(2)</sup>

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Rice and barley are among the most significant crops worldwide and are important genetic models for the grass family. Abiotic stresses such as heat and drought cause substantial crop loss worldwide threatening food security, and climate change is exacerbating their frequency and intensity. In the CLIMBER project, to explore the role of auxin and cytokinin in plant response to stress, we are generating and characterizing rice and barley plants expressing specific hormone biosensors to evaluate hormonal signaling and identify tissue domains in the developing inflorescence where hormone levels change under stress conditions.

In parallel transcriptomic analyses of developing barley inflorescences under control and heat-stress conditions are underway and will be integrated with analogous data from rice when available. The resulting data will be analyzed with innovative approaches to identify molecular mechanisms that are either shared, or distinct between the relatively distantly related species under study, and generate testable hypotheses regarding the role and mechanism of action of phytohormones in developmental responses to stress. Beside auxin and cytokinin, strigolactones have also emerged as players in abiotic stress responses in rice and other species, but the role of this pathway is largely unexplored in barley. To fill this gap, we are characterizing allelic variants of a barley strigolactone pathway gene identified from screening the HorTILLUS TILLING population: phenotypic analyses of different barley lines suggest a role of this gene in plant architecture and complementation of the corresponding Arabidopsis mutant is underway to test for functional conservation between the two species.



## **666 - Molecular mechanisms controlling the interdependency between cell expansion and cell differentiation**

**Noemi Svolacchia** <sup>(1)</sup> - **Riccardo Di Mambro** <sup>(2)</sup> - **Sabrina Sabatini** <sup>(1)</sup>

**Sapienza University of Rome, Department of Biology and Biotechnology Charles Darwin, Rome, Italy** <sup>(1)</sup> - **University of Pisa, Department of Biology, Pisa, Italy** <sup>(2)</sup>

How topological and geometrical cell properties instruct cells on their fate is a fundamental question in developmental biology. Recently we showed that EXPANSIN 1 gene, by controlling cell wall extension, regulates cell terminal differentiation promoting the exit of cells from the root apical meristem cells (RAM). In particular, we observed that EXPA-dependent cell wall extension triggers cell differentiation. Despite the intrinsic relationship between cell expansion and cell differentiation, the molecular mechanisms underlying this interdependency are still not known. By using the RAM of *Arabidopsis thaliana* as a model system to track cells developmental stages, we focused on genes regulating cell wall mechanical properties. Our preliminary data suggests that cell wall elasticity and rigidity influence root development by controlling the rate between cell differentiation and cell division, thus root growth. Indeed, by using cell state markers, we noticed that reducing cell wall elasticity and rigidity dampens cell differentiation and promotes cell division inputs. Our data represent the first evidence that cell wall modifications can instruct cells to divide or differentiate.

## **688 - The Role of myb-like Transcription Factor MYB ROOT in Root Growth and Development**

**Lena Elorduy Vergara** <sup>(1)</sup> - **Melina Altmann** <sup>(1)</sup> - **Patricia A. Rodriguez** <sup>(1)</sup> - **Ramakrishnan Pandiarajan** <sup>(1)</sup> - **Benjamin Weller** <sup>(1)</sup> - **Pascal Falter-Braun** <sup>(1)</sup>

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Root length depends on proximal meristem size, which is determined by the antagonistic interplay between the two phytohormones auxin and cytokinin (CK). By regulating its distribution and generating an auxin minimum, CK defines the meristem limiting transition zone (TZ) position. Cells stop dividing and start differentiating at this boundary, which ultimately defines root growth. In a previously published phytohormone protein interaction network we identified an yet uncharacterized myb-like transcription factor as an interactor of a key regulator of root length and activator of CK biosynthesis.

We demonstrate genetically that MYB Root is a negative regulator of root growth in *Arabidopsis thaliana*. Overexpression of this gene leads to significantly shorter roots in seedlings, an impaired gravitropic response, as well as an inhibition of root hair emergence, while a knockout mutation confers the opposite phenotypic effects.

We then investigated how MYB Root regulates root phenotypes by analyzing the expression of key genes in the CK and auxin signaling pathways. We find that MYB Root overexpression instantly affected transcription of various CK and auxin related genes, including ISOPENTENYLTRANSFERASE 1 (IPT1) and PINOID (PID) in seedling roots. Our data clearly establish MYB Root as an important regulator of root development.

## TOPIC:

### Plant ecosystems under environmental change

#### **Keynote Lecture**

**Plant ecosystems and environmental change: extreme events, resilience and adaptation mechanisms in nature**

**Giorgio Matteucci<sup>1</sup>, Alessio Collalti<sup>2</sup>, Negar Rezaei<sup>3</sup>, Alberto Battistelli<sup>3</sup>, Andrea Scartazza<sup>3</sup>, Ettore D'Andrea<sup>4</sup>**

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Global climate change is causing an increase in the frequency of extreme weather events. Storms, late frost, drought and heat waves are considered among the major drivers of current and future ecosystem dynamics in different regions. Furthermore, in many regions, increases in the interannual climatic variability have been recorded. Along millions of years, long-living organisms as trees have developed defence strategies and response mechanisms such carbohydrate reserves, resprouting, drought-deciduousness, serotiny and several others to cope with disturbance events that drive succession processes and regeneration dynamics. Nevertheless, in the last decades, impacts on ecosystems and on the recovery processes after extreme disturbance events are further aggravated by warming and, sometimes, by the concurrent occurring of extremes (e.g. drought after storms). In this framework, the capacity of trees and ecosystems to thrive and react to disturbance events could be impaired, posing risks to biodiversity and reducing the climate-change mitigation role of forests, with potentially positive feedbacks on global change. The key note will address the impact of extreme events on forests and the resilience and adaptation mechanisms of trees and ecosystems, with examples from recent research, including modelling results to explore potential future scenarios. A question will be asked: can humans do something to favour mitigation and adaptation?

## **Oral Communications**

### **129 - Leaf pigmentomics – A new approach for better understanding of seasonal pigment dynamics?**

**Fanny Petibon**<sup>(1)</sup> - **Guido L.B. Wiesenberg**<sup>(1)</sup> - **Mathias Kneubühler**<sup>(1)</sup> - **Michael E. Schaepman**<sup>(1)</sup> - **Michael W.I. Schmidt**<sup>(1)</sup>

**Department of Geography, University of Zurich, Zurich, Switzerland**<sup>(1)</sup>

Chlorophylls, carotenoids and anthocyanins are the main leaf pigment families, and fulfil vital physiological functions, including light harvesting, conversion, photoprotection and defence. To understand the response to environmental stresses of widely distributed deciduous tree species, monitoring the seasonal dynamics of pigments in leaves is essential. Traditionally, pigment content and composition are measured using spectroscopy targeting compound families. Technological development of novel optical sensors and chromatography systems with improved resolution allows unprecedented detail to monitor pigment dynamics at individual compound level.

We evaluated four methods to observe pigment composition, including (i) leaf optical properties measured in-situ using a contact probe with a field spectrometer, (ii) optical properties of bulk chemical extracts, (iii) compound-targeted standard chromatography methods, and (iv) our new pigmentomic approach. We compared the sensitivity of methods to detect seasonal dynamics of pigments in sun exposed and shaded leaves of deciduous trees.

Our new pigmentomic approach allows us to characterize 3.5 times more pigment metabolites than standard chromatography methods. The contribution of secondary pigment metabolites to the bulk signal was higher in spring and autumn (up to 9%) than in summer (1-2%) when leaves reached their full extend. Our presentation highlights that, for the first time, the inclusion of secondary pigment metabolites allow to not only identify pigment families, but individual pigment metabolites within families, and better describes seasonal dynamics of leaf chemistry. We conclude that calibration based on our pigmentomic approach improves the quality of spectral retrievals and enable us to better monitor in-situ plant-environment response with optical sensors.

## **244 - Unravelling foliar water uptake mechanisms and ecophysiological significance**

**Paula Guzmán Delgado** <sup>(1)</sup> - Alana Chin <sup>(1)</sup> - Jessica Orozco <sup>(1)</sup> - Emilio Laca <sup>(1)</sup> - Thomas Buckley <sup>(1)</sup> - Maciej Zwieniecki <sup>(1)</sup>

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Key words: cuticle, foliar water uptake, hydraulic resistance, recovery, stomata

Foliar water uptake, i.e. the absorption of water by leaf surfaces, is a widely spread phenomenon across plant lineages that has been overlooked for centuries. As such, the biological significance and mechanisms of foliar water uptake are poorly understood. To gain insight into this process, we carried out a series of experiments spanning cell, leaf and whole-plant measurements. Following a rehydration kinetics approach, we recently developed a methodological framework that allows for the quantification of basic hydraulic parameters, including resistance to rehydration via the surface of fog-treated leaves. We applied this framework to determine the differential contribution of the cuticle and stomata to foliar water uptake. We found that stomata can significantly contribute to water uptake, mainly in vapor phase, and to leaf rehydration. Preliminary results show that leaf surface hydraulic resistance is correlated with seasonal and spatial availability of atmospheric water and canopy height gradients of California native trees. Direct X-ray microtomography analyses revealed that leaf exposure to fog results in the re-expansion of redwood tracheids deformed by water stress. We also found that foliar water uptake can recover hydraulic conductance of drought-stressed almond leaves and enhances whole-plant physiological recovery in terms of increased water potential, stomatal conductance and photosynthetic assimilation, and of decreased leaf abscisic acid content. Collectively, these results indicate that foliar water uptake may be an adaptive trait with a critical role in sustaining plant physiological function. Research efforts should be hence devoted to better understand the foliar water uptake phenomenon, especially under the current global change scenario.

## TOPIC:

### Plant ecosystems under environmental change

#### ***Extended Elevator Pitches***

**139 - Widespread holm oak dieback in Mediterranean forests: the roles of carbon stress and hydraulic failure under recurrent drought events**

**Cecilia Brunetti<sup>(1)</sup> - Antonella Gori<sup>(2)</sup> - Francesca Alderotti<sup>(2)</sup> - Raffaella Balestrini<sup>(3)</sup> - Fabiano Sillo<sup>(3)</sup> - Dalila Pasquini<sup>(2)</sup> - Francesco Ferrini<sup>(2)</sup> - Mauro Centritto<sup>(1)</sup>**

**CNR, IPSP, Sesto Fiorentino, Italy<sup>(1)</sup> - DAGRI, University of Florence, Sesto Fiorentino, Italy<sup>(2)</sup> - CNR, IPSP, Turin, Italy<sup>(3)</sup>**

Mediterranean ecosystems are usually considered resilient to arid conditions, because of their capability to cope with and recover after severe stress events. However, in recent years, extensive tree dieback related to drought events have occurred in different Mediterranean forests. The aim of our research is to investigate the causes of widespread mortality of *Quercus ilex* observed in Tuscany. Physiological traits were measured through different seasons in an experimental site established in the Maremma Natural Reserve, characterized by areas with high mortality rates of *Q. ilex*. To investigate specific physiological and biochemical mechanisms underlying *Q. ilex* dieback, we have also conducted a pot experiment on three-years old seedlings subjected to progressive water stress followed by rewatering, whereas control plants were maintained in well-watered conditions. In both experiments, the following measurements were carried out: water relations, gas exchanges, chlorophyll fluorescence, carbohydrates, BVOCs, epidermal content of flavonols and chlorophyll. In addition, on shoots collected from plants of the pot experiment we performed target expression analyses focusing on genes involved in drought responses. Results of both studies led us to hypothesize that *Q. ilex* dieback observed in the Maremma Natural Reserve may be attributed to hydraulic failure. Although holm oak is considered an isohydric species subjected to carbon starvation caused by fast stomatal closure in response to water deficit, xylem embolism may occur under recurrent droughts compromising its ability to recover from severe stresses. Furthermore, both qualitative and quantitative changes in BVOC emissions were found under severe water stress. Considering the intrinsic high emission rates of monoterpenes of this species, variations in the production of these compounds may have implications for the atmospheric biochemistry in Mediterranean areas. In conclusion, our results contribute to elucidate possible mechanisms underpinning recent holm oak forest mortality and provide guidance for understanding Mediterranean forest diebacks under climate changing conditions.

**282 - Effects of lateral bud removal in growth and phenolics in male and female saplings of *Populus tremula* (L.) under simulated climate change**

**Norul Sobuj** <sup>(1)</sup> - **Virpi Virjamo** <sup>(2)</sup> - **Katri Nissinen** <sup>(3)</sup> - **Unnikrishnan Sivadasan** <sup>(3)</sup> - **Lauri Mehtätalo** <sup>(4)</sup> - **Line Nybakken** <sup>(5)</sup> - **Heli Peltola** <sup>(4)</sup> - **Riitta Julkunen-Tiitto** <sup>(4)</sup>

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The responses in growth and defense after tissue damage are highly variable in plants depending on species, damaged-tissue type and the intensity of damage. The prevailing abiotic conditions can also influence these responses. In this study, our aim was to examine how the removal of lateral vegetative buds affects the growth and accumulation of phenolics in saplings of the dioecious *Populus tremula* grown under simulated climate change. For three growing seasons, the saplings were grown under ambient conditions (control), elevated temperature (+2°C) and elevated UV radiation (30%) (UVB and UVA as its control), or a combination of these. In the fourth growing season, all saplings were grown under ambient conditions. The bud removal was performed twice – in summer and autumn – in the third year. The responses of growth and the accumulation of phenolics to the bud removal were measured at the end of the fourth growing season. Removal of 5% of the lateral buds resulted in higher leaf, stem and total plant biomass in both sexes of *P. tremula* saplings, compared to intact plants. The effects were greater in the temperature-treated plants, especially in the temperature-treated females. The concentrations of flavonoids and condensed tannins were higher in the bud-removed individuals. The concentration of condensed tannins was also higher in the males than in the females, opposite to the concentration of phenolic acids. There was no significant interaction between bud removal and UVB treatment on either growth or phenolics. Our results suggest that plants can allocate resources to both growth and defense simultaneously in response to tissue loss, and that global warming can modify the responses to some extent.

**285 - Inhibition of xylem cellular activity blocks recovery from drought induced embolism in poplar – insights from micro-CT analysis.**

**Francesca Secchi <sup>(1)</sup> - Chiara Pagliarani <sup>(2)</sup> - Francesco Petruzzellis <sup>(3)</sup> - Silvia Cavalletto <sup>(1)</sup> - Giulia Tonel <sup>(1)</sup> - Tadeja Savi <sup>(3)</sup> - Giuliana Tromba <sup>(4)</sup> - Claudio Lovisolo <sup>(1)</sup> - Maria Margherita Obertino <sup>(1)</sup> - Andrea Nardini <sup>(3)</sup> - Maciej Zwieniecki <sup>(5)</sup>**

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Perennial plants affected by drought-induced embolisms may benefit from mechanisms allowing for fast recovery of lost xylem hydraulic capacity to reduce prolonged impact on photosynthetic activity upon rehydration. Recent findings suggest that under water stress, a coordinated cascade of chemical and transcriptional adjustments occurs during stress. These processes while not changing xylem embolism level effectively prime stem for recovery by accumulation of sugars and ions in the apoplast. In this study, we test if chemical treatments either affecting stem pH (in vivo infiltration of xylem with pH buffer) or stem metabolic activity (xylem infiltration with NaVO<sub>3</sub>, NaCN, or plant exposure to CO gas), can reduce sugar accumulation, thus hindering or delaying recovery during rehydration. The ortho-vanadate treatment (NaVO<sub>3</sub>), aimed to block proton transporters and ATP metabolism, was selected for visual, in vivo analysis of embolism recovery using X-ray-microCT in comparison to untreated plants. Application of NaVO<sub>3</sub> led to an impediment in removal of embolisms formed during drought, despite the full recovery of leaf hydration to pre-stress conditions, while almost full removal of embolism was observed in control plants. Results suggest that embolism removal is an energy dependent process that requires accumulation of sugars in the apoplast. Image analyses indicated that the recovery process is spatially coordinated, with embolism formation accruing from inside out and recovery from outside in; thus, underlining the importance of xylem proximity to phloem (carbohydrates source).



**521 - The role of endogenous salicylic acid in endophytic bacterium *Bacillus subtilis*-mediated drought tolerance in wheat plants**

**Oksana Lastochkina**<sup>(1)</sup> - **Sergey Ivanov**<sup>(2)</sup> - **Regina Khafizova**<sup>(2)</sup> - **Evgenia Visheva**<sup>(1)</sup> - **Bulat Kuluev**<sup>(1)</sup> - **Alsu Lubianova**<sup>(1)</sup>

**Ufa Federal Research Center of the Russian Academy of Sciences, Institute of Biochemistry and Genetics, Ufa, Russian Federation**<sup>(1)</sup> - **Ufa Federal Research Center of the Russian Academy of Sciences, Institute of Chemistry, Ufa, Russian Federation**<sup>(2)</sup>

*Bacillus subtilis* 10-4 is a plant growth-promoting endophytic bacterium that induces resistance/tolerance in plants to a spectrum of pathogens and abiotic stresses including drought. This study analyzed *B. subtilis* 10-4-induced systemic tolerance in two *Triticum aestivum* L. (wheat) ecotypes differing in drought adaptive strategies (drought-tolerant (DT) cv. Saratovskaya55 and Ekada70 - Volga steppe ecotype; drought-sensitive (DS) cv. Salavat Yulaev, Omskaya35 - forest-steppe West Siberian ecotype). It was revealed the most pronounced protective effect of *B. subtilis* 10-4 under drought both at the cellular (decreasing drought-caused cell's osmotic and oxidative damages) and the whole organism levels (without inhibition of plant growth processes) manifest in DT wheat cultivars. The defense-related PR-1 genes were expressed and endogenous salicylic acid (SA) was accumulated in *B. subtilis* 10-4-treated wheat plants, suggesting activation of the SA-dependent signaling pathways by *B. subtilis* 10-4. The accumulation of endogenous SA and PR-1 gene expression were faster and stronger in DT plants treated with *B. subtilis* 10-4 under normal (non-stressed) conditions than that in DS plants. These findings were correlated with positive regulation by *B. subtilis* 10-4 the plant growth parameters under drought in early stages of ontogenesis. Further, it was revealed that joint application of *B. subtilis* 10-4 with the SA biosynthesis inhibitor 1-Aminobenzotriazole (1-ABT) resulted in notably decreasing the level of *B. subtilis* 10-4-induced endogenous SA accumulation in wheat plants of both DT and DS ecotypes/ The findings indicates that *B. subtilis* 10-4-induced increase in the endogenous SA performs as a hormonal intermediate in the manifestation of the positive physiological effect of *B. subtilis* 10-4 on wheat plants of these two different ecotypes under drought condition.

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## TOPIC:

### Plant ecosystems under environmental change

#### **Posters**

##### **595 - Beneficial multivariate masting: Inter-annual variability of global seed crops in a changing climate**

**Jessie Foest** <sup>(1)</sup> - **Andy Morse** <sup>(1)</sup> - **Mark Green** <sup>(1)</sup> - **Andrew Hacket-Pain** <sup>(1)</sup>

**Department of Geography and Planning, School of Environmental Sciences, University of Liverpool, Liverpool, United Kingdom** <sup>(1)</sup>

Many plant populations produce remarkably different quantities of seed each year, varying on a continuum from virtually zero seeds to bumper seed crops. This behaviour is thought to reduce the individual costs of reproduction - e.g., fewer seeds are lost to predators and/or pollination becomes more efficient. Not all seed crops are expected to fluctuate to the same extent or in the same way; patterns vary as a function of species and environment. However, few studies have explored how temporal patterns differ within and across species in a global spatio-temporal context. Improving our understanding of temporally variable reproductive strategies ("masting") is crucial, as resource pulses associated with large seed crops have profound effects on the establishment of seedlings, forest dynamics, and trophic cascades.

We used a newly compiled dataset (MASTREE+) to characterise reproductive patterns of plant populations across time and space. Our data contained 1784 time-series (6-69 years, between 1874 and 2020), measuring the annual production of seeds, fruits, cones or flowers at the population-level. It captures the annual reproductive output of 517 wild herbaceous and woody plant species in Europe (36.5% of time-series), North America (35.1%), and other world regions (28.4%). Using hierarchical clustering and econometric time series analysis methods, we find distinct reproductive strategies within and across species, alongside temporal trends in mean seed production and inter-annual variability. Our findings may be used to understand the evolution of masting (including relationships with other life-history traits), identify the physiological mechanisms regulating temporal variability, as well as improve forecasting models of seed production. Upcoming research will explore how the observed temporal patterns in fecundity relate to environmental change.

## **2 - Consequences of climate change and plant invasions on nitrogen acquisition of native woody seedlings**

**Simon Judy** <sup>(1)</sup>

**Plant Interactions Ecophysiology Group, University of Konstanz, Konstanz, Germany** <sup>(1)</sup>

Global change, such as climate change and/or biological invasions of species, affects plant fitness and competitiveness, especially in highly vulnerable seedlings, resulting in severe effects on forest ecosystems in the future. Facing these challenges and the accompanying ecosystem changes, more detailed knowledge of the basic processes and underlying mechanisms is required to understand ecosystem functioning, and thus develop and evaluate strategies for sustainable ecosystem management to secure ecosystem services for modern society. Most studies on plant interactions with regard to nitrogen acquisition and/or partitioning have focused on inorganic N, and only recently organic N acquisition of plants has come into the focus of research. Furthermore, plants have developed different mechanisms to optimise the utilisation of limited nitrogen (N) resources; however, the complex interactions between different species, particularly long-living woody species, with regard to the competition for N in the rhizosphere are currently only little understood. The research presented here provides new insights into the understanding of the processes involved in the regulation of belowground competition for nitrogen in temperate forests. We studied different tree species and their N acquisition strategies (i. e. inorganic and organic N forms) in competition. Further results from experiments including important abiotic factors provide evidence that these N uptake strategies strongly depend on the environment. Moreover, N acquisition in the rhizosphere of forests can also be influenced by invasive tree species.

## **19 - The study of the Lemna minor as a bioindicator of some petroleum pollutants in the freshwater ecosystems**

**Lidia Bondareva**<sup>(1)</sup> - **Nataliia Fedorova**<sup>(1)</sup>

**Federal Scientific Center of Hygiene named after F.F. Erisman, Analytical Methods of control, Mytishi, Russian Federation**<sup>(1)</sup>

The long-term monitoring of the state of the fresh-water ecosystem of the river Yenisei revealed the statistically reliable content of heavy metals (Fe, Zn, Cd, Cu, U etc.) in the water, bottom sediments, phyto- and zooplankton, and muscle mass of commercial fish consuming different types of food (benthos eaters, predators and herbivorous fish). The values of the indices of the ecological state of the Yenisei river were estimated to vary from 2,38 to 2,85 in the areas under study. A conclusion was made that the studied transects of the river Yenisei had a good ecological potential with a moderate level of ecological risks. The total index of risk for the water, taking into account the reference doses, amounts to 0.16 for the water, and to 0,47 for the flesh of commercial fish. The total index of risk for the population consuming fresh water and fish from the Yenisei river amounts to IR=0.63. The obtained value of the index is, in general, of no danger for the population health. Since the cancerogenic substances were not accurately revealed, we estimated non-cancerogenic nonthreshold risks. The non-cancerogenic nonthreshold risks for particular substances under consideration did not exceed the allowable level of 0.05 and were equal to 0.017. The estimation revealed that the ratio of developing reflectory-olfactory effects to the allowable value was equal to 0.01, and the ratio of the total noncancerogenic risk to the allowable level was 0.34. Here, the integrated indicator was 0.35, which did not exceed the regulatory level ( $II \leq 1$ ). In general, the risks with regard to all the indicators analyzed did not exceed the allowable levels and did not require additional measures of regulating the water quality.

## 20 - TRITIUM CONTENT OF SOME AQUATIC PLANTS OF THE MIDDLE YENISEI ECOSYSTEM

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The Mining and Chemical Combine (MCC) located in Zheleznogorsk on the right bank of the Yenisei river 50 km downstream from Krasnoyarsk was for a long time a source of radioactive contamination of the environment in Krasnoyarsk krai. Analysis of published data on the radiation-chemical condition of middle Yenisei showed that, before the shutdown of all the reactors in May 2010, there were several probable potential sources of tritium in water of the Yenisei river: tritium from the catchment territory contaminated as a result of nuclear tests; tritium from the catchment territory contaminated as a result of aerosol discharges from MCC; tritium in water discharged from reactor cooling systems; tritium in discharged low-level liquid wastes formed in the course of operation of the radiochemical plant, etc.

The following main results were obtained in this study. The  $^3\text{H}$  concentration in water of the main Yenisei waterway is  $4\text{--}6\text{ Bq L}^{-1}$ , which can be used as the basic background value of the tritium content of Yenisei water after the shutdown of the last nuclear reactor in 2010. The  $^3\text{H}$  concentration in the Yenisei bottom sediment samples taken after 2010 did not exceed  $\sim 6\text{ Bq L}^{-1}$  ( $\sim 2\text{ Bq kg}^{-1}$  wet weight). The  $^3\text{H}$  concentration in aquatic plants growing in the MCC observation zone, determined after the shutdown of the last reactor, was  $6\text{--}8\text{ Bq kg}^{-1}$  wet weight, and for the samples taken upstream from the MCC observation zone (Esaulovo village) and at a distance of 15 km downstream from the MCC discharge site it was  $2\text{--}3\text{ Bq kg}^{-1}$  wet weight.

## 75 - EVALUATION OF THE RADIATION LOAD OF MOSSES COLLECTED IN 2018 FROM NP DJERDAP

**Jelena Stanojković<sup>(1)</sup> - Ana Čučulović<sup>(1)</sup> - Marko Sabovljević<sup>(2)</sup> - Milorad Vujičić<sup>(2)</sup>**

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Mosses are bioindicators of environmental pollution for radionuclides and other pollutants. Depending on the moss type and age, their morphological and physiological characteristics, locality and substrate, altitude it has been shown that mosses adsorb <sup>137</sup>Cs. Cesium-137 is a fission product, the most significant and dangerous radionuclide, which has been released in the accident in Chernobyl (1986). The cesium ion is the chemical and biochemical homologue of potassium, it follows its metabolism in an organism.

Samples of mosses (30 samples, 10 species) were collected in the 2018 in the region of the NP Djerdap from two regions (Dobra and Djerdap). The Djerdap National Park is located in the north-east of Serbia, on the border with Romania. Radioactivity measurements were performed using an HPGe gamma-ray spectrometer. The specific activity of the artificially produced radionuclide <sup>137</sup>Cs was measured via  $\gamma$ -line at the energy of 661.6 keV. Measured activity was converted into doses with the assumption that all emitted particles (gamma and beta) were absorbed in the tissue that accumulated <sup>137</sup>Cs.

The absorbed dose strength is investigated moss samples from the Dobra region was from 0.042 mGy/year to 0.364 mGy/year, average 0.177 mGy/year, while in moss from the Djerdap region it was from 0.104 mGy/year to 0.713 mGy/year, average 0.104 mSv/year. Moss from Dobra region received a larger dose than moss from Djerdap region. The absorbed dose strength is investigated moss samples from the National Park Djerdap was from 0.042 mGy/year to 0.364 mGy/year, average 0.236 mGy/year.

The strength of the absorbed dose on the territory of NP Djerdap in moss are lower than the doses that cause changes in the reproduction cycle of plant and animal species (0.4 to 1 Gy a year) and lethal doses (4 Gy and 0.4 Gy a year).

**97 - Dynamics of intercellular water transfer in the roots of intact Zea mays L. plants under elevated concentrations of atmospheric CO<sub>2</sub>**

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In this study, the effect of atmospheric carbon dioxide concentration increase on the dynamics of radial intercellular water transfer in the root suction zone of intact maize plants was evaluated. To this end, a unique growth chamber, associated with <sup>1</sup>H NMR PGMF (proton nuclear magnetic resonance with a pulsed gradient of the magnetic field) equipment, was used. As the atmospheric CO<sub>2</sub> concentration increased up to 800 ppm and higher, and the intensity of water transfer in the roots significantly decreased. The average effective water diffusion coefficient ( $D_{ef}$ ) and the water permeability in root cells ( $P$ ) decreased by approximately 30-35% within 5-6 hours after the increase in CO<sub>2</sub> concentration. At a higher concentration of CO<sub>2</sub>, 1200 ppm, the rate of decrease in water permeability increased. After a day of exposure to elevated CO<sub>2</sub>, the intensity of water transfer was partially restored but remained below the control level (before CO<sub>2</sub> enrichment) over the next 7 days. Inhibitory analysis showed that root cell aquaporins made a significant contribution to the observed decrease in the intensity of water transport in the roots. The decrease in water permeability of root cells under elevated CO<sub>2</sub> concentrations possibly occurs due to the regulatory decrease in water conductivity of aquaporins via shoot-to-root long-distance signaling.

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## **99 - Plant acclimation to relative humidity modify the relationship between leaf structure and function in lettuce crop**

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When the air is warm and relative humidity is low, the atmosphere has a high demand for water, which can increase water used by plants. This effect, related to climate change, is already impacting agricultural and forest systems. When subjected to drought, plants can limit water loss by closing their stomata; however, this strategy makes them less effective at removing carbon from the atmosphere, thus reducing the ability to offset climate changing condition. Not only stomatal movements (opening/closing), but also their morphology and frequency, influence plant reaction to environmental conditions. In the past few years, it has been proposed that there is a coordination between structure and function in leaves and that such coordination change along gradients of water availability. However, most of the studies concern forestry and little is known in horticultural species, where improving water fluxes and in particular WUE under a changing environment is fundamental to ensure a sustainable production. In the present study, we investigate the relationship between structural and functional leaf traits in lettuce plants grown in growth chambers under two conditions of VPD (Vapour Pressure Deficit), namely VPD 0.69 (RH 80 %) and 1.76 (RH 60 %) kPa. Plants were irrigated to field-capacity and weighted every-day in order to record daily ET. Photosynthetic light-curves and chlorophyll “a” fluorescence analyses were utilised to assess plants physiological behaviour in response to different air moist condition. Morpho-anatomical analyses on leaves were conducted to characterize the hydraulic resistances and specific traits such as stomata, vein and functional anatomical traits in the mesophyll. Results showed that changes in VPD induce a fine tuning of the coordination between anatomical and eco-physiological traits in lettuce plants. It is noteworthy that this coordination changes between different parts of the leaves, directly or not exposed to air humidity and lights (sun or shade leaves).



## 121 - Identifying allelopathic compounds emitted by *Pittosporum undulatum* in Eucalypt forests

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Changing climatic conditions have played a major role in the evolution of plants and may be are modifying the composition, structure and functionality of native plant communities, favoring invasive species. Studies conducted in south-eastern Australia have reported *Pittosporum undulatum*, a native tree, to be an aggressive invader of Eucalyptus forests. The invasive capacity of *P. undulatum* is linked to its high competitive ability and dense canopy. We tested the hypothesis that the negative impact on floristic diversity is due to the release of allelopathic compounds that inhibit the germination and growth of other plants. To test this we compared the germination of *Pittosporum undulatum*, *Eucalyptus ovata* and lettuce on paper or on soil collected from under Eucalyptus and *Pittosporum* canopies. Seeds were watered with leachates made from fresh *P. undulatum* leaves, litter collected from under *P. undulatum* or Eucalyptus trees, or water.

*P. undulatum* seeds germinated more slowly than the other two species, however their growth rate was faster. Mortality rate of *E. ovata* seedlings was very high immediately after germination. While there were no significant treatment effects on rates of germination, there were some differences in morphology.

We also compared BVOCs (Biogenic Volatile Organic Compounds) using SPME fibers in air collected in Eucalyptus forests with and without invading *P. undulatum*. The main BVOCs in *P. undulatum* infested were  $\alpha$ - and  $\beta$ -pinene. The main difference between the two sites was the concentration of sesquiterpenes, which were much more abundant in areas without *Pittosporum*.

Our results suggest that the high invasiveness of *P. undulatum* is mainly related to morphological and physiological characteristics rather than to allelopathic compounds emitted by this species.

## 157 - Low-oxygen induced effects on maize roots

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Plants worldwide have to cope with flooding events and the resulting energy disruption caused by the lack of oxygen. For adaptation, plants evolved two strategies: the low-oxygen escape and the low-oxygen quiescence strategy. Maize has shown to be an intermediate candidate for both strategies. These strategies are well studied so far but the question was raised, whether and how the plants sense the low oxygen.

On one side, the discovered N-End-Rule Pathway (NERP) is a possible mechanisms. Here, transcription factors for regulation of responsible gene expression are stabilized under hypoxia and degraded via the proteasome under normoxia. The ethylene responsive factors group VII (ERF VII) have shown to be a putative group of transcription factors.

On the other side, the production of reactive oxygen species (ROS) is another possible mechanism. ROS is produced even under physiological conditions via the aerobic pathway. They function as signaling molecules in plant growth, cell development and programmed cell death. Most stressors lead to a protective “oxidative burst”. Low-oxygen has shown to be responsible for ROS-induced oxidative stress. These radicals cause lipid, nucleic and protein oxidation and furthermore total cell damage. Increased ROS levels can be reduced by ROS-scavenging molecules (ascorbate and glutathione) or enzymes (superoxide dismutase SOD, catalase CAT, ascorbate peroxidases APX, glutathione peroxidases GPX, and class III peroxidases). The peroxidases might act as stress indicators since their levels increase rapidly after stress induction. Analyses of peroxidase involved in biotic and abiotic stress are well-studied but the impact of low-oxygen, e.g. induced by flooding, on class III peroxidases is still rarely investigated.

This is the first study on plasma membrane bound proteins, especially class III peroxidases, in maize roots under low-oxygen stress. A combination of physiological (e.g. ADH, ethylene), transcriptional (qPCR, RNA Seq) and proteomic analysis (shotgun, 2D-PAGE/MS, activity) were performed.

## **294 - Melatonin Interposed Lead Absorption: The Anatomical Changes in *Amaranthus cruentus* L.**

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*Amaranthus cruentus* is an important leafy vegetable that is majorly consumed and cultivated largely even in sewage sludge environment with high level of heavy metals. Melatonin is a hormone which helps to stimulate vegetative growth of plant under stress. The study aimed at the effect of melatonin on lead absorption of *A. cruentus* in relation to their anatomical changes. Sterilized seeds of *A. cruentus* were primed in varying concentration of Melatonin (0 and 400 $\mu$ M to represent M0 and M400) for 24hours. Primed seeds were planted into 3kg of sterilized soil. Plants were exposed to Lead concentration (0, 10 and 20Mm to represent P0, P10 and P20) once a week till fruiting stage. Eleven (11) weeks after planting, Lead concentration at the leaves and roots were analyzed using Atomic Absorption Spectrometry (AAS). Leaves were sectioned transversely, stained and viewed under microscope at 200x. Melatonin significantly ( $P<0.05$ ) reduced Lead concentration at the root (33%) and leaves (67%) in treatment AcM400P20 and AcM400P10 respectively. Root vascular bundles of treatment AcM400P20 were 70% wider than its control (AcM0P20). A significant ( $P<0.05$ ) thickened of the root epidermis were observed in plant treatment AcM400P20 however, increased number of staggered vascular bundles were observed at the leaves of all plants treated with melatonin. Melatonin sequesters, localized and immobilized Lead at the root of *A. cruentus* which were reflected in the thickened of the root epidermis hence, restricted translocation of Lead to the leaves for healthy survival and protection of photosynthesis apparatus. Melatonin distorted the concentric arrangement of the leaves vascular bundles. Size of root epidermis and cuticle of *A. cruentus* were inversely related and presence of trichomes at adaxial and abaxial helped to maintained leaves turgidity which were responses of *A. cruentus* to Lead stress.

Key words: *A. cruentus*, anatomical structures, Lead concentration, melatonin

### **310 - Effect of *Conocarpus erectus* L. Biochar on Different Attributes of Cauliflower Grown on Nickel Contaminated Soil**

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Nickel toxicity is a worldwide yield limiting factor. Biochar had been used to reduce the toxic effects and to immobilize nickel in plants. Biochar also increases the bioavailability of nickel in plants. In this study the treatment of hardwood biochar prepared from *Conocarpus erectus* L. at various pyrolysis temperatures was used to check the bioavailability of nickel in nickel contaminated soil. Cauliflower (*Brassica oleracea* var. botrytis) was used an indicator plant to check the bioavailability of nickel after the application of immobilizing agents. Also, the different attributes of cauliflower (*Brassica oleracea* var. botrytis) such as biochemical, physiological and growth were observed by immobilizing agents and nickel bioavailability. There was significant increase in morphological, biochemical, physiological responses and antioxidant enzymes of cauliflower. The application of hardwood biochar prepared at the pyrolysis temperature 550 °C and 650 °C showed significant results in the length of shoots and roots, fresh and dry mass of shoots and roots, number of leaves, total surface area, biochemical parameters of cauliflower (*Brassica oleracea* var. botrytis) as compared to all other treatments in nickel contaminated soil. The activity of antioxidants was maximum at 450 °C. Application of hardwood biochar in the soil significantly decreased the concentration of nickel in DTPA extracts and in the roots and shoots of cauliflower (*Brassica oleracea* var. botrytis) at 550 °C pyrolysis temperature as compared to soil which was not amended. Therefore, it is suggested that application of hardwood biochar from *Conocarpus erectus* L. prepared at 550°C can significantly made nickel immobile in soil and bioavailable to plant in the nickel contaminated soil.

### **325 - Influence of irradiation and canopy level on surface properties and transpiration of *Fagus sylvatica* and *Quercus petraea* leaves**

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Growing conditions at different tree canopy positions may significantly vary and lead to foliar changes even within the same tree. Anatomical changes regarding leaf surface properties can help us understand how plants protect themselves against multiple biotic and abiotic stress factors. In this study we selected two model tree species (i.e., *Quercus petraea* and *Fagus sylvatica*) grown at one of their southernmost European distribution areas (Central Spain). Focusing on leaf surface properties and transpiration leaves samples were collected from lower canopy positions and also from fully irradiated, top canopy leaves and shaded top canopy leaves. Artificially shaded leaves grew for months within a bag made of netting fabric before they sprouted. At the end of summer, leaves were collected and several parameters were analyzed. Bagging induced different effects depending on the parameters measured, some of which showing a more plastic response than others. The factors responding more plastically to bagging were specific leaf area, thickness, transpiration and calcium, potassium and nitrogen (N): phosphorus (P) ratios. Other parameters differed according to the species, but also showed a plastic behavior, namely: stomatal density and wax concentration per unit area. Contact angle measurements revealed small changes, similarly to some of the leaf concentration of magnesium, sulphur, P or N. In addition, the mesophyll of beech leaves was deformed following bagging, but neither transpiration nor soluble waxes showed the damage). Different responses to bagging were also recorded among species and may be related to the ecophysiology of beech (a highly shade-tolerant species with a high foliar index) compared to sessile oak (with a higher irradiation requirement and drought tolerance).

### **331 - Chlorophyll content and chlorophyll fluorescence analysis in *V. myrtillus*, *V. vitis-idaea* and *V. uliginosum* populations growing in different forest types in Latvia**

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Chlorophyll fluorescence analysis is a useful tool for examination of environmental effects, diurnal and seasonal patterns of photosynthesis and can help to predict plant growth.

The aim of the research was to determine chlorophyll content and chlorophyll fluorescence parameters with non-destructive methods in different forest types. Information about growth, adaptation and vitality was obtained for three species of the genus *Vaccinium*: *V. myrtillus*, *V. vitis-idaea* and *V. uliginosum*.

Chlorophyll content was estimated using a chlorophyll meter SPAD-502, and chlorophyll fluorescence parameters - by Handy PEA chlorophyll fluorimeter during the growing season in 2018 and 2019. All three species were measured in Vacciniosa, Myrtillosa forest types, and in addition *V. myrtillus*, *V. vitis-idaea* were measured in Hylocomiosa.

Leaf chlorophyll content was lowest in June, at the beginning of the growing season and decreased in September in bilberries and bog bilberries. Chlorophyll content in the evergreen species *V. vitis-idaea* increases over the growing season and in September continued to increase.

$F_v/F_m$  varied between 0.76 and 0.83 in all species. ANOVA Single Factor Analysis did not show differences in  $F_v/F_m$  between different forest types in all species.  $F_v/F_0$  and  $PI_{ABS}$  continued to increase in September in *V. vitis-idaea*. There were significant differences in  $PI_{ABS}$  in *V. myrtillus* and *V. vitis-idaea* in different times during growing season, but in different forest types differences in  $PI_{ABS}$  were not found. A significant difference in  $PI_{ABS}$  was found between *V. myrtillus* and *V. vitis-idaea*.

The examined species are well adapted to all the investigated forest types. This supports previous genetic analysis results, which found that Baltic state populations of these species were not genetically differentiated. Nevertheless, our results indicate that species biology and seasonal conditions affect leaf chlorophyll content and chlorophyll fluorescence parameters.

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### 337 - Peas—a genetic resource for sustainable protein production in the Arctic?

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Peas are an important source of plant protein for humans and animals. The past few years an increased interest in peas as an alternative protein source, both within the food and fodder industry, have been observed in many countries. Peas have historically been a major cultivated crop in the Nordic countries. The low temperatures and long days in the Arctic areas of the Nordic countries however require specific adaptation of crops. On the other hand, taking climate change into account, increasing temperatures will gradually allow a cultivation of crops not earlier possible to cultivate at these more northern locations, one example is peas.

At NordGen, a common genebank for all the Nordic countries, a large number of pea accessions are conserved, including both cultivars, landraces and breeding material. Arctic Peas is a cooperation research project aiming to identify germplasms of peas well adapted either for breeding or immediate cultivation in the Arctic region and adjacent areas. Within the project 50 accessions have been evaluated in search for important traits at four contrasting Nordic locations at latitudes ranging from 55° to 69° north. Among the evaluated traits are flowering time, maturation time and yield as well as protein content in search for different expressions at locations with clear distinction in daylength, temperature and climate.

A sustainable Arctic protein production would benefit local farmers' communities and strengthen food security. If such a production should be able to implement, a set of suitable cultivars adapted for northern growing conditions are needed together with the gain of experience and knowledge about pea cultivation in more northern areas. As peas are self-sufficient with nitrogen the dependency of N<sub>2</sub>-fertilizers will be reduced and subsequently the carbon pollution by nitrogen-fertilizer production.

#### 416 - Cork oak endophytes - How can they be impacted by climate changes?

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Climate changes present a risk for many ecosystems in the world. Mediterranean region is described as one of the most vulnerable ecosystems, as decreased precipitation may impact water availability, and warming may increase fire risk and impact plant health. This region contains large areas of cork oak forests, an evergreen tree species, with high ecological and socio-economical value, mainly due to biodiversity conservation and production of cork. Although cork oak is well adapted to warm locations, an increasing decline has been reported for the Mediterranean region, which could be associated with climate changes, like recurrent drought events. Extreme climate events are described to weaken trees, change microbial communities, and potentiate the colonization by opportunistic pathogens. To understand the impact of climate in cork oak sustainability and health, endophytic fungal communities of cork oak forests from different bioclimates were analyzed. Twigs and leaves were collected from cork oak trees, naturally subjected to different precipitation and temperature levels (bioclimates), in Portugal. DNA from endophytic fungal communities from these plant tissues were extracted and ITS2 barcode region was sequenced by Illumina MiSeq technology. The influence of climate on these communities, as well as correlations with presence of opportunistic pathogens are described.



#### 430 - Role of ABA in stomatal responses to dehydration in resurrection plants

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Desiccation tolerance (DT) is the capacity of an organism to lose up to 95% of its total cellular water content for long periods and then fully recover its metabolic activity after rehydration. In vascular plants, DT is frequent in spores or seeds, but is rare in vegetative tissues. This occurs only in a small but taxonomically diverse group of species that are termed resurrection plants. Absciscic acid (ABA) is an important phytohormone that regulates stomatal closure and drought-response genes, amongst other important functions. However, differences in the sensitivity of stomatal guard cells to ABA have been reported across land plant lineages. In gymnosperms and angiosperms stomatal closure is regulated by ABA levels, whereas in the case of lycophytes and ferns the response to endogenous ABA varies among species. We hypothesise that as part of their particular strategy resurrection plants may have developed a different stomatal control by altering its responsivity to ABA. To check this, we compared the response to exogenous ABA application on excised leaves of phylogenetically related pairs of species (resurrection vs. sensitive) of angiosperms and ferns. Specifically, stomatal closure and water loss kinetics were measured. Furthermore, the endogenous ABA content was analysed. Resurrection plants showed higher constitutive ABA levels and different stomatal regulation in comparison to the sensitive species. The application of exogenous ABA affected all angiosperms but not ferns. Overall, our results suggest that ABA may not be directly implicated on the regulation of stomatal closure in DT angiosperms, but might be involved in the regulation of the expression of certain groups of genes crucial for DT.

#### **431 - Global warming: friend or foe for the survival of the *C. quitensis* antarctic ecotype?**

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Antarctica is one of the last pristine environments in which endemic organisms have progressively specialized to deal with very harsh weather conditions, living often at their physiological limit. Only two endemic vascular plants, *Colobanthus quitensis* and *Deschampsia antarctica*, have been able to establish and survive in Antarctica, more precisely in the Peninsula. Their colonization has increased over the last five decades as a consequence of the rise in temperature due to recent global warming. In this scenario, a new target in modern cell physiology is the study of the genetic and molecular traits of their adaptation to the rapidly changing environmental conditions which may permit the disclosure of molecular biomarkers for efficient climate change monitoring. To this, we performed differential proteomic analysis on *C. quitensis* plants grown in natural conditions (OUT samples) compared to plants grown for one year inside small greenhouses open on the top (OTC samples) which determine an increase of about 4 °C during midday, mimicking the effect of global warming. Interestingly, we found that photorespiration could play an important role in reducing oxidative stress and ROS-mediated photodamage improving protection against photoinhibition. On the other hand, plant resistance to abiotic and biotic stresses seems to be strongly favored by the interaction with rhizosphere microorganisms. Recently, we performed a metatranscriptomic analysis on *C. quitensis* leaves revealing the presence of many microbial species (fungi, bacteria, algae and viruses) associated to the aerial part of the plant too. Their role in the adaptation of *Colobanthus* to rapidly changing environmental conditions has been also investigated, comparing the metatranscriptomic data of OUT and OTC samples. Interestingly, we found higher fungal metabolic and transcriptional activities in plants grown under warmer conditions and many of them are involved in the biosynthesis of compounds functional to plant growth.

#### 438 - Too dry to survive: lethal hydraulic failure can be predicted on the basis of leaf water content

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Forecasts on plant drought-driven mortality, as a consequence of current climate changes, highlight the need of indicators for monitoring vegetation fitness and, when possible, mitigate the negative effects of drought on vegetation and crop productivity. The reliability of current predictive models is affected by shortcomings on physiology of plant die-back and, then, die-off. The most recent findings strongly suggest that hydraulics play a critical role in plant ability to cope with drought and hydraulic failure has been recognized the primary cause of drought-driven vegetation die-off.

In the present study we assessed the predictive power of the leaf water status for monitoring the drought-driven die-back in two Mediterranean native species: *Salvia ceratophylloides* Ard. (Sc) and *S. officinalis* L. (So). The study species showed significant differences in relative water content (RWC) thresholds inducing the loss of cell rehydration capacity (PLRC) and leaf hydraulic conductance ( $K_L$ ). However, because Sc showed significantly higher leaf saturated water content values than So, the different RWC values for inducing leaf hydraulic impairment corresponded, actually, to similar leaf water content values. Moreover, very robust linear correlations were recorded between the electrolyte leakage measurements, as a proxy of leaf membrane damages, and the  $K_L$ , so suggesting that the cell membrane integrity, as major driver of the leaf extra-xylem water pathway, is critical for  $K_L$  decline during dehydration. Our results pointed out the relevant role of the leaf water status for driving the hydraulic decline in response to water shortage, suggesting that leaf water content may be a very useful parameter for predicting the risk of leaf hydraulic impairment and, then, the potential die-back of species under severe drought. Moreover, novel results on the relevance of cell membrane integrity for leading the leaf hydraulic vulnerability during dehydration are shown.

#### **456 - A scalable approach to monitor pigment seasonal dynamics and intraspecific variation in leaf pigments**

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Chlorophylls and carotenoids are the most widespread and abundant leaf pigment families, and fulfil vital physiological functions, including photosynthesis, and photoprotection. Leaf pigments are thus important biochemical indicators of plant performance and acclimation. Traditionally, pigment content and composition are measured using spectroscopic methods targeting whole compound families. Newer optical sensors and chromatography systems with improved resolution now allow the monitoring of pigment dynamics at unprecedented detail, revealing individual compounds across levels of biological organization.

We evaluate four methods to observe pigment composition: (i) optical measurements of leaves in situ taken with a contact probe coupled to a field spectroradiometer, (ii) light absorption by bulk chemical extracts, (iii) compound-targeted standard chromatography-optical detection methods, and (iv) our new untargeted chromatography-optical detection approach. We compared the sensitivity of the methods to detect seasonal dynamics of pigments and intraspecific variability in sun-exposed and shaded leaves of deciduous trees.

We find that in situ spectral measurements can detect intraspecific variability in leaf chemistry within and between individual trees. Our new untargeted approach allows us to characterize 5.3 times more pigment metabolites than previous chromatography methods. Pigment metabolites contribute more to the bulk signal during spring and autumn (up to 9%) than in summer (1-2%) when leaves are fully extended and mature, and thus better describe seasonal dynamics of leaf chemistry. We conclude that calibration based on our approach improves the quality of spectral retrievals and enable us to better monitor in-situ plant-environment responses with optical sensors.

## TOPIC:

### Plant epigenetics

#### Oral Communications

##### 15 - Evolutionary and functional impact of epigenetic variations in forest trees facing climate change

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Forest die-off is reported all around the world due to heat and drought stress episodes. As fixed and long living organisms subjected to repeated environmental stresses, trees have developed mechanisms enabling them to cope with fluctuating environmental conditions. Recently, epigenetics has been proposed as a hub of integration linking physiological response and environmental constraints that needs evaluation in trees. Our objective is to evaluate the potential of epigenetics and more specifically DNA methylation to significantly participate to phenotypic plasticity in trees in response to stress and the potential use for trees breeders and forest managers.

Using a transgenomic approach, we have shown in shoot apical meristem of poplar trees that DNA methylation controls genes involved in the developmental plasticity such as phytohormone genes in response to environmental constraints (temperature, drought). We have also start to investigate how these epigenetic variations could be transmitted to primed organs, participate to stress memory and trees priming facing recurrent water stress.

Using reverse genetic approach (RNAi lines), we found that poplar trees with altered methylation profiles are affected for their tolerance to drought and display spontaneous lesion mimic responses suggesting a “priming system to pathogen attack”. We have identified genes and transposable elements targeted during this process and highlight a trade-off situation between plasticity and genome integrity in meristematic cells.

Finally, using a population epigenomic approach, we are evaluating the adaptive potential of DNA methylation variations in natural trees populations. Altogether, our data highlights functional and evolutionary roles of DNA methylation in natural population of trees in a context of climate change giving promising perspectives for tree breeding.

**175 - Loss of function of an arabidopsis ortholog of the mammalian MRG15 adaptor protein connecting splicing to chromatin leads to defective abscisic acid signaling**

**Alba Rodríguez Díez** <sup>(1)</sup> - Paula Duque <sup>(1)</sup>

**Instituto Gulbenkian de Ciência, Plant Molecular Biology Laboratory, Oeiras, Portugal** <sup>(1)</sup>

Alternative splicing, the process by which different transcripts are produced from the same pre-mRNA, has emerged as a key contributor to stress tolerance and is known to be greatly affected by environmental changes. Interestingly, the chromatin landscape also undergoes rearrangements to adjust the response to stress, with a functional connection between alternative splicing and chromatin having been found in animal systems. We are addressing the hypothesis that differential alternative splicing in plants is regulated by stress-induced chromatin changes. The link established in animals stems from adaptor proteins that upon reading a given chromatin mark recruit a specific splicing factor to the nascent pre-mRNA. Here we show that a loss-of-function mutant for the adaptor MRG15 ortholog in *Arabidopsis thaliana* exhibits an impaired response to the stress phytohormone abscisic acid (ABA) during early seedling development. The ABA sensitivity defect is corroborated at the molecular level by RT-qPCR analyses showing altered expression of ABA signaling marker genes in the mutant. Large-scale transcriptome analysis via RNA-seq under control and ABA conditions will reveal the genes and alternative splicing events under control of the MRG15 ortholog protein, with these loci being further analyzed at the chromatin level. This work is expected to disclose a role for crosstalk between chromatin and the splicing machinery in ABA-mediated plant stress responses.

## TOPIC:

### Plant epigenetics

#### **Extended Elevator Pitches**

**43 - Histone modification and activation by SOC1-like and drought stress-related transcription factors regulate AcSVP2 expression during kiwifruit winter dormancy**

**Rongmei Wu** <sup>(1)</sup> - Erika Varkonyi-Gasic <sup>(1)</sup> - Tianchi Wang <sup>(1)</sup> - Andrew Allan <sup>(1)</sup>

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The transcription factors SHORT VEGETATIVE PHASE (SVP)-like and DORMANCY ASSOCIATED MADS-BOX (DAM) have been shown to regulate winter dormancy in woody perennials. In kiwifruit, the AcSVP2 gene affects the duration of dormancy in cultivars that require high chill. In this study, we examined the expression, function and regulation of AcSVP2 in a low-chill kiwifruit *Actinidia chinensis* 'Hort16A'. Analysis of expression in shoot buds showed that AcSVP2 transcript was elevated in dormant buds during winter months and declined prior to bud-break. Overexpression of AcSVP2 in transgenic *A. chinensis* delayed bud-break in spring. A reduction in the active trimethylation histone marks of the histone H3K4 and acetylation of histone H3 contributed to the reduction of AcSVP2 expression towards dormancy release, while the inactive histone marks of trimethylation of the histone H3K27 and H3K9 in AcSVP2 locus did not show significant enrichment at the end of winter dormancy. Screening of 101 transcription factors in an assay with a 2.3kb promoter region of AcSVP2 found that kiwifruit SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1)-like genes are able to activate the AcSVP2 promoter. We further identified additional transcription factors associated with drought/osmotic stress and dormancy which may regulate AcSVP2 expression.

## **286 - Key transcripts and epigenetic signatures underlying the somatic embryogenesis process in different grapevine genotypes**

**Silvia Dal Santo <sup>(1)</sup> - Emanuele De Paoli <sup>(2)</sup> - Chiara Pagliarani <sup>(3)</sup> - Alessandra Amato <sup>(1)</sup> - Paolo Boccacci <sup>(3)</sup> - Cristian Del Fabbro <sup>(2)</sup> - Mario Pezzotti <sup>(1)</sup> - Giorgio Gambino <sup>(3)</sup> - Irene Perrone <sup>(3)</sup> - Mirko Celii <sup>(4)</sup>**

**Department of Biotechnology, University of Verona, Verona, Italy <sup>(1)</sup> - Department of Agri-Food, Environmental and Animal Sciences, University of Udine, Udine, Italy <sup>(2)</sup> - Institute for Sustainable Plant Protection, National Research Council of Italy, Torino, Italy <sup>(3)</sup> - Department of Agri-Food, Environmental and Animal Sciences, University of Udine, Udine (Italy) <sup>(4)</sup>**

Somatic embryogenesis (SE), i.e. initiation of embryos from plant somatic tissues, represents one of the most suitable tools for in vitro manipulation of the *Vitis* genus. Genetic engineering approaches for breeding purposes and/or functional genomics studies in grapevine strictly rely on the availability of embryogenic calli for *Agrobacterium*-mediated plant transformation, as well as for the regeneration of DNA-free gene edited plants via protoplast culture. A reliable technique allowing the production of somatic embryos and regenerated plantlets from several cultivars is the essential requirement for the widespread application of the “next-generation breeding techniques” in grapevine.

The somatic embryogenesis process is affected by many factors and the most important is undoubtedly the genotype. Appreciated and economically important *V. vinifera* genotypes as well as important rootstocks employed in viticulture show a low somatic embryogenesis competence, making fundamental the understanding of the molecular mechanisms underlying this recalcitrance.

In this work, the behavior of two grapevine genotypes characterized by a divergent SE aptitude, ‘Sangiovese’ and ‘Cabernet Sauvignon’, was investigated during in vitro SE by profiling mRNA, small RNA and DNA methylation changes via high-throughput sequencing technologies. Differential gene expression analyses showed that the recalcitrant genotype (‘C. Sauvignon’) redirects metabolic resources towards the production of defense transcripts already in the early steps of in vitro culture, triggering a process that could be antagonistic to the formation of embryogenic tissues. Moreover, novel insights into the epigenetic control of the SE process were achieved. An increased level of CHH methylation on transposable elements (TEs) is a consistent feature of embryogenic calli, regardless of the genotype, whereas DNA methylation at CG and CHG contexts contributes to explain the differences among genotypes. Finally, further analyses performed on different classes of TE allowed to localize for the first time the embryogenesis-associated hypermethylation.



## TOPIC:

### Plant epigenetics

#### **Posters**

##### **551 - Pinpointing the regulation in epigenetic gene regulation**

**Franziska Turck** <sup>(1)</sup>

**Max Planck Institute for Plant Breeding Research, Plant Developmental Biology, Köln, Germany** <sup>(1)</sup>

In recent years, we and others have reported progress on the research question how PRC complexes find their target genes in plants. Notably, transcription factors, belonging to the B3 domain transcription factor or the telomere repeat binding MYB-families recruit PRC complexes with distinct chromatin modifying activities. Cognate cis-motifs of PRC recruiting transcription factors distribute along target regions, which includes a high occurrence within gene bodies. We propose that target genes compete for PRC complexes, which cooperate to stabilize the epigenetic state. In this scenario, the relative concertation of cis-motifs defines the epigenetic stability of target genes thus contributing to a coordinated regulation of Polycomb Group target genes.

**679 - miRNA-mediated phosphate starvation mechanisms involved in embryogenic competence maintenance in tamarillo (*Solanum betaceum* Cav.) calli**

**Daniela Cordeiro** <sup>(1)</sup> - **Jorge Canhoto** <sup>(1)</sup> - **Sandra Correia** <sup>(1)</sup>

**Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal** <sup>(1)</sup>

Somatic embryogenesis (SE) is a developmental process by which somatic plant cells reprogram acquiring competence to develop into embryos, and later on into whole plants. It is a valuable tool for the rapid and large-scale micropropagation of numerous economically important species, an efficient regeneration system for genetically modified plant production and an important experimental system for studies on plant embryogenesis and cellular developmental plasticity. Due to the relevance of this regeneration process, there is a great interest in the analysis of the gene regulatory networks underlying it. Recently, the generation of genome-wide profiles of microRNAs (miRNAs) and their target genes has pointed out several of these molecules as key factors controlling SE induction and embryo development. We used previously obtained high-throughput sequencing data (RNAseq and small RNAseq), generated from tamarillo (*Solanum betaceum* Cav.) SE-induced cell lines to guide our investigation into the functional characterization of specific miRNAs-target pairs that showed differential expression in cell lines and tissues with distinct embryogenic ability. We found that while specific miRNA-target regulatory nodes were indicated as involved in the acquisition and expression of embryogenic competence, others seem to be related to the loss of that capacity during subcultures. So far, miR399 and miR827 showed to be up-regulated in calli that lost their embryogenic capacity throughout subcultures comparatively to embryogenic ones. Concomitantly their targets, involved in phosphate-transport responses in the cell (PHOSPHATE2 and PHOSPHATE TRANSPORTER 5), are down-regulated in long-term calli. Furthermore, since sugar mediates phosphate starvation responses, our hypothesis is that the long exposure to high sucrose rates is responsible for the loss of embryogenic competence in tamarillo throughout subcultures. More results are being obtained on the functional validation of both miRNAs. Nevertheless, the results so far obtained corroborate the hypothesis that phosphate metabolism is determinant for embryogenic competence commitment and expression during SE.

### **317 - Short exogenous peptides regulate the growth and expression of genes of *Nicotiana tabacum* L.**

**Larisa Fedoreyeva** <sup>(1)</sup> - **Neonila Kononenko** <sup>(1)</sup> - **Tatiana Dilovarova** <sup>(1)</sup> - **Boris Vanuyshin** <sup>(1)</sup>

**Institute of Agricultural Biotechnology RAS, department of cell biology, Moscow, Russian Federation** <sup>(1)</sup>

Peptides form an extensive and diverse signaling regulatory system that controls the growth and development of animals and plants. Dipeptides GlyGly and GlyAsp and amino acid Gly at a concentration of  $10^{-7}$  M significantly stimulate the growth and development of *Nicotiana tabacum* L. In the presence of peptides, the root system looks more developed, the length of the main root increased by about 40%. It should be noted that peptides stimulate of forming new lateral roots. Root hair formation is controlled by transcription factors such as bHLH, WER, CPC, GL2. Peptides and glycine inhibit the expression of a transcription factor GTL1 that inhibits root hair growth.

FITC labeled peptides penetrate into the roots of tobacco and accumulate in the apical zone of the root cap and epidermal root cells. After incubation of the roots with FITC-peptides, fluorescence was detected in the cell walls, cytoplasm and nucleus. The fluorescence intensity in different root cells was different. This may be due to the different competence of cells of different zones of the root for the interaction, penetration and accumulation of peptides.

The studied peptides modulate the expression of genes of KNOX and GRF families. The profile of induction or repression of gene expression by the same substances in tobacco is different.

The pronounced biological activity of these substances at such a low concentration presumably due to the fact that they perform a regulatory signaling function in the cell and mainly epigenetic nature.

DNA methylation is an important epigenetic mechanism that controls the genetic processes in cells. The short exogenous peptides can selectively affect the expression of methyltransferase DNA genes. Peptides increase expression gene CMT3 (~ 30%), but reduce (~ 4-fold) the expression of DRM2.

The study was performed in the framework of the state assignment AAAA-A17-117091460012-8 and partially RFFI № 18-016-00150

### 327 - Genetic and epigenetic uniformity assessment of offspring of barley regenerants

**Renata Orłowska**<sup>(1)</sup> - Katarzyna Anna Pachota<sup>(2)</sup> - Piotr Tomasz Bednarek<sup>(3)</sup>

**Plant Breeding and Acclimatization Institute-National Research Institute, Department of Plant Physiology and Biochemistry, Radzików, Poland**<sup>(1)</sup> - **Plant Breeding and Acclimatization Institute-National Research Institute, Department of Plant Physiology and Biochemistry, Radzików, Poland**<sup>(2)</sup> - **Plant Breeding and Acclimatization Institute-National Research Institute, Department of Plant Physiology and Biochemistry, Radzików, Poland**<sup>(3)</sup> email: [r.orlowska@ihar.edu.pl](mailto:r.orlowska@ihar.edu.pl)

The genomes of many eukaryotic organisms contain no more than 5% of genes. The rest of the genome is occupied by various types of distributed sequences, including transposable elements (TEs). These genome elements can move from one genome location to another, leading to variation in organization and genome content. The general classification of TEs divides them into two classes: class I - retrotransposons and class II - DNA transposons. Abiotic stress, such as the plant in vitro cultures, can lead to genomic shock, which may result in, among others, activation of TEs. As a consequence that activation, contributes to the tissue culture-induced variation in regenerants and somaclonal variation in offspring of regenerants. Thus TEs activated during in vitro conditions may impact genetic/epigenetic variation in plants that were derived via sexual reproduction as offspring of regenerants.

Here we present the use of Methylation Sensitive Transposon Display (MSTD) as the method to investigate the level of variation between offspring of barley doubled haploid.

Seeds of spring barley (genotype 2dh/8) four regenerants were used to obtain offsprings. Fresh leaves of offspring seedlings served as a source of the genomic DNA. The molecular analyses were based on the MSTD method, which is a modification of the metaFLP technique. One primer complementary to the TE sequence and the other Acc65IKpnI was used in the analyses. The polymorphism of methylation sites (CG, CXG) flanking five families of TEs: Ty1-copia (Bare1), Ty1-gypsy (Bagy1, Sabrina), TRIM (Cassandra), LARD (Sukkula), CACTA (Balduin, Caspar) was analysed. The MSTD analysis resulted in 276 markers shared by both Acc65I/MseI and KpnI/MseI platforms and amplified by ten selective primer pairs.

The most polymorphic DNA profiles were observed for primers based on the CACTA TEs families. Differences in the level of polymorphism between offspring derived from different regenerants were also observed. Differences in the level of sequence variation and changes in DNA methylation were also observed. It seems that the observed differences in the level of polymorphism between the plants studied can be associated with both the regenerant from which the offspring were obtained and the activity of TEs in the barley genome.

### **335 - Dual role of non-coding tandem-repeats integrating epigenetic silencing with environmental response**

**Diego H. Sanchez** <sup>(1)</sup>

**IFEVA (CONICET-UBA), Facultad de Agronomia (FAUBA), Universidad de Buenos Aires (UBA), Buenos Aires, Argentina** <sup>(1)</sup>

Non-coding tandem-repeats are ubiquitous genomic features, but knowledge about their biological relevance is scarce particularly in plants. Here, we present data demonstrating that specific tandem-repeats may display a central function in the intersection between epigenetic silencing and environmental gene expression. We show that promoter tandem-repeats within the imprinted SDC locus of *Arabidopsis thaliana*, known targets of transcriptional-gene-silencing and largely mediating epigenetic suppression and imprinting, are also necessary and sufficient for heat-mediated SDC induction with distinct anatomical pattern in vegetative tissues. Our results demonstrate that these tandem-repeats exhibit a bona fide dual role, acting not only in epigenetic silencing at normal temperatures but also in transcriptional activation upon adverse stress. These observations imply an unusual form of transcriptional regulatory mechanism under abiotic stress, but we provide empirical data suggesting that it may also be operative in the regulation of other *A. thaliana* genes.

### **382 - dsRNA synthesis method in vivo for RNA interference regulation of the *Nicotiana benthamiana* phytoene desaturase gene**

**Tatiana Frolova<sup>(1)</sup> - Elena Filipenko<sup>(1)</sup> - Alexey Kochetov<sup>(1)</sup>**

**Institute of Cytology and Genetics SB RAS, Plant Genetics, Novosibirsk, Russian Federation<sup>(1)</sup>**

The use of genetically modified organisms in agriculture is a rather acute issue due to the unproven safety of this approach. An alternative way to regulate the expression of genes is the RNA interference – the silencing of certain genes using short RNA molecules, including exogenous dsRNA.

For plants, there is the possibility of penetration of exogenous dsRNA into the plant protoplast with subsequent phenotypic manifestation (for example, the accumulation of a metabolite in tissues, leaves color changing, the emergence of resistance to the pathogen). However, in vitro synthesis of dsRNA sequences is very expensive, especially for the most promising fragments of several hundred nucleotides in length.

The aim of this work is to develop an approach to the synthesis of dsRNA fragments in vivo in bacterial *E. coli* cells using plasmid L4440, specially designed for the expression of dsRNA. Phytoene desaturase is a key enzyme responsible for the production of chlorophyll in the leaves. The silencing of this gene has a clear phenotypic manifestation accompanied by the leaves whitening.

In the laboratory of Genetic Engineering of the Institute of Cytology and Genetics SB RAS we obtained *E. coli* HT115 strain carrying the L4440 plasmid with an integrated sequence for silencing tobacco phytoene desaturase using RNA interference. Currently, it is planned to perform the experiments on the induction of phytoene desaturase silencing on model plants of *N. benthamiana* using various fractions based on a bacterial suspension: overnight culture, bacterial pellet and supernatant, cell lysis fraction, total RNA and isolate dsRNA. Also, we planned to use different plant organs, such as abaxial leaf surface, apical meristems and root soaking.

**471 - Gene dosage compensation of rRNA transcript levels in Arabidopsis thaliana lines with reduced ribosomal gene copy number**

**Francesca Lopez** <sup>(1)</sup> - **Antoine Fort** <sup>(1)</sup> - **Luca Tadini** <sup>(2)</sup> - **Aline V. Probst** <sup>(3)</sup> - **Marcus McHale** <sup>(1)</sup> - **James Friel** <sup>(1)</sup> - **Frédéric Pontvianne** <sup>(4)</sup> - **Paolo Pesaresi** <sup>(2)</sup> - **Ronan Sulpice** <sup>(1)</sup> - **Peter McKeown** <sup>(1)</sup> - **Galina Brychkova** <sup>(1)</sup> - **Charles Spillane** <sup>(1)</sup>

**Ryan Institute, National University of Ireland, Galway, Ireland** <sup>(1)</sup> - **Dipartimento di Bioscienze, Università degli studi di Milano, Milano, Italy** <sup>(2)</sup> - **GReD, Université Clermont-Auvergne, Clermont-Ferrand, France** <sup>(3)</sup> - **LGDP, Université de Perpignan Via Domitia, Perpignan, France** <sup>(4)</sup>

The 45S rRNA genes (rDNA) are amongst the largest repetitive elements in eukaryotic genomes. rDNA consists of tandem arrays of rRNA genes, many of which are transcriptionally silenced. Silent rDNA repeats may act as 'back-up' copies for ribosome biogenesis and have nuclear organization roles. Through Cas9-mediated genome editing in the Arabidopsis thaliana female gametophyte we reduced 45S rDNA copy number to a plateau of ~10%. Two independent lines had rDNA copy numbers reduced by up to 90% at the T7 generation, named Low Copy Number (LCN) lines. Despite drastic reduction of rDNA copies, rRNA transcriptional rates and steady-state levels remained the same as wild type plants. Gene dosage compensation of rRNA transcript levels was associated with reduction of silencing histone marks at rDNA loci and altered Nucleolar Organizer Region 2 organization. While overall genome integrity of LCN lines appears unaffected, a chromosome segmental duplication occurred in one of the lines. Transcriptome analysis of LCN seedlings identified several shared dysregulated genes and pathways in both independent lines. Cas9 genome editing of rRNA repeats to generate LCN lines provides a powerful technique to elucidate rDNA dosage compensation mechanisms and impacts of low rDNA copy number on genome stability, development, and cellular processes.

## **515 - Role of BPM1 protein in a control of methylation patterns of CML41 and FWA genes through RdDM pathway in *Arabidopsis thaliana* L.**

**Tamara Vuk** <sup>(1)</sup> - Lucija Markulin <sup>(1)</sup> - Andreja Škiljaica <sup>(1)</sup> - Mateja Jagić <sup>(1)</sup> - Nataša Bauer <sup>(1)</sup> - Dunja Leljak-Levanić <sup>(1)</sup>

**Faculty of Science, University of Zagreb/Department of Biology, Zagreb, Croatia** <sup>(1)</sup>

*Arabidopsis thaliana* BPM1 protein belongs to the MATH-BTB family of proteins containing two domains; MATH (Meprin and TRAF Homology) and BTB (Bric-A-Brac, Tramtrack, Broad Complex). The best-described function of MATH-BTB proteins is within cullin3-based E3 ligases where MATH domain serves as an adapter and binds substrates destined for degradation via 26S proteasome.

Co-immunoprecipitation and mass spectrometry analysis revealed interaction of BPM1 with DMS3 and RDM1, components of a DDR complex involved in RNA-directed DNA methylation (RdDM) mechanism. The DDR complex recruits polymerase V to chromatin and is indirectly responsible for positioning of methylation machinery at specific genomic locations. The interactions between BPM1 and the aforementioned components of DDR complex are independent of the MATH domain, indicating that DMS3 and RDM1 are not substrates directed for degradation.

To further elucidate the role of BPM1 protein in RdDM, two known targets of RdDM, genes CML41 and FWA were selected for methylation pattern analysis by bisulfite conversion, PCR amplification and sequencing. The obtained sequences were processed using CyMATE software. Methylation patterns for each individual methylation position, as well as for each methylation context were compared between wild type, BPM1 overexpressor and a mutant with a dysfunctional DMS3 protein. The results showed significantly higher CHH methylation in BPM1 overexpressing plants and a loss of CHH methylation in DMS3 mutant.

Our current goal is to use chromatin immunoprecipitation to identify novel targets of RdDM whose transcription or methylation are influenced by BPM1 and to further correlate methylation patterns with expression profiles in different tissues and mutants.



#### **548 - Epigenetic regulation of desiccation tolerance loss during germination of *Pisum sativum* L. seeds**

**Ksenia Strygina** <sup>(1)</sup> - **Ekaterina Krylova** <sup>(1)</sup> - **Elena Khlestkina** <sup>(1)</sup> - **Galina Smolikova** <sup>(2)</sup> - **Sergei Medvedev** <sup>(2)</sup>

**N.I. Vavilov All-Russian Institute of Plant Genetic Resources, Postgenomic studies lab, St. Petersburg, Russian Federation** <sup>(1)</sup> - **St. Petersburg State University, Department of Plant Physiology and Biochemistry, St. Petersburg, Russian Federation** <sup>(2)</sup>

In higher plants' life cycle, the transition from seed dormancy to germination represents a critical stage of survival. Before the plants can resume vegetative development, massive reprogramming of the transcriptome and attendant signaling pathways is required. As result silencing of seed maturation gene and the activation of vegetative growth genes occur. The most important hormonal signal is a balance between abscisic acid and gibberellins, but other hormones such as auxins, brassinosteroids, ethylene, cytokinins, and jasmonates, also involved. A network of transcription factors known as the LAFL as well as DELAY OF GERMINATION1 are negative regulators of seed germination and should also be repressed before seedling development. The repression is associated with chromatin remodeling complexes Polycomb Repressive Complex 1 and 2, as well as the PICKLE and PICKLE-RELATED2 proteins. Here we discuss the epigenetic modifications, including the DNA methylation in promoter regions of key genes that control the seed transition from dormancy to germination in *Pisum sativum* L. The object of study is the embryonic axes isolated from germinating *P. sativum* seeds before and after initiation the root growth. Before radicle protrusion, the seeds are tolerant to desiccation, but they completely lose that property at the post-germination stage. The sequences of key genes (LEC1, LEC2, ABI3, ABI5, FUS3, DOG1) were identified based on reference genes of *Arabidopsis thaliana* (L.) Heynh. and *Glycine max* (L.) Merr. The analysis of the methylation pattern of the promoters of the identified genes was carried out using MethPrimer followed by cloning of gene sequences. The work was supported by grant no. 20-16-00086 from the Russian Science Foundation with using the equipment of Research Park of Saint Petersburg State University.

**562 - Ionising radiation-induced DNA methylation changes in Arabidopsis thaliana plants exposed to gamma radiation over multiple generations**

**Pol Laanen**<sup>(1)</sup> - Eline Saenen<sup>(2)</sup> - Mohamed Mysara<sup>(3)</sup> - May Van Hees<sup>(2)</sup> - Robin Nauts<sup>(2)</sup> - Ann Cuypers<sup>(4)</sup> - Nele Horemans<sup>(1)</sup>

**SCK CEN & Hasselt University, Biosphere Impact Studies & Centre for Environmental Research, Mol, Belgium**<sup>(1)</sup> - **SCK CEN, Biosphere Impact Studies, Mol, Belgium**<sup>(2)</sup> - **SCK CEN, Microbiology, Mol, Belgium**<sup>(3)</sup> - **Hasselt University, Centre for Environmental Research, Hasselt, Belgium**<sup>(4)</sup>

As sessile organisms plants have to adapt to environmental changes including enhanced ionising radiation (IR). Epigenetic modifications such as DNA methylation changes might play a pivotal role in these acclimation processes. Whole genome DNA methylation modifications as a result of IR exposure have been reported previously warranting a more detailed study. Here, the effect of  $\gamma$ -radiation on the induction of differentially methylated regions (DMRs) across the genome is studied over multiple generations of Arabidopsis thaliana plants.

Three generations (Parent (P0), generation 1 (S1), and generation 2 (S2)) of seven-day old A. thaliana plants were exposed to either of 2 different radiation conditions (30 or 110 mGy/h) or to natural background radiation (control condition) for 14 days. P0 consisted of previously non-exposed plants, whereas S1 and S2 plants had already received a similar irradiation in the previous one or two generations, respectively. Whole genome bisulfite sequencing was used to identify DMRs, including the methylation context, in each generation and for all radiation conditions. An intra- and intergenerational comparison of the DMRs, including their association with genes and transposable elements (TEs), was made.

The results show that practically all changes occurred in the CpG methylation context. A clear increase of IR-induced DMRs was observed over the 30 mGy/h generations, most occurring in S2. Counterintuitively, no significant differences were observed in those exposed to 110 mGy/h. Many DMRs were associated with TEs, most of which were hypermethylated, likely increasing genetic stability. Additionally, many DMRs were associated with genes related to development as well as a number of (a)biotic stress responses, including DNA repair, and RNA splicing. Our findings indicate that DNA methylation likely plays an important role in the response to IR and possibly in stress adaptation.

**579 - Ionising radiation-induced DNA methylation changes in Arabidopsis thaliana plants exposed to gamma radiation over multiple generations**

**Pol Laanen** <sup>(1)</sup> - **Eline Saenen** <sup>(2)</sup> - **Mohamed Mysara** <sup>(3)</sup> - **May Van Hees** <sup>(2)</sup> - **Robin Nauts** <sup>(2)</sup> - **Ann Cuypers** <sup>(4)</sup> - **Nele Horemans** <sup>(1)</sup>

**SCK CEN & Hasselt University, Biosphere Impact Studies & Centre for Environmental Research, Mol, Belgium** <sup>(1)</sup> - **SCK CEN, Biosphere Impact Studies, Mol, Belgium** <sup>(2)</sup> - **SCK CEN, Microbiology, Mol, Belgium** <sup>(3)</sup> - **Hasselt University, Centre for Environmental Research, Hasselt, Belgium** <sup>(4)</sup>

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## 655 - Transcriptomic and epigenetic changes in Arabidopsis autoimmune mutants vs. targeted PTI- and ETI-responses

Lisa Amelung <sup>(1)</sup> - Linn von Pein <sup>(2)</sup> - Sebastian Schröder <sup>(3)</sup> - Jan Knop <sup>(1)</sup> - Julia Kehr <sup>(4)</sup> - Stefan Hoth <sup>(5)</sup>

PhD student, Universität Hamburg/Molecular Plant Physiology, Hamburg, Germany <sup>(1)</sup> - PhD student, Universität Hamburg/Molecular Plant Genetics, Hamburg, Germany <sup>(2)</sup> - Master student, Universität Hamburg/Molecular Plant Physiology, Hamburg, Germany <sup>(3)</sup> - Professor, Universität Hamburg/Molecular Plant Genetics, Hamburg, Germany <sup>(4)</sup> - Professor, Universität Hamburg/Molecular Plant Physiology, Hamburg, Germany <sup>(5)</sup>

Pathogen effectors target regulators of PAMP-triggered immunity (PTI) to inhibit defense in plants. However, cytosolic immune receptors guard these positive regulators to initiate effector-triggered immunity (ETI) upon recognition of an effector. We have established saul1 mutants as a model for autoimmunity in Arabidopsis thaliana. These plants show reduced growth, defense gene expression and lesions in all aboveground organs when shifted to lower temperatures.

We have used RNA-Seq and Whole Genome Bisulfite Sequencing (WGBS) to resolve the gene expression as well as DNA methylation changes in the induced autoimmune mutant. Analysis showed that only a few genes were regulated after 1-2 hours, whereas thousands were differentially expressed after 96 hours. Correlation to alterations in DNA methylation status as well as further comparison to other autoimmune mutants is currently ongoing and will help to reveal the mechanisms behind gene regulation in autoimmunity.

To compare these findings to changes resulting from bacterial infections, Arabidopsis thaliana was stressed with different strains of Pseudomonas syringae DC3000. To further identify the relation of transcriptomic and epigenetic changes to PTI and/or ETI in vivo, an inducible-effector-system was established and is currently under investigation. This system will help identifying key-regulated genes of specific immune reactions and their associated DNA methylation patterns.

## TOPIC:

### Plant epigenetics 2

#### **Keynote Lecture**

##### **Long non-coding RNAs in epigenetic regulation**

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In eukaryotes, three-dimensional genome organization is critical for transcriptional regulation of gene expression. Long noncoding RNAs (lncRNAs) can modulate chromatin conformation of spatially related genomic locations within the nucleus and are emerging regulators of the epigenetic landscape at specific loci. We have identified the lncRNA APOLO (AUXIN REGULATED PROMOTER LOOP) that show cis regulation of the 3D conformation of gene promoter. More recently, we showed that APOLO recognizes multiple distant independent loci in the *Arabidopsis thaliana* genome. APOLO recognizes its targets likely by short sequence complementarity and the formation of DNA-RNA duplexes (R-loops). The invasion of APOLO to the target DNA decoys the plant Polycomb Repressive Complex 1 component LHP1, modulating local chromatin 3D conformation. In addition, we found other APOLO-like lncRNA embedded in a cluster of co-regulated genes. Indeed, the long noncoding RNA (lncRNA) MARneral Silencing (MARS), localized inside the *Arabidopsis thaliana* marneral cluster, controls the local epigenetic activation of its surrounding region in response to ABA. MARS modulates the POLYCOMB REPRESSIVE COMPLEX 1 (PRC1) component LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1) binding throughout the cluster in a dose-dependent manner, determining H3K27me3 deposition and chromatin condensation. In response to ABA, MARS decoys LHP1 away from the cluster and promotes the formation of a chromatin loop bringing together the MARNERAL SYNTHASE 1 (MRN1) locus and a distal ABA-responsive enhancer. Hence, lncRNAs are emerging as novel regulators of 3D chromatin conformation rearrangements involved in plant epigenetic regulations.

## TOPIC:

### Plant epigenetics 2

#### **Extended Elevator Pitches**

##### **405 - Detection of neighbors and transcriptional reprogramming: does chromatin accessibility count?**

**Alessandra Boccaccini <sup>(1)</sup> - Claire Paltenghi <sup>(1)</sup> - Ruben Benstein <sup>(2)</sup> - Markus Schmid <sup>(2)</sup> - Christian Fankhauser <sup>(1)</sup>**

**CIg, University of Lausanne, Lausanne, Switzerland <sup>(1)</sup> - Umeå Plant Science Centre, Umeå University, Umeå, Sweden <sup>(2)</sup>**

Plants can sense the proximity of other plants by the reduction of the ratio between the Red (R) and Far Red (FR) components of the light spectra. The FR light is reflected by leaves decreasing the R/FR ratio (LRFR) and creating a shaded environment, which is not tolerated by sun-lover plants such as *Arabidopsis thaliana*. The molecular mechanism underlying the response of *Arabidopsis* to dense vegetation has been extensively studied and it is known that remodeling of gene expression happens to allow a series of growth adaptations known as Shade Avoidance Syndrome (SAS) (Fiorucci and Fankhauser, 2017). The LRFR-mediated gene expression is ensured by the activity of a well-established network of transcription factors (TFs). The binding sites for TFs are commonly found in accessible regions of chromatin (Sullivan et al., 2014), but if a chromatin remodeling happens during SAS is not understood.

Our aim is to determine how chromatin accessibility changes during the SAS, considering both temporal and spatial regulation, by ATAC-sequencing. This allows us to identify not only which and when the known regulatory regions changes nucleosome conformation during SAS, but also to find new regulatory elements that haven't been considered yet.

Moreover, we want to assess if auxin plays a role in this regulation. In fact, the SAS is initiated by a rapid and transient increase of auxin biosynthesis, which drives the expression of different groups of genes. The newly synthesized auxin is detected after 1h of LRFR (Kohnen et al., 2016), but not during the second day of LRFR (Pucciariello et al., 2018). Our intent is to understand if this initial boost in auxin biosynthesis can prime the gene expression, acting on nucleosome occupancy and making genes more accessible for transcription, facilitating their expression over the days, also in absence of a new peak of auxin biosynthesis.

## TOPIC:

### Plant evolution and development

#### **Keynote Lecture**

##### **Evolution of plant cell proliferation control: Redox matters**

**Sabine Zachgo,**

**University of Osnabrück**

In multicellular plant development, final organ size and whole plant body architecture is controlled by the spatiotemporal regulation of cell proliferation and cell differentiation processes, integrating the response to environmental stimuli. TCP transcription factors are known key regulators of angiosperm cell proliferation processes. What are the TCP functions in early diverging land plants and how did they contribute to adaptive processes enabling land plant colonization?

We generated knockout mutants for MpTCP1, the single TCP-P clade gene in the liverwort *Marchantia polymorpha* and characterized its function conducting cell proliferation and morphological analyses. Loss of MpTCP1 activity leads to decreased thallus growth through reduced cell proliferation, indicating a conserved, ancestral TCP-P function in growth regulation. Interestingly, MpTCP1 regulates a complex downstream network of ROS producing and removing enzymes and thereby likely modulates H<sub>2</sub>O<sub>2</sub> levels. The identification of a single, and highly conserved Cys in the DNA-binding TCP domain was intriguing and its function was further investigated by redox-EMSAs. Loss of MpTCP1 activity also causes an enhanced thallus pigmentation. Biochemical analyses identified novel plant pigments in *Marchantia*, namely aminochromes that might function in UV protection.

Our data indicate a crucial ROS function in the nucleus for the regulation of transcription factor activity. We apply 3D-dSTORM superresolution microscopy to detect and quantify the colocalization of proteins of the transcription machinery. Thereby, we aim to investigate ROS and their effects in the nucleus below the defraction limit.

## TOPIC:

### Plant evolution and development

#### **Oral Communications**

##### **163 - Durum Wheat Pan-Transcriptome as a Bridge to Unravel Tetraploid and Hexaploid Wheat Gene Function and Evolution**

**Danara Ormanbekova**<sup>(1)</sup> - **Marco Maccaferri**<sup>(1)</sup> - **Sven O. Twardziok**<sup>(2)</sup> - **Davide Scaglione**<sup>(3)</sup> - **Vera Vendramin**<sup>(3)</sup> - **Simone Scalabrin**<sup>(3)</sup> - **Giuseppe Sciara**<sup>(1)</sup> - **Simone Corneti**<sup>(1)</sup> - **Matteo Bozzoli**<sup>(1)</sup> - **Andrea Massi**<sup>(4)</sup> - **Michele Morgante**<sup>(3)</sup> - **Klaus F.X. Mayer**<sup>(2)</sup> - **Roberto Tuberosa**<sup>(1)</sup>

**University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy**<sup>(1)</sup> - **PGSB, Plant Genome and Systems Biology, Helmholtz Center Munich, Neuherberg, Germany**<sup>(2)</sup> - **IGA Technology Services, University of Udine, Udine, Italy**<sup>(3)</sup> - **Società Produttori Sementi, Bologna-Syngenta, Argelato, Italy**<sup>(4)</sup>

This study presents the transcriptome analysis of 13 elite durum wheat varieties representatives of the worldwide cultivated germplasm. cDNA libraries were produced from roots, seedling leaves and developing grains. Based on the reference genome sequence assembly of durum wheat cv. Svevo, 75.0, 70.5 and 74.5% of high-confidence Svevo genes were expressed in grain, leaf and root, respectively. Principal Component Analysis (PCA) analysis showed a gene expression clustering led by tissues and varietal ancestry. Differentially up- and down-regulated gene clusters based on tissues and varieties were identified. Functional enrichment analysis for three Gene Ontology terms showed that differentially expressed genes were significantly enriched in transport, kinase activity, binding, enzyme activity and protein metabolism. Variance expression analysis projected on the Svevo assembly revealed the chromosome regions that drove the major expression variation patterns. Clustering the gene expression profiles and the cultivar's expression profiles evidenced several gene expression patterns related to their co-ancestry, particularly for the grain. Towards a more complete assembly of a pan-transcriptome in durum, the cultivar-specific reads that could not be mapped on the Svevo genome (4-30% referred to Svevo Illumina sequencing data) are being de novo assembled. Further, using the transcriptome of the 13 varieties in relation to bread wheat reference genome (cv. Chinese Spring IWGSC RefSeq) we are currently investigating the gene loss/deletion during the polyploidisation events. Moreover, the availability of the genome assemblies of the 10+ Wheat Genomes Project, which includes cultivars that represent genetic diversity, will allow us to infer strong allele fixation events (allopolyploidisation bottleneck).



## 252 - Evolution and development of carnivorous plant leaves

**Gergo Palfalvi** <sup>(1)</sup> - **Mitsuyasu Hasebe** <sup>(1)</sup>

**National Institute for Basic Biology, Okazaki, Japan, Department of Basic Biology, The Graduate School for Advanced Studies, SOKENDAI, Okazaki, Japan** <sup>(1)</sup>

Carnivorous plants acquired the ability to attract, capture and digest small animals for a fitness-increasing nutritional supply with novel leaf modifications. Such leaf modifications, or traps include e.g mucilaginous glands on the leaf surface, fast moving snap-traps, or pitchers. Very little known about the development of such organs and how it infers with the basic leaf body plan or even with the carnivory itself. To understand such processes, we approached in two different ways.

First, we used two species from the Droseraceae family, namely the Venus flytrap (*Dionaea muscipula*) and the spoon-leaved sundew (*Drosera spatulata*) to perform transcriptomic analysis on the leaf developmental series. This helped us to decipher how carnivory infers with leaf development and in more general, how carnivory might evolved in the context of leaf development.

Secondly, we used the Albany pitcher plant, *Cephalotus follicularis*, which has the unique ability to produce photosynthetic flat leaves and carnivorous pitcher leaves based on different environmental cues. This system enables us to conduct comparative experiments between the two leaf types, while the genetic background is fixed. To get a cellular resolution of the leaf- and pitcher initiation, we developed a single cell transcriptomic pipeline using green plant tissues. The cellular level transcriptomic data let us infer the pitcher-specific cell types and their appearance in the developmental process.

#### **444 - Studying the evolution of gene networks through *Marchantia polymorpha***

**Tomás Werner** <sup>(1)</sup> - Sara Coelho <sup>(1)</sup> - Sara Laranjeira <sup>(1)</sup> - Rómulo Sobral <sup>(1)</sup> - M. Manuela R. Sobral <sup>(1)</sup>

**CBFP, Universidade do Minho, Braga, Portugal** <sup>(1)</sup>

The establishment of new interactions within a gene regulatory network drives the emergence of new biological functions and morphologies. New interactions are often associated with repeated events of gene duplication that increase the genome complexity. Exploring the plant evolutionary path and understanding the function of a given gene is thus challenging due to redundancy within multigene families. The analysis of gene function in early land plants allows the study of ancestral gene functions, due to the existence of fewer duplicated gene copies, and enables a better understanding of the establishment of gene regulatory networks. The DDR transcriptional module is composed by three MYB proteins- DIVARICATA (DIV), RADIALIS (RAD) and DIV-and-RAD-Interacting Factors (DRIF) - and it controls several development processes in angiosperms, such as flower asymmetry in *Antirrhinum majus* and fruit development in tomato. To elucidate early functions of these proteins, we are studying DIV and DRIF homologs of *Marchantia polymorpha*, a liverwort that is used as a basal plant model. To unravel how the DDR module was first established, transgenic plants with gene function knocked out by the CRISPR/Cas9 editing system, with promoter::reporter gene fusions and with ubiquitous expression have been generated and their phenotypes are being studied. The results of this study could yield a better understanding of the evolution of gene regulatory networks and of how this module functions.

This work was supported by Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior through national funds (Programa de Investimento e Despesas de Desenvolvimento da Administração Central) with the project grant PTDC/BIA-PLA/1402/2014 and by a Research Unit grant UID/MULTI/04046/2019 to BioISI

## TOPIC:

### Plant evolution and development

#### **Extended Elevator Pitches**

**92 - Parental conflict or cell polarity establishment?**

**Mechanistic insight in MAP kinase signaling in the plant embryo**

**Martin Bayer** <sup>(1)</sup>

**Max Planck Institute for Developmental Biology, Dept. of Cell Biology, Tübingen, Germany** <sup>(1)</sup>

In flowering plants, asymmetric cell divisions are controlled by a MAP kinase signaling pathway including the MAP3K YODA. In the early Arabidopsis embryo, the YODA-dependent pathway is activated by two distinct mechanisms - an evolutionarily conserved receptor complex and a Brassicaceae-specific protein that activates YODA independent of receptor activation.

We will present mechanistic insight in the evolution of this MAP kinase signaling pathway as well as the regulation of YODA signaling on a molecular level and discuss specific parental contributions in this context.

## TOPIC:

### Plant evolution and development

#### **Posters**

##### **615 - cT-DNA in *Linaria vulgaris* L. is multicopy, inverted and homogenized**

**Ivan Vladimirov** <sup>(1)</sup> - **Olga Pavlova** <sup>(2)</sup> - **Denis Bogomaz** <sup>(1)</sup>

**Peter the Great St.Petersburg Polytechnic University, Peter the Great St.Petersburg Polytechnic University, Saint-Petersburg, Russian Federation** <sup>(1)</sup> - **Beagle Ltd, Peter the Great St.Petersburg Polytechnic University, Saint-Petersburg, Russian Federation** <sup>(2)</sup>

*Linaria vulgaris* is an example of a plant containing a vertically inherited sequence of *Agrobacterium rhizogenes* T-DNA (called cT-DNA, «cellular T-DNA») in the genome. The fixation of cT-DNA sequences in genomes, carrying the pathogenic program of genetic colonization, induce interest even only by the fact of such plants survival.

Deep studying of general features of cT-DNA brings us closer to understanding the causes and mechanisms of its fixation in plants genomes. We combined multiple long-range PCR with genome walking for studying extended structure of cT-DNA. Using digital PCR method, we estimated copy number of cT-DNA elements. NGS with low covering allows us to develop a set of microsatellite markers, also used for copy number estimation.

According to new data, cT-DNA elements in *L. vulgaris* form an inverted complex repeat of two simple direct repeats. After cT-DNA integration, cT-DNA sequence duplication events took place at least two times. The phenomenon of concerted evolution (spontaneous alignment of similar multicopy sequences) of cT-DNA sequences as well as some details of this process have been shown for the first time. We have shown, that *L. vulgaris*, as well as other cT-DNA containing species, has inverted structure of repeats. This fact indicates possible existence of some general causes and mechanisms of cT-DNA fixation in plant genomes during evolution.

## 9 - How to make a complex organ: on the evolution of carpel developmental regulators

Kimmo Kivivirta <sup>(1)</sup> - Denise Herbert <sup>(1)</sup> - Annette Becker <sup>(1)</sup>

Justus-Liebig-University, Department of Plant Biology, Giessen, Germany <sup>(1)</sup>

A major evolutionary innovation in the plant lineage is the angiosperm carpel, their unifying character and most complex plant organ, composed of many clearly distinct tissue types to ensure reproductive success. Several theories on carpel origin exist as it has been a matter of debate since many decades. Many components of the gene regulatory network (GRN) governing carpel development and their genetic interactions have been described, but mainly in *Arabidopsis thaliana* only. To elucidate the origin and evolution of carpel GRNs and to identify novel carpel development regulators, we follow two approaches: thorough phylogeny reconstructions to identify the age of the GRN components and phylotranscriptomics to identify evolutionary conserved co-regulated clusters of orthologs. For the latter, we generated transcriptomes from laser micro-dissected carpel developmental stages and compared co-expressed gene clusters of orthologous genes from phylogenetically distant species. Our data so far indicate that developmental processes present already in the most recent common ancestor of seed plants, such as reproductive meristem termination or adaxial/abaxial polarity specification requires few interacting transcription factors, which are purged after whole genome duplications (WGD). In contrast, developmental processes associated with derived carpel characters, such as the transmitting tract in *A. thaliana* require larger numbers of interacting transcription factors which were retained as duplicates after WGD. Further, we will present recent results on the evolution of carpel gene regulatory networks and novel regulators of carpel development.

### **36 - Phylogenetic relationship among wild and cultivated grapevine in Sicily: a hotspot in the middle of the Mediterranean basin**

**Roberto De Michele** <sup>(1)</sup> - **Francesca La Bella** <sup>(1)</sup> - **Alessandro Gristina** <sup>(1)</sup> - **Ignazio Fontana** <sup>(1)</sup> - **Davide Pacifico** <sup>(1)</sup> - **Giuseppe Garfi** <sup>(1)</sup> - **Antonio Motisi** <sup>(1)</sup> - **Dalila Crucitti** <sup>(1)</sup> - **Loredana Abbate** <sup>(1)</sup> - **Francesco Carimi** <sup>(1)</sup>

**Institute of Biosciences and Bioresources, CNR, Palermo, Italy** <sup>(1)</sup>

Grapevine (*Vitis vinifera* ssp. *sativa*) is a perennial crop especially important for wine and fruit production. The species is highly polymorphic with thousands of different varieties selected by farmers and clonally propagated. However, it is still debated whether grapevine domestication from its wild ancestor (*V. vinifera* ssp. *sylvestris*) has been a single event or rather it occurred on multiple occasions during the diffusion of its cultivation across the Mediterranean. Located in the center of the Basin, Sicily is its largest island and has served as a hotspot for all civilizations that have crossed the Mediterranean throughout history. Hundreds of unique grapevine cultivars are still cultivated in Sicily and its surrounding minor islands, though most of them are menaced by extinction. Wild grapevine is also present with isolated populations thriving along riverbanks. With the aim to evaluate the phylogenetic relationships among Sicilian varieties, and to assess the possible contribution of indigenous wild populations to the genetic makeup of cultivated grapevine, we analyzed 170 domestic cultivars and 125 wild plants, collected from 10 different populations, with 23 SSR markers. We also compared our data with published dataset from Eurasia. Results show that Sicilian wild populations are related to the cultivated Sicilian and Italian germplasm, suggesting events of introgression and/or domestication of local varieties.

## 78 - Dissecting heavy metal detoxification in the early land plant *Marchantia polymorpha*

Claudio Varotto<sup>1</sup>, Martina Leso<sup>1</sup>, Erika Bellini<sup>2</sup>, Matteo Buti<sup>4</sup>, Alessandro Saba<sup>3</sup>, Luigi Sanità di Toppi<sup>2</sup>, Mingai Li<sup>1</sup>

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The recent isolation of the phytochelatin synthase (PCS) from *Marchantia polymorpha* demonstrated that the enzyme is active and could therefore contribute to metal detoxification. Its actual contribution in metal tolerance in vivo remains an open question.

In this study, we obtained by CRISPR/Cas9 genome editing two independent mutant alleles of PCS, *Mppcsge\_1* and *Mppcsge\_2*. The mutants resulted extremely sensitive to treatment with the non-essential heavy metal cadmium (Cd), but tolerant to zinc (Zn). Correspondingly, the mutants were completely devoid of phytochelatin biosynthetic activity, as demonstrated by thiol-peptide quantification. The first comprehensive characterization of the responses to a range of 10 heavy metals (5 essential and 5 non-essential) was carried out in vivo, defining the relative toxicity of the different metals. Noteworthy, mutant plants were highly tolerant to the metalloid arsenic (As), suggesting a major role of phytochelatin synthase in *M. polymorpha* aimed at detoxification of divalent, non-essential metals. Whole transcriptome analysis of WT and one of the mutant lines identified a range of differentially expressed genes in different metabolic pathways that clearly distinguish the mutant even in the absence of metal stress and of a visible phenotype.

These results demonstrate that the phytochelatin-mediated detoxification of metals is an ancestral trait in land plants and suggest that phytochelatin synthase may participate to metal homeostasis in normal conditions. The mutant genotypes obtained further represent highly sensitive bioindicators of Cd contamination, and as such they could find application in biomonitoring.

## 234 - Evolutionary genomics of cotton fiber development reveals signatures of domestication

**Bhupendra CHAUDHARY** <sup>(1)</sup> - **Dhananjay Pandey** <sup>(2)</sup>

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Extra-long and spinnable fiber traits of cotton have been selected under domestication of an agronomically inferior wild ancestor, followed by millennia of human-mediated selection. We measured temporal expression alterations in the fiber cells of wild and domesticated cotton, using a microarray platform that interrogates 42,429 unigenes. The distribution of differentially expressed genes across developmental stages was significantly different, and the prolonged fiber growth in the cultivated form is associated with the up-regulation of profilin cell-wall structural genes. Profilins play prominent roles in cell-wall maintenance through actin sequestering and cytokinesis. Evidently, evolutionary variations in the profilin exon/intron architecture were accomplished by 'exon/intron-gain' and insertion/deletion during sequence-exonization. Further, ectopic expression of cotton GhPRF1 gene in tobacco resulted in the hyperactivation of the apical meristem and early flowering phenotype with increased flower number in contrast to the WT plants. The GhPRF1 transduced the key positive flowering regulator AP1 gene via coordinated expression of FT4, SOC1, FLC1 and FT1 genes involved in the apical-to-floral meristem signalling cascade. These results provide a novel temporal perspective on apical meristem determinacy and flower development adding to our understanding the importance of profilin structural genes in the regulation of important phenotypic traits during plant development.



## 267 - Plant DNA viruses: possible triggers of horizontal gene transfer events

**Emanuela Noris** <sup>(1)</sup> - **Marco Catoni** <sup>(2)</sup> - **Anna Maria Vaira** <sup>(1)</sup> - **Slavica Matic** <sup>(1)</sup> - **Laura Miozzi** <sup>(1)</sup> - **Gian Paolo Accotto** <sup>(1)</sup>

**National Research Council of Italy (CNR), Institute for Sustainable Plant Protection (IPSP), Torino, Italy** <sup>(1)</sup> - **School of Biosciences, University of Birmingham,, Birmingham, United Kingdom** <sup>(2)</sup>

The exchange of genetic material among unrelated species, known as horizontal gene transfer (HGT), has a prominent role in the evolution of living organisms. Viruses are potential triggers of HGT, as they introduce their genetic material into the host cells, are prone to recombine if mixed infections occur, and can colonize different hosts. The growing availability of host and viral genome sequences has allowed to identify gene flow events between viral and host genomes in different eukaryotic lineages, in either “virus-to-host” or “host-to-virus” direction. However, in spite of the proposed role of viruses in driving HGT in eukaryotes that can harbor the same viral pathogens, virus-mediated HGT events have not been monitored in real time yet. We reported rapid, spontaneous, and surprisingly efficient production of chimeric molecules composed of virus and plant DNA sequences during the infection of beet plants by a DNA virus of the Geminiviridae family, i.e. Beet curly top Iran virus (BCTIV). These hybrids can replicate and move systemically in plants with the help of the progenitor virus and are encapsidated within BCTIV virions. Since these chimeric elements containing beet plant sequences can also multiply in other plant species, where transcription of the beet-derived DNA can occur, we have documented the initial steps of a possible virus-mediated horizontal transfer of DNA between plant species. Mechanisms leading to the formation of these chimeric molecules and their gene transfer potential are currently under investigation.

## **268 - The comprehensive de novo annotation of Pack-TYPE transposons provides new insights into their importance for plant genome evolution**

**Jack Gisby<sup>(1)</sup> - Marco Catoni<sup>(1)</sup>**

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Pack-TYPE DNA transposons are plant genetic elements that can incorporate DNA from multiple chromosomal loci into their internal sequence. During transposition, excision events mediated by transposases allow them to relocate, capture and duplicate exons from host genes, providing an exceptional source of genetic variation in plants and contributing to evolution of new genes. Due to the variability of Pack-TYPE transposons DNA sequences, their annotation in plant genome is challenging. Previous research investigating these elements was mostly based on BLAST of terminal sequences and manual curation of terminal site duplications and internal protein hits. Therefore, we have developed an R software package, available through the Bioconductor project, for the automated annotation of Pack-TYPE transposons called packFinder. This package provides a standardised workflow for their annotation and analysis, with minimal setup and expertise required. Benchmarking of the tool demonstrates that it can annotate the majority of Pack-CACTA and Pack-MULE elements discovered in previous publications in addition to previously unknown elements, with a minimal false-positive rate. We used PackFinder to generate a comprehensive and accurate annotation of Pack-TYPE transposable elements in several plant genomes, and we will present new insight about the contribution of these elements to plant gene and genome evolution.

### **300 - Evolution and morphology of the anther glands in an early branching clade of papilionoids (Leguminosae-Papilionoideae)**

**Viviane Gonçalves Leite**<sup>(1)</sup> - **Simone Pádua Teixeira**<sup>(2)</sup> - **Vidal Freitas Mansano**<sup>(1)</sup>

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The ADA clade is an early-branching of Papilionoideae and consists of three subclades: Angylocalyceae, Dipterygeae and Amburanae. Its members can exhibit an unusual condition within Leguminosae that is the presence of anther glandular appendages. Thus, this study aims to check the distribution, morphology and evolutionary history of glandular appendages in the anthers of 33 species of the three subclades. Flower buds of dried specimens were processed for surface and anatomical analyses. In the Dipterygeae subclade the eight analyzed species of *Dipteryx* and three analyzed species of *Pterodon* exhibit an anther appendage with a secretory cavity. In *Taralea*, some species have a glandular appendage with a secretory cavity (*T. cordata*, *T. crassifolia* and *T. nudipes*) and others do not (*T. rigida*, *T. reticulata* and *T. oppositifolia*). In *Monopteryx* (*M. inpa* and *M. uacu*) no secretory cavity was found in the anther appendages but a phenolic apical tissue. A secretory cavity is also present in the anther appendage of the Amburaneae subclade species (*Cordyla madagascariensis*, *Myrocarpus frondosus*, *Myroxylon balsamum*, *M. peruiferum*) but absent in *Amburana acreana*, *A. erythrosperma*, *A. cearensis*, *Dussia tessmanni* and *Petaladenium urceoliferum*. In the Angylocalyceae subclade *Alexa gandiflora*, *A. superba*, *Angylocalyx pynaertii*, *A. talbotii* and *Castanospermum australe* no secretory cavity was found in the anther appendage. Our data suggest that a secretory cavity present in the anther appendages was acquired in the common ancestor of *Dipteryx* + *Pterodon* + *Taralea* and lost in some species of this last one. An apical phenolic appendage may be acquired in *Monopteryx*, which is sister group to the *Dipteryx*, *Pterodon* and *Taralea*. The presence of secretory cavity in the anther appendage is a condition distributed in the many species of the subclades Dipterygeae and Amburaneae (lost in some others) and absent in the subclade Angylocalyceae (CNPq, Fapesp, Capes).

**Keywords:** ADA clade, Angylocalyceae, Amburaneae, Dipterygeae, Fabaceae, Gland, Secretory structure.

## 462 - Preservation of genome integrity during male gametogenesis in *Physcomitrium patens*

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The extant bryophyte *Physcomitrium patens* serves as a model to study land plant evolution in general and evolution of reproduction in particular. Transposable elements (TEs) occupy up to a half of the *P. patens* genome and their activation particularly in reproductive organs has to be tightly controlled, since it could affect fertilization success and survival of offspring.

Germline TEs reactivation in plants was demonstrated by our group and others for the flowering plant model *Arabidopsis thaliana*. There, expression of TEs in the pollen vegetative nucleus precedes the formation of small interfering (si) RNAs, later accumulating in the sperm cells and thus reinforcing transposon silencing in sperm cells. Apparently, TEs in *A.thaliana* act as a double-edged sword: if their expression is compartmentalized, they may serve beneficial function.

Similarly to *A.thaliana* siRNAs, piRNAs are assigned TE silencing function in animal germlines. piRNA biogenesis is assisted by a group of Lotus domain containing proteins (LDCPs). Deletion of the LDCP genes, or genes coding for the proteins interacting with LDCPs, is known to cause sterility in animal models.

Taking together aforementioned evidence, we hypothesize that small RNA-based mechanisms of TE silencing in gametes may be analogous across different kingdoms of life and exploit similar protein domain repertoire. Our microarray data indicate activation of TEs in moss antheridia. In turn, a group of *P.patens* LDCPs, although not homologous to animal LDCPs, is overexpressed in antheridia and seem promising candidates for safeguarding the genome during gametogenesis in *P.patens*. We generated *Ppldcp3* mutants and observed fold times less production of sporangiophores in comparison with wild-type plants and no mutant spores were capable to germinate. We are seeking the mechanistic explanation of the observed phenomenon using advanced microscopy and omics approaches.

**472 - Transcriptome profiling of indole-3-butyric acid-induced adventitious root formation in *Olea europaea* L. – a focus in the induction phase**

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Adventitious root (AR) formation is a limiting step in vegetative propagation of some agronomical interesting woody-plant species. Olive (*Olea europaea* subsp. *europaea* L.) is one of the most important fruit trees for the agricultural economy of Mediterranean region, that comprises several cultivars with reduced ability for AR formation. Aiming to have a broader view into the molecular mechanisms underlying AR formation in olive, the transcriptomic changes that occur during the induction phase were analysed. In vitro growing plants of cv. 'Galega vulgar' were used to establish the rooting assays. The indole-3-butyric acid (IBA) was used as root induction factor. Samples were collected at 6, 24 and 72 hours post-induction (hpi) from IBA-treated and non-treated microshoots. Three biological repetitions were considered per timepoint and treatment. Truseq stranded mRNA and small RNA libraries were synthesized and further sequenced by using Illumina Paired-End reads and Illumina Single-reads 1x50bp, respectively. Trimmed mRNA reads were mapped against the *O. europaea* cDNA RefSeq. The edgeR package was employed to identify differential expressed genes (DEGs), and the KEGG Orthology-Based Annotation System (KOBAS) was used to perform a functional classification and pathway assignment of the up/down-regulated DEGs. Conserved mature miRNAs were found by BLAST trimmed sRNA sequences against miRBase (v. 21). An EDGE analysis of mature miRNAs was done to identify differentially expressed miRNAs. Potential target genes were identified through psRNATarget software. The achieved results show that AR formation in olive is a biological process whose complexity starts at very early stages (6 hpi), depending on multiple signalling and metabolic pathways, mostly related to oxidation-reduction process, regulation of transcription and auxin signalling pathway. In addition, transmembrane transport mechanisms appear as playing key roles during the induction phase. Regulation of gene expression through a mechanism mediated by miRNA was identified in genes involved in key pathways.

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## 510 - Integrating stomatal physiology and morphology: the evolution of stomatal control

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Stomata regulate the uptake of carbon dioxide (CO<sub>2</sub>) for photosynthesis and loss of water via transpiration. The balance of water-loss and CO<sub>2</sub>-uptake has played a key role in the evolution of plants, and will be increasingly important in a drier hotter world. The conductance of CO<sub>2</sub> and water vapour across the leaf surface is determined by stomatal morphology (the number, size, and spacing of stomatal pores) and physiology (the regulation of pore aperture). The proportion of the epidermis allocated to stomata and the evolution of amphistomaty are linked to the physiological function of stomata. Moreover, the relationship between stomatal density and [CO<sub>2</sub>] is mediated by physiological stomatal behaviour; species with less responsive stomata to light and [CO<sub>2</sub>] are most likely to adjust stomatal initiation. Many studies have investigated stomatal physiology or morphology in isolation, which may lose the 'overall picture' as these traits operate in concert to produce distinct mechanisms for stomatal control. Consideration of the interaction between stomatal morphology and physiology is critical to our understanding of plant evolutionary history, plant responses to climate change, the efficacy of the stomatal method to reconstruct palaeo-[CO<sub>2</sub>] and the development of more productive climate-resilient crops.

## **565 - Speciation history within the complex of European pine taxa**

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Closely related species that share their evolutionary history and background genetic variation are hard to delimit. However, such species are valuable as they offer unique insights in speciation mechanisms, maintenance of species integrity despite the homogenizing effect of gene flow and molecular signatures of adaptive divergence. Here, we aimed to disentangle the phylogenetic relationships of closely related pines from the *Pinus mugo* complex and *P. sylvestris* and examine the alternative scenarios of speciation within this group. Clear species delineation would greatly aid our understanding of divergence history at the genomic level and improve the search of regions under selection that may play a significant role in the maintenance of species integrity and local adaptation. We used sequence variation data at dozens of neutral genes, phylogeny reconstruction and a coalescent modelling framework to test the presence and magnitude of reticulation events in evolutionary history of pines. We found great overlap of neutral variation within *P. mugo* complex and signatures of multiple instances of interspecific gene flow during species divergence. We rejected pure hybrid speciation model for *P. uliginosa* and the best fitting model revealed *P. mugo* and *P. uncinata* as a sister species with basal *P. uliginosa* and asymmetric migration between them after divergence. The magnitude of interspecies gene flow differed greatly between species, and it was consistently stronger from representatives of *P. mugo* complex to *P. sylvestris* than in the opposite direction. The results indicate the prominent role of reticulation evolution in forest trees and provide a genetic framework to study species integrity maintained by selection and local adaptation.

**667 - A PHABULOSA-controlled genetic pathway regulates Ground Tissue patterning in the Arabidopsis root**

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In both animals and plants, development involves anatomical modifications. In the root of *Arabidopsis thaliana*, maturation of the Ground Tissue (GT) - a tissue composed by one cortex layer and one endodermis layer - is a paradigmatic example of these modifications. Indeed, during post-embryonic maturation, in a time window spanning from 7 to 14 days post germination (dpg), a second cortex layer, namely the Middle Cortex (MC), is generated by asymmetric cell divisions in the endodermis of about 80% of primary roots. As it reported, the cell cycle regulator CYCLIN D6;1 (CYCD6;1) plays a central role in this process, as its accumulation in the endodermis triggers the formation of MC. The phytohormone gibberellin (GA) is a key regulator of the timing of MC formation, as alterations in its signalling and homeostasis result in precocious endodermal asymmetric cell division. However, little is known on how GA signalling and homeostasis are regulated during GT maturation. Here, we show that the HOMEODOMAIN LEUCINE ZIPPER III (HD-ZIP III) transcription factor PHABULOSA (PHB) is a master regulator of MC formation, controlling the accumulation of CYCD6;1 in the endodermis in a cell non-autonomous manner. We show that PHB activates the GA catabolic gene GIBBERELLIN 2 OXIDASE 2 (GA2ox2) in the vascular tissue, thus regulating the stability of the DELLA protein GIBBERELLIN INSENSITIVE (GAI) - a GA signalling repressor - in the root and, hence, CYCD6;1 expression in the endodermis.



## TOPIC:

### Plant metabolism and bioactive compounds

#### **Keynote Lecture**

**Crop improvement for healthy diet. Tomato as a model system.**

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A unique aspect of the rich color diversity of plants is that many of the pigments involved are also important nutrients. However, the so-called “eat right with color” is not always a good standard for judging food’s nutritional value. A great advance in our knowledge of the metabolic pathways of pigments, such as carotenoids and flavonoids, has been made recently. This knowledge needs to be extended to plant breeders so that nutrient-rich vegetables and cereals can be selected. In the present lecture, the tomato plant will be used as a model to integrate fundamental knowledge of plant physiology and biochemistry and its extension to plant improvement. In addition to this basic knowledge, new genetic manipulation techniques, such as gene editing and “de novo” domestication, will help in designing more nutritious crops.

## **Oral Communications**

### **22 - Why are some sugars toxic to plants?**

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Plants use a number of different nucleotide sugars as building blocks for cell wall polymers or glycoproteins. During germination, development, growth or under stress, plants remodel for instance the cell wall and thereby releases sugars, which are taken up into the cytosol. In contrast to animals, which often secret such sugars, plants have established a powerful recycling system to reactivate sugars to nucleotide sugars. Recycling of sugars involves two steps: first a sugar specific 1-kinase and second a universal sugar pyrophosphorylase (USP), which accepts many different sugar-1-phosphates. The enzymes involved in the recycling process are tightly controlled in their activity. A knockdown in USP leads to dwarf plants and a knockout in USP is lethal. Some mutants in sugar-1-kinases render plants sensitive to a particular sugar, a phenomenon termed sugar toxicity. By using physiological and molecular approaches, we dissect sugar toxicity to understand the mechanisms. Here we present data on L-arabinose and D-galactose toxicity as a typical example.

**61 - Molecular characterization of delta12-fatty acid acetylase and delta12-oleate desaturase in the polyacetylene biosynthetic pathway from medicinal herb *Bidens pilosa***

**Kin-Ying To<sup>(1)</sup> - Po-Yen Chen<sup>(1)</sup> - Mi-Jou Hsieh<sup>(1)</sup> - Yung-Ting Tsai<sup>(1)</sup> - Hsiao-Hang Chung<sup>(2)</sup> - Lie-Fen Shyur<sup>(1)</sup> - Cheng-Han Hsieh<sup>(1)</sup>**

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*Bidens pilosa* is an erect annual plant that is commonly used as an herbal tea component or traditional medicine for treating various disorders including diabetes. To date, around 200 secondary metabolites have been identified from *B. pilosa* including polyacetylenes, flavonoids, phenylpropanoids and terpenes. Polyacetylenes have two or more carbon-carbon triple bonds or alkynyl functional groups mainly derived from fatty acid and polyketide precursors. Here we report the cloning of full-length cDNAs encoding  $\Delta^{12}$ -fatty acid acetylase (designated as BPRFAA) and  $\Delta^{12}$ -oleate desaturase (designated as BPROD), which we predicted to play a role in the polyacetylene biosynthetic pathway, from *B. pilosa*. Subsequently, 4 expression vectors including pBPRFAA, pBPROD, pOD-RNAi and pControl (empty vector) were constructed and transformed into *B. pilosa* via the *Agrobacterium*-mediated method. Over 10 putative transgenic lines were obtained from each construct. Genomic PCR analysis confirmed the presence of transgene and selection marker gene in the obtained transgenic lines. Southern hybridization indicated the T-DNA insertion in some transgenic lines was single copy. Furthermore, 4 to 5 FAA genes and 2 to 3 OD genes were detected in wild-type (WT) plants. Quantitative real time-PCR revealed that FAA1, FAA4, FAA14, FAA15, OD1 and OD5 had higher expression levels than the WT plants. Western blot analysis revealed the OD protein expression in selected transformants. High-performance liquid chromatography profiling was used to analyze the 7 index polyacetylenic compounds and fluctuation patterns were found. Furthermore, stable inheritance of the transgene was clearly demonstrated in selected transformants by progeny assay.

## **191 - Tonoplast cytochrome b-561 controls ascorbate homeostasis in Arabidopsis plants exposed to high light**

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Ascorbate is a water-soluble antioxidant which fulfills manifold functions in plants. As a substrate of peroxidases and oxidases, it plays essential roles in redox signalling and ROS scavenging. As a substrate of violaxanthin de-epoxidase, it is involved in thermal dissipation in high light. Ascorbate is also important in stabilizing 2-oxoglutarate-dependent dioxygenases involved, among the others, in hormones and anthocyanins biosynthesis. Not surprisingly, ascorbate is present at millimolar concentrations in virtually any cell compartment, including the apoplast, though little is known about its transport. Enzymatic systems that keep ascorbate reduced are known in the cytosol, chloroplasts, peroxisomes and mitochondria, but apparently absent in vacuoles and the apoplast. Here we show that a transmembrane cytochrome residing on the tonoplast controls ascorbate homeostasis in Arabidopsis plants exposed to high light.

Cytochromes b-561 are transmembrane electron-transport proteins with two ascorbate binding sites facing opposite sides of the membrane. Among the four cytochromes b-561 of Arabidopsis, one is localized on the tonoplast (TCytb). We performed patch-clamp recordings on large vacuoles isolated from Arabidopsis mesophyll cells, which clearly showed that application of ascorbate on the cytosolic side elicited measurable currents in the presence of the artificial electron acceptor ferricyanide on the luminal side, and viceversa. Moreover, monodehydroascorbate could act as the physiological electron acceptor in the cytosol, when ascorbate was present in the vacuole. These are the first electron current recordings ever reported for plant membrane redox systems in native conditions. Ascorbate-elicited currents were absent in vacuoles isolated from tcytb ko-mutants suggesting that vacuolar and cytosolic ascorbate pools could equilibrate through TCytB. The tcytb ko-mutants showed distinct ascorbate-related phenotypes when exposed to excessive illumination, including exaggerated ascorbate and anthocyanins accumulation in leaves and delayed flowering. All results point to a role of the cytochrome b-561 TCytB in controlling ascorbate homeostasis in plants.

## 240 - The Multifaceted Networks Regulating Suberin Metabolism in Plants

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Lipophilic barriers allow plants to thrive in terrestrial habitats as they minimize their water loss, facilitate exchange of gases and solutes, and protect them against environmental cues. Suberin, one such barrier, is a heteropolymer consists of polyaliphatic and polyphenolic domains. The spatial and temporal coordination of both domains is essential for the proper establishment of a complete suberin lamellae. The polyaliphatic domain mainly composed of a mixture of very-long-chain C20 to C26 fatty acids, primary alcohols and glycerol; while the polyaromatic is mostly made up of hydroxycinnamic acid derivatives derived through the phenylpropanoid pathway. Suberin forms a typical lamellar structure deposited in-between the plasma membrane and the cell wall of specialized tissues as tree bark, seed coat, root endodermis cell layer, skin of potato tubers, and russeted and/or reticulated fruit species like apple, pear, quince, and melon. Here, we present two cases of novel regulatory networks involved in suberin biosynthesis. The first describes the functional characterization of SUBERMAN, a novel MYB-type transcription factor that initiates the deposition of suberin lamellae sealing the cell walls of endodermis cells in the Arabidopsis root. We demonstrate that roots with altered SUBERMAN expression exhibit substantial changes in their suberin profiles accompanied by modified transcriptional programs associated with metabolism of phenylpropanoids, suberin, lignin and cuticular lipids, as well as those related to root transport activities and cell wall modification. The second case designates the revealing of regulatory metabolic networks involved in suberization processes accompanying the establishment of unique reticulated structures decorating the skin of melon fruit varieties. The multilevel investigation of structural attributes, chemical composition, and gene expression profiles on a set of reticulated skin melons provide important insights into the molecular and metabolic bases of fruit reticulation and its association with suberization processes.

## 527 - Localization of phenolic compounds in barley leaves can be modulated by irradiance and CO<sub>2</sub>

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Phenolic compounds (PhCs) present in barley (*Hordeum vulgare*) leaves are a key factor in enhancing plant tolerance to stress. Environmental stress is often accompanied by an increase in reactive oxygen species (ROS) and thus, oxidative stress. PhCs reduce the presence of ROS by screening UV irradiation and acting as antioxidants. Here we examine the accumulation and localization of phenolic compounds in two barley varieties with different levels of tolerance to oxidative stress. Plants were grown in either low or high irradiance levels and exposed to varying levels of [CO<sub>2</sub>]. We present a novel method for measuring the presence of phenolic compounds in leaves using fluorescence microscopy and image analysis, in addition to liquid chromatography coupled with mass spectroscopy detection. Results confirm that high intensity irradiation has a strong effect on the accumulation of PhCs and further show that elevated [CO<sub>2</sub>] provides a similar effect in terms of PhC-localization and accumulation. Under high irradiation and elevated [CO<sub>2</sub>], PhCs localize near leaf surfaces, while under low irradiance conditions, a greater allocation of PhCs is seen deeper in the mesophyll. Overall, the mesophyll cells had a greater response to environmental input than the epidermal cells. Different profiles of PhCs were seen between the two barley varieties, with the oxidative-sensitive variety accumulating mainly hydroxybenzoic acids and the oxidative-tolerant variety accumulating hydroxycinnamic acids. This may suggest that hydroxycinnamic acids play a more prominent role in preventing or ameliorating oxidative stress in barley.

## TOPIC:

### Plant metabolism and bioactive compounds

#### ***Extended Elevator Pitches***

**184 - Inside of allelopathy of invasive Japanese and Bohemian knotweed: rhizome extracts stimulate programmed cell death and affect cell structure and function in young radish roots**

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Allelopathy serves as an important strategy for successful dominance of invasive plants. Once the allelopathic compounds are released in the soil, the production of reactive oxygen species is strongly increased in the nearby plants. While the growth suppression is a well-known effect of the exposure to the allelochemicals, little is known about the cellular mechanisms behind it. Therefore, the aim of our study was to analyze ultrastructural changes and programmed cell death in the exposed roots of seedlings.

Radish (*Raphanus sativus*) seeds were treated with aqueous extracts of Japanese knotweed (*Fallopia japonica*) and Bohemian knotweed (*F. xbohemica*) rhizomes with concentrations of 1% and 10% (w/v). Morphological (length of root and shoot, biomass) and oxidative stress related parameters (total antioxidative capacity, lipid peroxidation via the content of malondialdehyde, localization of H<sub>2</sub>O<sub>2</sub>) were analyzed. Cellular changes in root tips were characterized with light and transmission electron microscope. Activity of the programmed cell death related enzymes was measured fluorometrically using substrates for proteases with caspase- and metacaspase-like activities.

Our study showed that knotweed extracts caused the concentration-dependent significant increase of total antioxidative capacity, a slight increase of malondialdehyde content and stimulated accumulation of H<sub>2</sub>O<sub>2</sub>. These changes might be the reasons for strong reduction of radish root length recorded in our study. Knotweed extracts affected especially the structure of root cap and root apical meristem. Cells were of abnormal shape or even disintegrated and several organelle changes occurred, especially evident in mitochondria and endoplasmic reticulum. The ultrastructural signs of programmed cell death were in correlation with the activities of proteases with caspase-like activities, which were significantly higher in roots of treated seedlings. Understanding these allelopathic mechanisms is of high importance in managing knotweeds and other plant invaders.

## **452 - Heterologous expression of cyanobacterial Orange Carotenoid Protein (OCP2) as a soluble carrier of ketocarotenoids in *Chlamydomonas reinhardtii***

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Photosynthetic organisms evolved different mechanisms to protect themselves from high irradiances and photodamage. In cyanobacteria, the photoactive orange carotenoid protein (OCP) acts both as a light sensor and quencher of excitation energy. It binds ketocarotenoids and, when photoactivated, interacts with phycobilisomes, thermally dissipating the excitation energy absorbed by the latter, and acting as efficient singlet oxygen quencher. Here, we report the heterologous expression of an OCP2 protein from the thermophilic cyanobacterium *Fischerella thermalis* (FtOCP2) in the model organism for green algae, *Chlamydomonas reinhardtii*. Robust expression of FtOCP was obtained through a synthetic redesigning strategy for optimized expression of the transgene. FtOCP2 expression was achieved both in a strain previously selected for efficient transgene expression (UVM4) and in a bkt background, characterized by the constitutive expression of an endogenous  $\beta$ -carotene ketolase, normally poorly expressed in this species, resulting into astaxanthin and other ketocarotenoids accumulation. Recombinant FtOCP2 was successfully localized into the chloroplast. Upon purification it was possible to demonstrate the formation of holoproteins with different xanthophylls and ketocarotenoids bound, including astaxanthin. Moreover, isolated ketocarotenoid-binding FtOCP2 holoproteins conserved their photoconversion properties. Carotenoids bound to FtOCP2 were thus maintained in solution even in absence of organic solvent. The synthetic biology approach herein reported could thus be considered as a novel tool for improving the solubility of ketocarotenoids produced in green algae, by binding to water-soluble carotenoids binding proteins.



## TOPIC:

### Plant metabolism and bioactive compounds

#### Posters

**613 - Characteristic and unique methyl jasmonate induced volatile defense responses in the ancient plant *Selaginella martensii***

**Tana Wuyun** <sup>(1)</sup> - **Lu Zhang** <sup>(2)</sup> - **Hanna Hõrak** <sup>(3)</sup> - **Eero Talts** <sup>(1)</sup> - **Bin Liu** <sup>(1)</sup> - **Ülo Niinemets** <sup>(1)</sup>

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Methyl jasmonate (MeJA) induces a series of defense responses in higher plants, but less information is available for evolutionarily old plants. We studied exogenous MeJA responses of the spikemoss *Selaginella martensii* that has diverged from the common ancestor of land plants 400 million years ago and although jasmonate pathway is present in spikemosses, its activation and regulation is poorly known. MeJA concentrations between 10 mM and 50 mM were used to study quantitative relationships between the induction of volatile organic compounds (VOCs) and MeJA doses. High-resolution proton transfer reaction time-of-flight mass spectrometer (PTR-TOF-MS) and gas-chromatography coupled to mass-spectrometric detection (GC-MS) were used to identify and monitor the kinetics and magnitude of release of specific VOCs. Comparative transcriptome analysis was done to reveal the underlying molecular defense mechanisms. We found that *S. martensii* was relatively insensitive to MeJA treatment compared to higher plants, yet it emitted a characteristic and unique VOC blend in a dose-dependent manner. Similar to several other ancient plants, *S. martensii* genome includes special microbial terpene synthase-like genes (MTPSLs) and the expression of these genes was related to the MeJA-driven change in the VOC emission. The quantitative MeJA dose vs. emission relationships in the ancient plant *S. martensii* together with modifications in underlying gene activities advance the understanding of the evolution of MeJA-dependent defenses.

## **621 - Black sapote (*Diospyros digyna* Jacq): phytochemical characterization and antioxidant properties of seed, pulp and peel extracts**

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Black sapote (*Diospyros digyna* Jacq.), called also 'black persimmon' or 'chocolate pudding fruit', is a tropical fruit belonging to the Ebenaceae family. Despite the fruit is commonly consumed in subtropical regions, it may be mistakenly considered unattractive by European consumers due to its particular aspect. Since black sapote is a climacteric fruit that becomes rapidly perishable after harvesting, its use as food is almost exclusively limited to the countries of origin. Moreover, the optimal transport and storage conditions are not actually well known and the local production in European area may be the only alternative to preserve fruit quality. Thanks to the climate changes that affected our planet during the last century, some Italian geographical areas have developed subtropical-like climatic conditions suitable for the cultivation of tropical fruits also in Europe.

The aim of this work is the evaluation of the phytochemical profile and antioxidative properties of extracts from peel, pulp and seed of *Diospyros digyna* fruits grown in Sicily. UV-Vis assays (Folin-Ciocalteu and BL-DMAC) revealed the presence of high amount of antioxidant compounds. HPLC-DAD-ESI-MS/MS analysis identified 29 different compounds distributed between the edible and non-edible parts. Cellular Antioxidant Assay showed that the extracts prevented lipid peroxidation in HepG2 cells with higher efficacy of peel and pulp with respect to seed extracts. Moreover, our results suggested that the observed antioxidant protection involved both redox active properties (as measured by ABTS, DPPH and FRAP assays) of the extracts and up-regulation of genes encoding for antioxidant enzymes (MnSOD, CuZnSOD, GPx and CAT) in cells as evaluated by qRT-PCR. Taken together, our data provided evidence on the potential of *D. digyna* pulp to be consumed as food, and on the possible valorisation of its main agronomic by-products, such as peel and seeds, for nutraceutical preparations.

**631 - Lights and shades in the way for cannabinoids biosynthesis: a focus on the variability of THCAS-like genes and their possible involvement in the chemical phenotype of Cannabis sativa L.**

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Cannabis sativa L. has long been cultivated for its pharmaceutical significance due to the accumulation of phytocannabinoids in female inflorescences, and is now emerging as a low input alternative crop for its promising use in several industries.

Its predominant compounds are THCA and CBDA, followed by CBGA and CBCA, which accumulate at different extents depending on the strain and growth stage.

Despite years of breeding aimed at eliminating narcotic potential from industrial varieties, some consistently accumulate traces of residual THCA in their inflorescences, often to levels close to 0.20% dry weight, the limit set by E.U. for granting subsidies.

Although the cannabinoid synthases have been characterized enzymatically, many molecular aspects remain to be clarified.

Besides the presence of functional rather than non-functional THCA and CBDA synthase genes, resulting in the accumulation of THCA and/or CBDA, or in the prevalence of CBGA when both non-functional forms are present in the genome, there are some sequences related to the THCAS genes and therefore named THCAS-like, the function of which is still controversial. In this work, complete THCAS, CBDAS and THCAS-like coding sequences have been isolated from thirteen genotypes consisting in either hemp or medical Cannabis varieties. A set of highly specific primer pair was developed and used to analyze their transcriptional profiles in Cannabis inflorescences, also characterized for cannabinoids content by HPLC-UV. Based on results, hypotheses are given to explain the presence of low THCA levels in hemp varieties that do not express any functional THCAS gene.

**636 - Acylserotonins – a new class of plant lipids with antioxidant activity and potential pharmacological activities**

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During analysis of components of baobab seed (*Adansonia digitata*) oil, several new fluorescent compounds were detected in HPLC chromatograms that were not found previously in any seed oils investigated by our group. After preparative isolation of these compounds, structural analysis by NMR, UHPC-HR-MS, GC and spectroscopic methods was applied and allowed identification of these compounds as series of N-acylserotonins containing saturated C22 to C26 fatty acids with minor homologues of C27 to C30. The main homologue was N-lignocerylserotonin and the content of odd-C atom number fatty acids was unusually high among the homologues. The suggested structure of the investigated compounds was additionally confirmed by their chemical synthesis. Synthetic N-acylserotonins showed pronounced inhibition of membrane lipid peroxidation of liposomes prepared from chloroplast lipids, especially when the peroxidation was initiated by a water-soluble azo-initiator. Comparative studies of the reaction rate constants of the N-acylserotonins and tocopherols with a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in solvents of different polarity revealed that N-acylserotonins showed similar activity to  $\delta$ -tocopherol in this respect. The described compounds have been not reported before either in plants or in animals. This indicates that we have identified a new class of plant lipids with antioxidant activity that could have promising pharmacological activities.

## 649 - Characterization of a novel algal saprophyte with degrading activity towards *C.vulgaris* cell wall

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Microalgae have emerged as a renewable source for biofuel as well as pharmaceutical and nutraceutical products. However, several factors such as low productivity, expensive harvesting procedures and difficult metabolite extractability limit their full utilization at industrial scale. Similar to the successful employment of enzymatic arsenals from lignocellulolytic fungi to convert lignocellulose into fermentable sugars for bioethanol production, likewise specific algalytic formulations could be used to improve the extractability of lipids from microalgae to produce biodiesel. Currently, the research areas related to algivorous organisms, algal saprophytes and the enzymes responsible for the hydrolysis of algal cell wall are still little explored. Here, an algal trap method for capturing actively growing microorganisms was successfully used to isolate a filamentous fungus, that was identified by whole genome sequencing, assembly and annotation as a novel isolate. The fungus was able to assimilate heat-killed *Chlorella vulgaris* cells and its enzymatic arsenal was identified by nanoLC-MS/MS. The culture filtrate contained a diverse array of enzymes: proteases and exo-glycosidases. The treatment of *C. vulgaris* with the filtrate of the fungus improved the release of chlorophylls and lipids from algal cells by 42.6% and 48.9%, respectively. The improved lipid extractability from *C. vulgaris* biomass treated with the fungal filtrate highlights the potential of algal saprophytes in the bioprocessing of microalgae for the sustainable production of biofuel or bioactive compounds.

## **10 - Profiling the Volatile Metabolome in Pear Leaves with Different Resistance to the Pear Psylla *Cacopsylla bidens* (Šulc) and Characterization of Phenolic Acid Decarboxylase**

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Pear Psylla is the most important pest of pear in all pear-growing regions, in Asian, European, and the USA. Pear psylla damages pears in several ways: High-density populations of these insects can cause premature leaf and fruit drop, diminish plant growth, and reduce fruit size. In addition, their honeydew promotes sooty mold on leaves and russetting on fruit. Pear psyllas are also considered vectors of pear pathogens such as *Candidatus Phytoplasma pyri* causing pear decline that can lead to loss of crop and tree vigor, and sometimes loss of trees. Psylla control is a major obstacle to efficient Integrated Pest Management.

Recently we have identified two naturally resistance pear accessions (Py.760-261 and Py.701-202) in the Newe Ya'ar live collection. GC-MS volatile metabolic profiling identified several volatile compounds common in these accessions but lacking, or much less common, in a sensitive accession, the commercial Spadona variety. Among these volatiles were styrene and its derivatives. When the resistant accessions were used as inter-stock, the volatile compounds appear in commercial Spadona scion leaves and it showed reduced susceptibility to pear psylla. Laboratory experiments and applications of some of these volatile compounds were very effective against psylla eggs, nymphs and adults.

The genes and enzymes involved in the specific reactions that lead to the biosynthesis of styrene in plant are unknown. We have identified a phenolic acid decarboxylase that catalyzes the formation of p-hydroxystyrene, which occurs as a styrene analog in resistant pear genotypes. The His-tagged and affinity chromatography purified *E. coli*-expressed pear PyPAD1 protein could decarboxylate p-coumaric acid, and ferulic acid to p-hydroxystyrene and 3-methoxy-4-hydroxystyrene. In addition, PyPAD1 had the highest activity towards p-coumaric acid.

Expression analysis of the PyPAD gene revealed that its expressed as expected, i.e. high when styrene levels, and psylla resistance were high.

## **26 - Ethylene and auxin regulation on aroma pathways during tomato (*Solanum lycopersicum* L.) fruit ripening**

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As knowledge of the role of auxin and ethylene-auxin interaction in climacteric fruits ripening has been highlighted, the hormonal regulation of secondary metabolism changes in climacteric fruits, including those related to aroma compounds biosynthesis, needs to be better understood. In the present study, the influence of auxin and ethylene-auxin interaction on the volatile organic compounds (VOC) metabolism was evaluated during tomato (*Solanum lycopersicum* L.) fruit ripening. Tomato fruits cv. MicroTom and Cherry at mature green stage were randomly separated into four groups according to hormonal treatments: CTRL (without treatment); ETHY (ethylene treatment); IAA (indole-3-acetic acid treatment); ETHY+IAA (both hormones treatment). Color shift and ethylene emission were daily determined. VOC profiles and transcription of genes related to their synthesis were analyzed in specific ripening stages. The results showed that color shift and ethylene emission were accelerated by ethylene and delayed by auxin treatment. ETHY+IAA fruits shown a delay of both parameters in the first days after harvest, however, the climacteric peak was advanced in relation to the CTRL. At breaker stage, MicroTom IAA fruits presented the most different VOC profile when compared to the others. ETHY+IAA fruits presented profiles more similar to ethylene-treated fruits. Cherry fruits treated with ETHY+IAA presented VOC profiles closer to IAA treated fruits, while ETHY treated fruits presented similar profiles to CTRL fruits. At red stage, MicroTom and Cherry treated with ETHY+IAA showed a closer profile to IAA treated fruits, suggesting that auxin effects overlap ethylene effects. VOC profiles of ETHY treated fruits were more similar to the profiles of the CTRL fruits. The transcription of genes related to the synthesis of important tomato volatile compounds, such as LoxC, evidenced their regulation by both hormones. As conclusion, ethylene and auxin regulate specific VOC pathways with consequences on specific volatile levels, impacting on tomato aroma formation during ripening.

#### **45 - Single-cell type study of ‘mustard oil bomb’ in plant immunity**

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Glucosinolates (GLS) are one of the most extensively studied specialized metabolites in Brassicales, which comprises of specialized chemical defense mechanism known as ‘mustard oil bomb’, aka glucosinolate-myrosinase system. This system protects against herbivore and pathogen attack when glucosinolates are hydrolyzed by myrosinases to release toxic degradation products. Glucosinolate profiles have been well-studied in seeds, leaves, flowers, siliques, roots, and different plant parts of Arabidopsis, but single single-type specific studies in guard cell, mesophyll cells, trichomes, and epidermal cells are not well documented. The objective of this study is to identify differential distribution of glucosinolate-myrosinase system at single cell-type resolution. The single-cell types generated from the same tissue are rare, and they provide important information on what takes place at cellular resolution. According to our metabolomics data, the glucosinolate profiles of guard cells (GC) differ from leaves and mesophyll cells (MC). These differences may be due to the different roles played by different glucosinolates in the specific cell-types, indicating glucosinolates have undiscovered roles in guard cells. To investigate the specifics of the biochemical pathway of glucosinolates, we used myrosinase-overexpressing mutants with a distinct phenotype. Detailed analyses indicated the GLS profiles in leaves and MC were similar, but different from GCs. We then tested GLS profiles in GC and MC responses to plant pathogen treatments. The results have provided insight into how the “mustard oil bomb” plays a role in plant immunity. This study has opened a new direction toward thorough understanding of how the metabolomic signatures are related to plant defense phenotypes at the single cell resolution.



## 60 - Plasmopara viticola infection impeded by Origanum vulgare vapour through its immunity priming potential in Vitis Vinifera

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The amount of synthetic pesticides applied in viticulture is relatively high compared to other agricultural crops, due to the high sensitivity of the grapevine (*Vitis vinifera* L.) to fungal diseases such as downy mildew (*Plasmopara viticola*). Alternatives to reduce fungicides are utterly needed to ensure a sustainable vineyard-ecosystems and consumer acceptance.

Essential oils (EOs) are amongst the most promising natural plant protection products due to their antibacterial, antiviral and antifungal properties. However, the efficiency of EOs depends highly on timing and method of application and the molecular interactions of host, pathogen and EO, which underlie the efficiency of EOs, is not understood. To circumvent the drawbacks of a direct application, the presented study aimed a) to evaluate whether a continuous fumigation of EO can control downy mildew and b) to decipher molecular mechanisms that are triggered in host and pathogen by EO application.

Therefore, we customized a climatic chamber, which permitted a continuous fumigation of potted vines with different EOs. Several experiments with vines, infected with *Plasmopara viticola* and subsequently exposed to continuous fumigation of different EOs with different concentrations and application times were conducted. Experiments were stopped when signs of infections were clearly present on the control after sporulation was induced. Strikingly oregano oil vapor treatment reduced downy mildew development to 95%. RNA analysis for differentially expressed genes yielded in a total of 4800 EO modulated transcripts in vines. Strikingly many genes linked to the plant immune system were triggered by EO vapour (ethylene synthesis, phenylpropanoids and flavonoid synthesis), which indicates for the first time, that the antifungal efficiency of EO is mainly due to the priming of resistance pathways inside the host plants. These results are of major importance for the production and research on biopesticides, plant stimulation products as well as for resistance breeding strategies.

**Keywords:** Plant defense; essential oil; *Plasmopara viticola*; Grapevine

**86 - Impact of light-emitting diode irradiation on the regulation of nitrogen balance, biomass and pharmacologically important alkaloids accumulation in medicinal plants *Catharanthus roseus* G.Don**

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*Catharanthus roseus* is an important medicinal plant which accumulates pharmacologically significant terpenoid indole alkaloids (TIA). TIA of *C. roseus* includes more than 130 substances. Major TIA of leaves are diuretic vindoline and catharantine. Ajmalicine are used for arterial hypertension treatment. Vinblastine and vincristine are well known as antineoplastic drugs used in chemotherapy of cancer diseases. Vinblastine and vincristine are the most valuable among *C. roseus* TIA.

The ability to create the special light spectrum with LEDs provides the ecologically friendly possibility to manipulate plant morphology. In our study *C. roseus* plant was a model crop for studying development regulation, dry biomass accumulation and TIA biosynthesis under experimental LED lighting. *C. roseus* plants were grown under LL and LED lighting with a PPFD 200  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  or 500  $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ . LED lighting systems generated Photosynthetically Active Radiation (PAR) with six different spectrum energy distribution and wavelength ratios in physiologically important wavelength ranges (Red/Blue, Red/Green and Red/Far-Red ratios)

It was shown that LED lighting had its greatest effect on vindolin and catharantine accumulation as well as Nitrogen Balance Indices in *C. roseus* plants when an intermediate Red/Blue ratio was applied. If Red/Blue ratios were too high or too low, alkaloid accumulation was reduced. Thus, the index can be considered as an indicator of the biosynthetic activity of alkaloids. One of the LED lighting treatment increased the yields of ajmalicine in leaves and roots about five- to sixfold in comparison to LL control. In the same time vinblastine lightly increased in leaves of *C. roseus* plants under LED with intermediate Red/Blue ratio in comparison to other LED treatment. On the other hand It was found the best LED lighting to stimulate vinblastine accumulation. We proposed that the stimulation the levels of dimerics alkaloids under this treatment was due to an increase of H<sub>2</sub>O<sub>2</sub>-peroxidase activity.

## 91 - An unusual chemical signature in Asteraceae pollen coat underpinned by the neofunctionalization of BAHD acyltransferases

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Evolution within the plant kingdom is highly correlated with the apparition of new metabolic pathways, and notably those leading to synthesis of the so-called specialized metabolites. Indeed, their great diversity confers to plants the ability to adjust to their environment. Evolution of the reproductive structures was a key element during terrestrialization and emergence of pollen was a major event as well as the appearance of pollen coat among Angiosperms. Pollen coat assumes a wide range of functions such as protection against biotic or abiotic stresses, stigma-pollen recognition mechanisms or pollinator attraction but nonetheless, with a variable chemical composition depending on species. However, tri-substituted spermidines are specific phenolamides highly conserved in the Eudicots pollen coat. Still, to date, no clear function was assigned to these compounds. Nevertheless, their maintenance since appearance in pollen coat suggests their major significance in evolution of the green lineage. Otherwise, phenolamides constitute a class of specialized metabolites resulting from the conjugation of amines with phenolic acids that have been widely studied for their involvement in development, reproduction or biotic stress tolerance. Concerning synthesis of spermidine derivatives in pollen coat, it has been attributed to acyltransferases belonging to the BAHD family that catalyze acyl-CoA dependent acylation. Characterized enzymes from *Arabidopsis thaliana* or *Malus domestica* were up to now the only representatives of enzymes with ability to catalyze acylation of secondary amino-group of an aliphatic polyamine. Here, we identified new pollen coat phenolamides specific to the Asteraceae family that differ from the usual spermidine derivatives. In chicory (*Cichorium intybus* L.), we cloned and characterized two genes encoding BAHD acyltransferases involved in the metabolic diversification observed in this Asteraceae family. Further insights related to these new metabolites could give additional clues on the role of pollen coat phenolamides in regard to plant evolution.

## 142 - Oxylipins formed during the lipoxygenase pathway

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Oxidized derivatives of polyunsaturated fatty acids (PUFAs) - oxylipins - are an important class of bioactive molecules involved in the processes of plant growth and development. They play an essential role both in the responses against biotic/abiotic stresses. Among the enzymatic pathways, the formation of oxylipins via the lipoxygenase pathway is one of the main one. Oxylipins formed during this pathway include the well-known jasmonates and their derivatives, as well as many other compounds such as hydroperoxy-, hydroxy, oxo- and epoxy derivatives of fatty acids, divinyl ethers, volatile aldehydes, etc. The first step in biosynthesis is catalyzed by the lipoxygenase generating either 9- or 13-hydroperoxide from PUFAs such as linoleic and alpha-linolenic acids. These derivatives serve as a substrate for enzymes of the CYP74 family (P450 superfamily): allene oxide synthases (AOSs), hydroperoxide lyases (HPLs), divinyl ether synthases (DESs), and epoxyalcohol synthases (EASs). We have identified novel oxylipins and unusual enzymes of their biosynthesis in different plants. Among them are the first 13-specific DESs (RaDES (*Ranunculus acris*), SmDES1, SmDES2 (*Selaginella moellendorffii*)), the unique double function enzymes and the first 9-AOS member of the CYP74B subfamily (CYP74B33, *Daucus carota*), whereas all other members of this subfamily are 13-HPL, responsible for the formation of "green note" C6-aldehydes and C12-aldoacids. The unusual properties of CYP74B33 are 1) its 9-specificity, unlike most 13-AOS, and 2) the ability to produce a significant amount of cyclopentenones along with  $\alpha$ -ketols. We have also showed the first examples of the complete conversion of AOS to HPL and EAS, HPL to EAS as well as DES to AOS by site-directed mutagenesis. Some more mutations led to changes of the CYP74s catalytic activities in different ratios: from partial to almost complete. The work was supported by grant 20-04-01069 from the Russian Foundation for Basic Research and MK-903.2020.4.

**162 - Bioactive molecules with antioxidant and hepatoprotective attributes resulted through green extraction of herbs originated from Romanian spontaneous flora**

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Medicinal plants of the spontaneous flora are continuously studied for potential health benefits, industrial applications and optimum processing techniques. Dandelion, mulberry and chicory already have a history in being used as key ingredients in making teas, salads, coffee substitutes, and refreshing beverages. Over the last years, various in vitro and in vivo studies have concluded that such plant extracts are beneficial in managing non-alcoholic liver diseases, diabetes and cardiovascular disease.

Currently, the research efforts are aiming at using renewable plant sources of bioactive compounds which can help in developing functional products with a positive impact on public health concerns. Given the abundance of these plants in the spontaneous flora combined with the industry's small interest in their therapeutic potential, we decided to study the bioactive compounds of dandelion (*Taraxacum officinale* L.) leaves and roots, mulberry (*Morus* L.) leaves, and chicory (*Cichorium intybus* L.) roots using green extraction methods.

The selected plant parts were subjected to alcoholic and acetic fermentation in apple cider and the obtained tinctures were tested for their chemical composition. The experimental results revealed that preparing tinctures of dandelion, mulberry, and chicory using apple cider as a solvent lead to a bioactive extract of polyphenols and polysaccharides. The identified compounds are listed with various benefits in the management of liver diseases, diabetes and cardiovascular disease given their reported antioxidant, anti-inflammatory, hypolipidemic, and hepato-protective attributes.

The green extraction of bioactive compounds from medicinal herbs represents an opportunity for researchers to develop functional products with valuable therapeutic impact on various diseases, therefore, further similar studies are welcomed to contribute to the general aim of improving one's health status and quality of life.

**Keywords:** apple cider, bioactive compounds, chicory, dandelion, green extraction methods, mulberry, tinctures

## 178 - Composition, biosynthesis and effect of environmental factors on cuticular wax in bilberry

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The outer surface of plants is covered by cuticular wax, which plays a role in non-stomatal water loss, protection from UV rays and plant defense. We have found differences in chemical composition and morphology while characterizing the cuticular wax in bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), bog bilberry (*Vaccinium uliginosum*) and crowberry (*Empetrum nigrum*) fruits using GC-MS. Triterpenoids were found to be dominant compounds in bilberry and lingonberry cuticular wax while fatty acids and alkanes dominated in bog bilberry and crowberry, respectively. All studied berry waxes showed high in vitro Sun Protection Factors (SPFs) depicting high UV-B absorbing capacities. Developmental and environmental factors are known to play an important role in cuticular wax biosynthesis. Therefore, we have characterized cuticular wax of glossy mutants of bilberry along with wildtype bilberry through developmental stages. The wax load between the mutant and wildtype bilberry was found to be almost similar, however the proportion of triterpenoids was higher; fatty acids, aldehydes and ketones, lower in mutant wax as compared to wildtype bilberry. Based on morphology and compositional analysis results, we proposed a correlation between glaucousness, ketones and rod like structures in bilberry. Peel specific expression of wax biosynthetic genes indicates their role in wax biosynthesis in bilberry. In studying the effect of environmental factors, we observed that the proportion of triterpenoids increases in bilberry cuticular wax as we move from northern latitudes to south. We have also done controlled phytotron experiments to study the effect of temperature and high UV-B radiation on the chemical composition of bilberry cuticular wax. Our studies bring new information on the biosynthesis and effect of environmental factors on composition, morphology of cuticular wax layer in bilberry.

[1] Trivedi P et al. 2019. Developmental and environmental regulation of cuticular wax biosynthesis in fleshy fruits. *Frontiers in Plant Science*, 10, 431

[2] Trivedi P, et al. 2019. Compositional and morphological analyses of wax in wild northern berry species. *Food Chemistry*, 295: 441-448

[3] Analysis of composition and biosynthesis of cuticular wax among wild type bilberry (*Vaccinium myrtillus*) and its glossy mutant (manuscript in preparation)

## 206 - Identification and characterization of key enzymes involved in the metabolism of secoiridoids in *Olea europaea*

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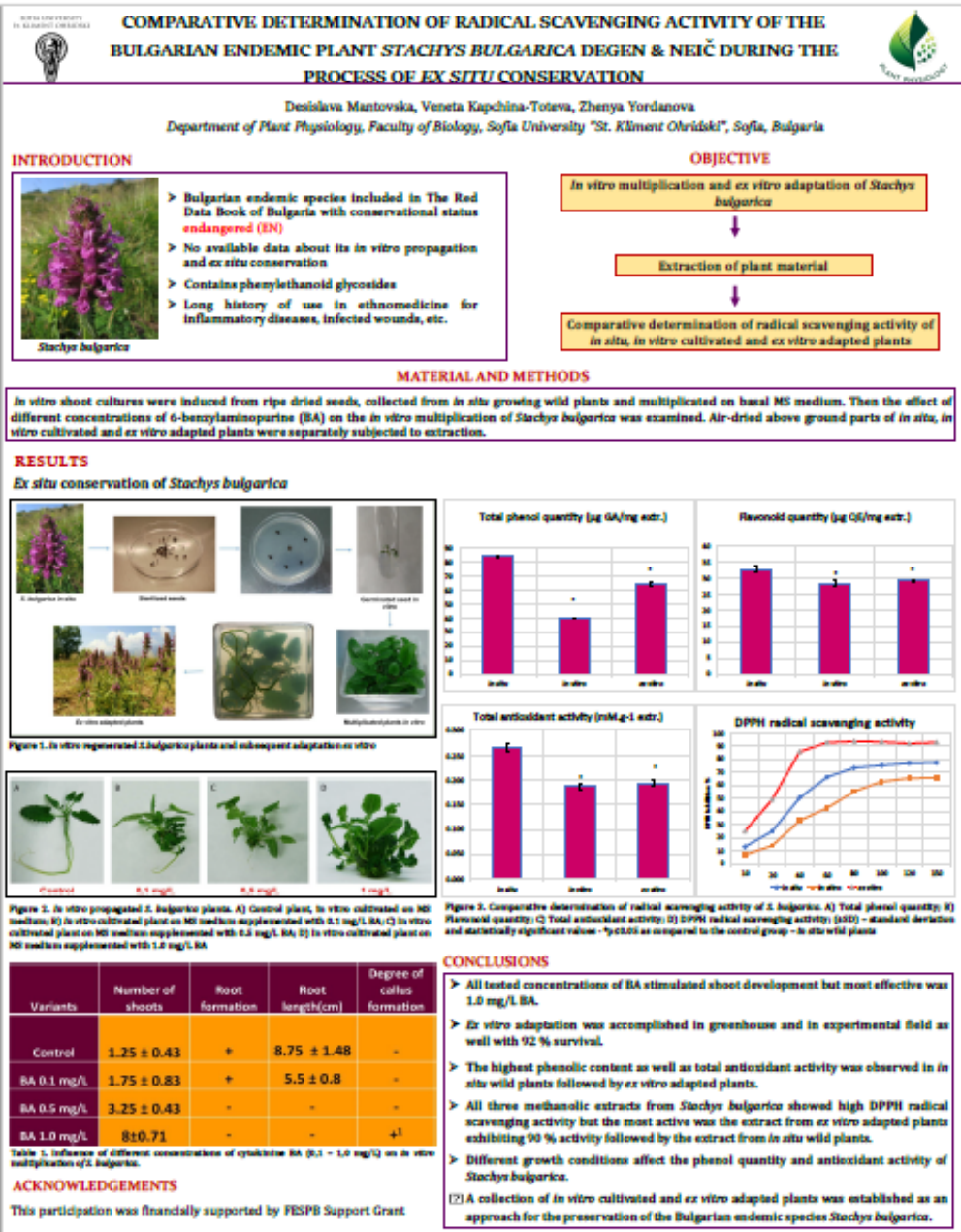
Olive tree is an emblematic crop and its cultivation in the Mediterranean basin goes back millennia. One of the most fascinating traits that evolution has gifted to olives is the production of certain secondary metabolites, known as oleosides. Many of the beneficial characteristics of olive oil in human health are attributed to these compounds and thus, there is an arising interest in order to explore their pharmaceutical applications. Due to extremely narrow taxonomic distribution of oleosides, our knowledge concerning the enzymatic hubs that govern the biotransformation of the olive secoiridoids is scarce. We have recently identified a  $\beta$ -glucosidase (OeGLU) that specifically deglycosylates oleuropein - the dominant secoiridoid in olive - producing the bioactive defensive aglycone form. The functional characterization of a gene should ideally be complemented with genetic analysis, however this approach using the recalcitrant perennial Oleaceae species is extremely difficult and time consuming. To overcome this drawback, we have introduced the Virus-Induced Gene Silencing (VIGS) approach to the olive tree. The successful of VIGS approach for OeGLU give us the opportunity to perform functional genomics analysis in olive. In order to clarify the functional activities of a number of genes involved in metabolism of secoiridoids, we combine the heterologous expression of candidate genes in planta with bioinformatics and VIGS approach in olive tree. Decrypting the "metabolic mystery" of olive and understanding the enzymatic hubs that govern this differentiated pathway by exploiting genes involved in secoiridoid metabolism would favor researchers of diverse fields and raise numerous biotechnological applications. Here, we present our work of characterizing olive enzymes/genes that are involved in both the anabolic and catabolic pathways of the bioactive secoiridoids.



207 - Comparative determination of phenolic content and antioxidant potential of the Bulgarian endemic plant *Stachys bulgarica* during the process of ex situ conservation

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## **226 - Hexanoic acid application in roots can modulate secondary metabolism and redox state-related genes in coffee leaves**

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Hexanoic acid (Hx) is a short, naturally occurring monocarboxylic acid that is a potent natural priming agent against pathogens. The mechanism that rely Hx impacts in plants are still very poorly understood, since most studies were focused on resistance mechanisms. Here we hypothesize that, similarly to what occurs in several plant systems, hexanoic acid can induce a long distance modulation in key genes of plant metabolism. For this, we evaluated by RNA-seq the leaf transcriptome of two *Coffea arabica* cultivars with contrasting resistance to pathogens, in response to the application of hexanoic acid in an eliciting concentration in nutrient solution. Hx modulate more genes in the disease-susceptible cultivar (121) than in the resistant cultivar (91 genes). A total of eight genes have significant similar transcriptional modulation in both cultivars, including genes related to redox balance, jasmonate signalling and the phenylpropanoid metabolism. Hx significantly repressed only an electron acceptor in chloroplasts. All other genes were upregulated. They include a glycosyltransferase associated to the salicylate-jasmonate signaling crosstalk, an ATPase, aldo keto reductases and genes related to the biosynthesis of hydroxycinnamic acids and terpenoids. These results demonstrate that the application of Hx in roots can alter the gene expression patterns of leaves, activating genes involved in redox regulation and synthesis of secondary metabolites. Funding: CAPES and FAPESP.

## 229 - Development of molecular markers for specific metabolic pathways using contrasting plant phenotypes

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The development of molecular markers from metabolic pathways that regulate the traits of interest can be a powerful tool for selection of valuable genotypes in breeding programs. Strawberry and raspberry are popular berry crops and improving their nutritional properties, in particular, increasing the content of bioactive compounds, is one of the main directions of modern breeding. We developed 20 microsatellite (SSR) markers from structural and regulatory flavonoids biosynthesis genes of *Fragaria* and *Rubus* species, located in the coding and non-coding gene sequences. These markers were tested on 48 *Fragaria* and *Rubus* genotypes, contrasting in the content of anthocyanins demonstrating high antioxidant activity, as well as ploidy and origin. The anthocyanin profile in berries of 11 raspberry and blackberry cultivars harvested in 2018 and 2019 were determined by HPLC-ESI-MS. A high proportion of the developed markers are transferable within and between *Fragaria* and *Rubus* genera and are polymorphic. Transferability and polymorphism of the SSR markers depended on location of their PCR primer binding sites and microsatellite loci in the genes, respectively. Our results suggest that boreal *Rubus* species are more related to *Fragaria* compared to raspberry and blackberry. The SSR markers representing transcription factors genes that regulate flavonoid biosynthesis showed high allelic variability and may be good candidates for marker-assisted selection of berry species. The developed genetic markers can be used both for genetic diversity and population genetics studies in *Fragaria* and *Rubus* species and in breeding programs for improving anthocyanin related traits. A similar approach can be used for other purposes, for example, we are screening woody plants with contrasting abiotic stress tolerance to develop markers of resistance to heavy metals and subsequent selection of genotypes for phytoremediation of contaminated soils.

## 243 - Diverse Photosynthetic Pathways in Dicotyledonous species – an Overview from Leaf Anatomical Characters

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Plant species are known to follow different photosynthetic pathways and their delimitation using leaf anatomical characters has been long established. Interestingly, most of these characters were determined using photosynthetic pathways in monocotyledons and they rarely conform to dicotyledonous plant features. Establishment of leaf anatomical characters that may be peculiar to dicotyledonous plant genera were investigated in three genera known to exhibit C<sub>3</sub> and C<sub>4</sub> photosynthetic metabolism (Euphorbia L., Boerhavia L., and Cleome L.) in addition to creating a possible grouping of the species in these genera along their respective photosynthetic pathways. Standard anatomical procedures were employed to investigate characters such as stomata index, stomata size, inter-stomatal distance, stomatal density, interveinal distance, intercellular air spaces, leaf thickness, mesophyll thickness, Kranz tissue, one cell distant count, maximum lateral cell count, vein density and vein distance. The data from this study highlight features that are at variance to what is known of monocotyledons such as interveinal distance of less than 166µm indicating C<sub>4</sub> dicotyledonous species while any higher values indicate C<sub>3</sub> species. In addition, our data suggests that the 'maximum lateral cell count' criterion of cells ranging from 2 to 6 indicates C<sub>4</sub> dicotyledonous species while numbers higher than this indicates C<sub>3</sub> species. The physiological implications of the identified characters are discussed. This study is probably the first known to report on anatomical characters that are delimiting for dicotyledonous species.

## **289 - Identification and regulation of genes involved in stilbene biosynthesis in *Vitis vinifera***

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**- TH Bingen - University of Applied Sciences, Bingen, Germany** <sup>(3)</sup>

Stilbenes have received widespread attention in medical research because of their many beneficial effects on human health. They have been detected in at least 72 plant species including crops like peanut and grapevine. While there are about 100 different stilbenes in plants of the *Vitis* genus and roughly 20 of these are known to be present in wine, only two enzymes of the stilbene pathway have been identified and characterized. Considering the various modifications of the basic stilbene skeleton and the transport of stilbenes, additional unknown genes have to be involved in these processes. An important step towards the discovery of such genes was the recent identification and characterization of the two grapevine transcription factors VvMYB14 and VvMYB15, specifically regulating the stilbene pathway. Overexpression of these transcription factors in *Vitis vinifera* and subsequent microarray analysis was used to initially identify the target genes of the transcription factors. Thereby, yet unknown genes of the stilbene pathway were analyzed and their regulation was studied in detail. Amongst others, a Laccase and three Glycosyltransferases were identified through their upregulation by VvMYB15. These genes were further analyzed with respect to their expression levels and correlation to stilbene accumulation in various developmental stages of the grape berry as well as in *Vitis vinifera* tissue infected with *Plasmopara viticola*. So far, these candidate genes show promising correlations with the MYB transcription factors and stilbene synthases. Furthermore, ectopic expression of these structural genes will allow the biochemical characterization of the respective gene products. Therefore, this research project will provide new insights into the molecular basis and the regulation of the stilbene pathway in plants. The basic results concerning the genetic differences of grapevine cultivars or species and the impact of environmental factors on stilbene metabolism could allow optimization of grapevine stilbene content via subsequent grapevine breeding approaches.

### **304 - Mass spectrometry-based metabolomics: A rapid technology for anticancer lead-finding from medicinal plant *Withania somnifera***

**Varsha Tomar** <sup>(1)</sup> - **Dr. Debabrata Sircar** <sup>(1)</sup>

**Indian Institute of Technology, Roorkee, Biotechnology Department, Roorkee, India** <sup>(1)</sup>

Quality assurance has been an important concern in the production and utilization of medicinal plants. Identification of quality associated biomarker metabolites (QABMs) play crucial role in quality control. *Withania somnifera* (Ashwagandha) extracts have long been used in traditional and modern medicine system to cure several human ailments. Withanolides are known for reported bioactivities. However, major bottleneck of isolating these bioactive metabolites from natural plant sources are, low abundance and occurrence of variations in content and composition of metabolites depending on the plant part. Mostly, plant extracts show bioactivity based on synergistic or antagonistic effects. Therefore, the idea of a holistic metabolomics approach is more rational to identify large number of metabolites at a single go. This study was planned to compare the metabolomics profile of different parts (root, stem, leaf and flower) of *W. somnifera* using high-throughput gas-chromatography and liquid-chromatography mass spectrometry-based metabolomics and to understand the mechanism of actions of important lead-metabolites as anticancer agent. Metabolomics data has shown that in addition to withanolides, phenolics, terpenes and sugars were pre-dominant metabolites detected in *W. somnifera*. Upon metabolomics, identified QABMs were tested for anti-proliferative activity against a number of human cancer cell lines. 'Withaferin A' was found to be a very important QABM exhibiting significant mechanism-based anti-proliferative activity. These data showed that mass spectrometry-based metabolomic approach is a powerful and efficient tool to discover large number of plant metabolites with unique bioactivities circumventing time-consuming fraction guided separation process.

### **308 - Supramolecular polymeric cyclodextrins as new elicitors for the production of plant bioactive compounds**

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The high nutritional, medicinal and economic value of the secondary metabolites that plants synthesize makes these compounds of great importance for cosmetic, food and pharmaceutical industries. These industries try to find production techniques to obtain secondary metabolites that are reproducible, simple and with low economic costs. Currently, the production of secondary metabolites can be carried out by extraction from plant raw material, by chemical synthesis and from in vitro plant cell cultures. In fact, in vitro plant cell cultures have the greatest advantages since the extracts obtained from them are homogeneous, and they have a growth in sterile conditions and indefinite maintenance, regardless of seasonal and weather conditions. However, when the production of these metabolites want to be extrapolated at the industrial level, it is necessary to optimize different parameters, and make a successful selection of elicitors to increase the production of a particular metabolite. Of the most successfully used elicitors to increase the production of trans-resveratrol in grapevine cell cultures, cyclodextrins and methyl jasmonate stand out. In particular,  $\beta$ -cyclodextrins have a chemical structure that makes them special, not only as elicitors, but also as compounds capable of containing, inside them, highly hydrophobic molecules such as trans-resveratrol. However, the use of cyclodextrins increases production costs, making their industrial exploitation economically unfeasible. Therefore, the development of recovery strategies for these molecules is necessary to give a viable solution to their industrial use. In this work, carboxymethylated and hydroxypropylated  $\beta$ -cyclodextrins have been used to form polymers using epichlorohydrin as a cross-linking agent, and these polymeric cyclodextrins were jointly used with methyl jasmonate to elicit grapevine cell cultures. Once elicitation experiments were finished, a magnet allowed the recovery of polymers and trans-resveratrol was extracted from them. Thus, all trans-resveratrol produced by the cells and secreted into the culture medium is trapped and recovered by the polymers.

### **313 - Volatomics profiling of papaya fruits to identify non-invasive marker for tracking different ripening stages**

**Komal Kushwaha** <sup>(1)</sup>

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Papaya (*Carica papaya* L.) is one of the most important fruit crops grown in the tropical and sub-tropical regions of the world. Being a climacteric fruit, papaya has a short postharvest life, which limits the nutritional value and economic return. Shipping in refrigerated containers cannot yet offer sufficient storage life without the use of fungicides. This work is focused on identifying signature volatile organic compound (VOCs) as non-invasive marker for tracking the ripening stages and nutritional profile under pre- and post-harvest conditions. VOCs were profiled using SPME GC-MS techniques. Total thirty-seven VOCs were detected from the papaya fruits, out of which five were identified as signature VOCs. Signature VOCs showed significant variation during ripening and post-harvest storage along with exhibiting a pattern correlation with nutritional profile. Sugars, amino acids, carotenoids, fatty acids and phenolics were profiled during pre and post-harvest storage. VOCs were discriminated using the Principal Compound Analysis. Correlation of VOCs with nutritional profile was established using pattern algorithm. These signature VOCs could serve as excellent candidates for sensing ripening stages and nutritional value of papaya by using non-invasive sensors. Farmers in this mobile phone generation could easily accept this technology.

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#### **Biography**

I, Komal Kushwaha, completed graduation and post-graduation in botany from Banaras Hindu University, India. Currently, I am senior research fellow in department of biotechnology, IIT Roorkee, India. I qualified GATE with 94.91 percentile in 2017.

### **321 - Colleters in Neotropical Lecythidaceae: structure and functional aspects of secretion**

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The term colleter refers to trichomes or emergencies positioned close to developing vegetative and floral meristems. Some functions can be attributed to colleters, such as protecting young tissues against desiccation and attack by harmful microorganisms due to the secretion of a viscous, mucilaginous and/or lipophilic exudate in these conditions. In Eudicotyledons, the occurrence of colleters is probably underestimated due to the scarcity of studies related to them and the difficulties in determining their function. The present work aims to report for the first time the occurrence of floral and leaf colleters in Neotropical Lecythidaceae and to describe their morphological and functional aspects. For this, light microscopy and scanning electron microscopy were used to analyze the distribution and structure of colleters close to vegetative and reproductive meristems at different stages of development of *Gustavia augusta* L., *Couroupita guianensis* Aubl., *Cariniana legalis* (Mart .) Kuntze and *Lecythis pisonis* Cambess. besides characterizing the secretion composition histochemically. The colleters of all species are elongated and sessile, having a central parenchymatic axis recovered by a secretory epidermis coated by a cuticle. They are located in the vegetative meristems close to the buds and at the base of young leaves and in the reproductive meristems at the base of bracts and bracteoles. The histochemical analysis showed a heterogeneity of the exudate present in the secretory cells of all analysed species - essentially indicating the presence of mucilage in association with lipophilic, phenolic, proteic and terpene compounds. The location of these colleters, the chemical nature of the exudate and the timing of secretion, point out their involvement with the protection of meristematic regions in vegetative and reproductive organs, performing a key role in the protection against desiccation, preventing water loss due to hydrophilic property of mucilage and pathogen attack due to the antimicrobial property of terpenes.



### **332 - Comparative study of precursors and acrylamide levels in sweet potato (*Ipomoea batatas* L.) fries prepared by different frying methods**

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The sweet potato (*Ipomoea batatas* L.) is regarded as the 7th most important food crop in the world and one of the main staple foods in tropical and subtropical areas. In the last decades, the plant has also become increasingly important in Europe, mostly consumed in fried form like fries or chips.

Acrylamide, a group 2a carcinogen (IARC), is known to be formed from natural precursors (reducing sugars and asparagine) during high-temperature treatment (> 120°C). Since sweet potato contains these compounds, these analyses are highly relevant in respect to human health.

The research questions were: How much do sweet potato varieties differ in the content of metabolites relevant for human consumption? Is air-frying better than fat-frying in terms of acrylamide levels in sweet potato fries?

Acrylamide, soluble sugars, asparagine and ascorbic acid were analysed in 4 different sweet potato varieties by HPLC and LC-MS.

Different varieties of sweet potatoes showed characteristic differences in their compositions regarding the measured metabolites. Surprisingly, the acrylamide content after the two frying techniques was not significantly different, whereas between the varieties the acrylamide content differed due to the variable contents of precursors. The highest acrylamide levels were found in Bonita, followed by Purple on second place, Murasaki and Covington showed the lowest amounts. In sweet potato, the acrylamide formation is mostly dependent on the asparagine content, whereas the sugar content is more relevant for organoleptic attributes.

In general, French fries made from regular potatoes versus sweet potatoes are in the same range concerning acrylamide content.

This research highlights the importance of extensive knowledge about the variability of metabolites in different varieties of sweet potatoes for obtaining good-quality fries with low acrylamide levels.

### 339 - Characterization of Monolignol Oxidoreductases from the Berberine Bridge Enzyme-like Protein Family in *Arabidopsis thaliana*

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Among the flavoprotein superfamily, the members of the berberine bridge enzyme-like (BBE-like) subfamily (pfam 08031) were found to be present in bacteria, fungi and plants. The family name derives from the so-called “berberine bridge”, a characteristic C-C bond formed during the cyclisation reaction of (S)-reticuline to (S)-scoulerine catalyzed by the berberine bridge enzyme.

Based on sequence similarities, BBE-like enzymes were identified to be present throughout the plant kingdom ranging from two in the moss *Physcomitrella patens* to 57 in western poplar (*Populus trichocarpa*). *Arabidopsis thaliana* harbors 28 genes encoding BBE-like proteins (AtBBE-like). Results obtained from microarray expression analyses suggest the involvement of AtBBE-like proteins in various stress-induced plant responses as well as developmental processes. Despite the similarities in sequences and structures, the biological functions of the individual AtBBE-like genes appear to be highly diverse, very specific, and non-systemic.

Biochemical characterization of AtBBE-like subgroup six, which consists of five genes (AtBBE-like 13, 15, 24, 25 and 26), showed high similarities in sequence and structure. The catalytic properties of AtBBE-like 13 and 15 were determined and the proteins were found to oxidize monolignols to their corresponding aldehydes [1]. In order to investigate the in planta functions of AtBBE-like subgroup six, we have, so far, generated tissue-specific reporter lines for all genes of AtBBE-like subgroup 6 as well as loss-of-function mutants for AtBBE-like 13. Employing these lines, we have documented expression patterns in seedlings and found the genes to be expressed mainly in roots, where the individual genes showed different expression patterns on the tissue level. Additionally, AtBBE-like 13 reporter lines showed altered expression in lateral roots when treated with indole-3-acetic acid (IAA). Phenotypic description of loss-of-function mutants, detailed descriptions of reporter lines and physiological experiments comparing mutant to wild type plants will widen our understanding of the AtBBE-like protein family.

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[1] Daniel et al. (2015) The Journal of Biological Chemistry 290: 18770–1878

**341 - The first step of benzoxazinoids biosynthesis in rye (*Secale cereale* L.) may occur both in the plant upper parts and roots.**

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Benzoxazinoids (BXs), secondary metabolites synthesized by several species of the Poaceae family, play an important role in biotic and abiotic stress resistance, and in allelopathy. Common rye is a species producing BXs at a particularly high level. Up to now, it was widely believed that the first step in BX biosynthesis (conversion of indole-3-glycerolphosphate to indole, controlled by Bx1 gene and its orthologues, encoding for indole-3-glycerol phosphate lyase – IGL), takes place in chloroplasts. The results of numerous experiments on rye BXs carried out by our group for several years clearly showed, however, that the synthesis of these compounds may occur both in the aerial parts of rye plants and their roots. We have detected the BXs [specifically: 2-hydroxy-4H-[1,4]benzoxazin-3-one, 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), DIBOA glucoside, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), DIMBOA glucoside and 6-methoxybenzoxazolinone] both in roots of plants cultivated under control conditions and after stress (i.e. co-cultivation with Berseem clover, subjecting to low temperature). They were also present in roots regenerated in vitro and in roots of kernels deprived of the upper parts immediately after they appeared. The final proof was the immunodetection of IGL in the plastidic fraction (most probably in leucoplasts) of these roots. Therefore, we postulate to revise the current opinion according to which the first stage takes place only in chloroplasts.

**342 - Secretory structures at flowers of *Baccharis platypoda* (Asteraceae): first report of colleters for the family**

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The rupestrian fields are environments with high altitudes and low-organic-matter soil, where the species are exposed to water and solar stress. *Baccharis platypoda* DC. occurs in this type of environment with a rigid and transparent secretion that covers the entire inflorescence. The objective of the study was to characterize the secretory structures and the chemical composition of the secretion. Inflorescences were collected from three male and three female individuals of *B. platypoda* occurring in the same proximity (Serra do Cipó, Minas Gerais, Brazil). Inflorescence samples were fixed in Karnovsky's solution, dehydrated in ethyl alcohol and processed for anatomical analysis in light and scanning microscopy. Histochemical analyzes were carried out by applying the reagents copper acetate/rubeanic acid for total lipids, Nile Blue sulfate for neutral lipids, ferric chloride for phenolic compounds, aluminum chloride for flavonoids, coriphosphine for pectin, Aniline Blue Black for proteins and red ruthenium for mucilage. For the chromatographic analyses, the fresh inflorescences were extracted with 25 mL of methanol and the extract was analyzed by gas chromatography coupled to mass spectrometry. The results were similar for both individuals. The microscopic analyzes show the presence of glandular trichomes and canals in the epidermis on the abaxial face of petals and sepals, nectaries in the bracts and secretory ducts in the axis of the inflorescence and petals, sepals and bracts. Trichomes are the secretory structures that produce the exudate in abundance and whose function is to protect the flower buds in development. Histochemical tests were positive for all substances except lipids and mucilage. Chromatographic analysis showed the presence of genkwanin and naringenin. Thus, it is concluded that the trichomes found to act as colleters. These structures help to protect the inflorescence against damage caused by excess light due to the presence of flavonoids.

### 347 - Light-spectrum modifies the diurnal rhythm of sulfate and glutathione metabolism in barely

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Spectral quality, intensity and period of light modify many regulatory and stress signaling pathways in plants. Circadian clock is an endogenous oscillator mechanism producing biological rhythms with approximately 24 hour periods, thus it is able to synchronize developmental and metabolic processes with daily changes in the environment. Glutathione (GSH) is one of the most important low-molecular weight thiol in the cellular redox system, which is used for both detoxification of so called reactive oxygen species (ROS) and for the transmission of redox signals. The activity of enzymes involved in GSH biosynthesis and sulfate metabolism are also reported to change according to a diurnal manner. The aim of this study was to evaluate whether supplemental FR (735 nm) and B (450nm) LED panels modify the transcription and diurnal rhythm of enzymes related to GSH- and sulfate metabolism in cold tolerant winter habit barley variety 'Nure'. Our results show that enrichment of the spectrum of white incident light by red/far-red and blue components decreased and increased the glutathione-dependent redox potential, respectively, and modified its diurnal rhythm. The transcription of the genes encoding enzymes of cysteine and glutathione metabolism was greatly induced by red/far-red light and decreased by blue light; however, its diurnal rhythm was only slightly affected. Thus, red/far-red light induces a more reducing environment and activates the antioxidants in contrast to the inactivating effect of blue light. In addition, nitrate reduction, which is interconnected by sulfate reduction, was similarly affected by modulated white light.

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### 357 - Investigation of ACTs involved in HCAA accumulation in Poaceae

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Hydroxycinnamic acid amides (HCAAs) are one of the major families of plant secondary metabolites and are known to function as defensive compounds in plants. The major HCAAs in genus *Hordeum* are coumaroylagmatine and feruloylagmatine, and are also used as the precursors of the barley-specific defense compounds. In barley (*H. vulgare*), these HCAAs are biosynthesized by agmatine coumaroyltransferase (ACT). In the case of other poaceous plants such as rice and wheat, the concentration of HCAAs are very low, though they possess ACT-like genes showing more than 80% identity with HvACT. To explain the significant difference in HCAA accumulation among the poaceous plants despite their possessing homologous ACT genes, we characterized the ACTs and analyzed transcript levels of the genes as well as HCAA contents in barley, wheat, and rice. In addition to the HvACT, TaACT-R-NIL and OsaHT that are assumed to be included in HCAA production, novel ACT homologs were cloned from barley and wheat. Moreover, an ortholog of HvACT was also isolated from *H. murinum*, a species of *Hordeum* that accumulates high level of coumaroylagmatine and feruloylagmatine. All the ACTs were heterologously expressed in *E. coli* and their enzyme activity were evaluated. As a result, HvACT showed the highest activity to p-coumaroyl-CoA, feruloyl-CoA and agmatine. The transcript levels were evaluated by real-time PCR using cDNAs prepared from barley, wheat and rice, and it was demonstrated that the transcript level of HvACT was the highest. The expression of HvACT peaked 48 hours after seeding and then decreased. This profile correlated to the occurrence coumaroylagmatine in barley. Our results indicate that both the ACT activity and transcript levels are responsible to the control of HCAA accumulation in graminea, and high level accumulation of HCAAs in barley attributes to higher activity and expression of HvACT.

### **358 - Expression profiles of the CaCPR1 and CaCPR2 in Hot pepper (*Capsicum annuum* L. cv. Bukang)**

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**(1)**

The cytochrome P450s (CYP) play important roles in development and defense system in plant. CYPs require NADPH-cytochrome P450 reductase (CPR) enzyme for their functions. CPRs are required for electron transfer from NADPH to cytochrome P450. There are two CPR genes in the hot pepper (*Capsicum annuum* L. cv. Bukang) genome which are CaCPR1 and CaCPR2. The CaCPRs expression levels were measured by quantitative real-time PCR in various development stages and stress conditions (Jasmonic acid, Salicylic acid, drought treatments). The CaCPR1 expression level was gradually increased during fruit ripening. The CaCPR2 gene was constitutively expressed in all the tested tissues but the expression level was lower than the CaCPR1. Under the stress conditions, both of the CaCPR1 and CaCPR2 expression levels were increased possibly because more CPR enzymes are needed against stress. To investigate the enzymatic properties, two CaCPRs were isolated from hot pepper and heterologously expressed in *Escherichia coli*. The enzymatic activities were assessed using protein and chemical substrates such as MTT and ferricyanide. In vitro assay showed that both CaCPR1 and CaCPR2 mediated electron transfer from NADPH to the substrates. These results suggest although CaCPR2 may play minor enzyme in normal condition, the CaCPR1 and CaCPR2 could be involved in responses of plants under stress conditions.

**372 - Agrobacterium-mediated transformation of cardoon cell cultures allows alteration of the phenylpropanoid pathway.**

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Cardoon (*Cynara cardunculus* L. var. *altilis*) is a traditional Italian vegetable crop, with high yields of seed oil with a good fatty acid profile (high oleic acid) and a vigorous lignocellulosic biomass characterized by valuable compounds that can be recovered both from the apical part (e.g. chlorogenic acid and other polyphenols) and from roots (inulin). To overcome the hurdles of seasonal availability and high biochemical variability of the biomass, standardized cardoon cell cultures represent an alternative to field cultivation. In this frame, we tested a biotechnological approach via *Agrobacterium*-mediated transformation to obtain stable transgenic cardoon cell lines with the aim of increasing the accessibility of the cellulose fraction and of boosting the production of high-value nutraceuticals. Our strategy revolves around the overexpression of the *Arabidopsis* MYB4 gene, encoding a transcriptional factor known to be a master regulator of the phenylpropanoid pathway. We provide a detailed characterization of the transgenic lines based on molecular, metabolic and transcriptomic comparisons with wild-type controls.

The research activities here described are part of the larger project BOBCAt (funded by Cariplo) devoted to optimize and scale-up the growth the use of cardoon cell cultures in economically and environmentally sustainable conditions, in line with the principles of Circular Economy.



### **379 - Identification of a novel miRNA regulating nicotine biosynthesis by targeting QPT in *Nicotiana tabacum***

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**(1)**

MicroRNAs (miRNAs) are a class of ~22 nt small noncoding RNAs, which regulate target genes expression post-transcriptionally through sequence-complementarity. Thousands of miRNAs have been discovered in many plants and animals up to now, and their regulatory roles in numerous biological processes have been uncovered. Nicotine is the predominant alkaloid in tobacco (*Nicotiana tabacum*), responsible for the addiction of smoking and functioning as one of the most effective plant defense metabolites in nature. It is produced in the roots and accumulated mainly in the leaves. Many important coding genes involved in nicotine biosynthesis had been identified, e.g. putrescine N-methyltransferase (PMT), quinolate phosphoribosyltransferase (QPT) Berberine Bridge Enzyme-Like Proteins (BBLs) and A622. However, little is known about whether miRNAs involved in nicotine biosynthesis. By small RNA sequencing for *Nicotiana tabacum*, we identified one miRNA (Nt-miR2635) may target QPT. The evolution analysis revealed this miRNA may be novel one, which have no homolog with other plants. The interaction between Nt-miR2635 and QPT have been verified by in vivo miRNA-mediated cleavage assay. Over-expressed Nt-miR2635 in *N. tabacum* significantly reduced the content of nicotine, around 42% - 72%, pointing to roles in nicotine biosynthesis. Meanwhile, the expression of QPT was also reduced a lot (around 48% - 67%). Our study uncovers a new role for miRNA in mediating nicotine biosynthesis.

### **388 - *Ophiopogon japonicus*: phytochemical authentication for a dermo-cosmetic development.**

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*Ophiopogon japonicus* tubers are used in Traditional Chinese Medicine and are currently traded for the treatment of pathological skins. Their supply has to be traced and secured to ensure safety and efficacy of the resulting dermo-cosmetic ingredient. They share botanical and morphological characteristics with other plant species, particularly *Liriope spicata*. To avoid falsifications, analytical authentication of these plants tubers is thus an essential step.

In this context, we performed a comparative analytical study of various samples of *Ophiopogon japonicus* tubers, from different supply regions. Phytochemical profiles obtained by ultra performance liquid chromatography coupled with tandem high resolution mass spectrometry (UPLC-MS/MS) were then directly compared with those of *Ophiopogon japonicus* and *Liriope spicata* specimens from referent collections.

Chromatograms obtained from referent samples revealed specific markers of *Ophiopogon japonicus* and *Liriope spicata*, in particular the range of homoisoflavonoids [1-5] and alkaloids. Identification of the species could be performed thanks to comparison of parent and fragment ions to components described in the literature.

Based on these data, our results demonstrated that one of the samples contains alkaloids not present in Asparagaceae (i.e. the family of *Ophiopogon japonicus* and *Liriope spicata*), thus suggesting falsification of this sample. Two other samples display trace amounts of homoisoflavonoids, a variation probably due to the cultivation region which does not totally refute *Ophiopogon japonicus*. The other samples have the specific markers of authentic specimen of *Ophiopogon japonicus*.

This analytical analysis of samples from different regions allowed us to authenticate samples of *Ophiopogon japonicus* comparatively to certified specimens. This approach will allow to secure future supplies of this starting raw material and to detect early some falsifications. This work is essential to guarantee safety and efficacy of the resulting dermo-cosmetic ingredient.

### 389 - CHARACTERIZATION OF VOLATILE COMPOUNDS IN GRUMIXAMA (*Eugenia brasiliensis* LAM.), NATIVE FRUIT FROM ATLANTIC FOREST.

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Plants can synthesize, accumulate and emit volatile organic compounds (VOCs) that act as (i) defense mechanisms against microorganisms and herbivores; (ii) in plant-plant communication; and (iii) to attract seed dispersing agents. In addition, VOCs directly influence the quality of fruits, acting as flavor molecules. Aromatic properties of the fruits depend (i) on the phenotypic plasticity of the plant; (ii) combination of VOCs among themselves and with other compounds; (iii) concentration; and (iv) the threshold of perception of the compounds. The objective of this work was to identify volatile metabolites that confer desirable sensory characteristics to grumixama fruit (*Eugenia brasiliensis* LAM.), a native species of Brazilian Atlantic Forest, assisting in the selection of candidates with potential for propagation. The fruits were obtained in ripe stage from eleven trees at the municipalities of Pariquera-Açu and Paraibuna, São Paulo. VOCs were analyzed by headspace-solid phase microextraction method (SPME) GC-MS. ~~Ninety two~~Ninety-two VOCs were identified, all of them with odor description in literature. Among them, the most abundant in both regions were terpenes. There are also: alcoholterpenes, alcohols, aldehydes, benzene compounds, ketones, esters, ethers and furanone. The multivariate analysis showed a significant difference between the VOC's profile of these regions. A greater number of VOCs (73) was observed in Pariquera-Açu, in which the plants were grown without any trait in wild condition. Paraibuna fruits presented less abundance of VOCs (35), however their representation in different organic classes were greater than Pariquera-Açu, including benzene compounds, aldehydes and furan. These results may have been caused by edaphoclimatic factors, which subject plants to different challenges and, consequently, different metabolic responses, among which the synthesis of volatiles is one of the most evident. Therefore, knowing the regulatory mechanisms and identifying the synthesis pathways for these compounds will provide subsidies for other researchers to work in their different areas.

#### **403 - *Sutherlandia frutescens* (L.) R.Br. metabolome and proteome profile in response to salt and nutrient stress**

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*Sutherlandia frutescens* (L.) R.Br. (Fabaceae) is an indigenous southern African medicinal plant with diverse pharmacological properties. *Sutherlandia* is renowned for its unique triterpenoid glycosides and flavonoids, sutherlandiosides and sutherlandins, respectively; and the presence of free amino acids including L-canavanine and gamma ( $\gamma$ ) aminobutyric acid (GABA). Wild populations show chemical heterogeneity that may be linked to varied environmental conditions. Some of these populations grow in nutrient poor soils and have a coastal habitat. We thus examined the metabolome and proteome in response to salt and nutrient stresses in in vitro plants of *S. frutescens* as phytochemical changes are also regulated by environmental stresses. Using GC-MS, increased levels of fructose, glucose and sucrose were detected. Proline, cadaverine, putrescine, and GABA were also elevated in plants exposed to stress. Through LC-MS-metabolomics, secondary metabolite changes were detectable in treated and non-treated plants. Sutherlandin C (725.19-m/z), D (725.19-m/z); and sutherlandioside A (653.42-m/z), B (653.45-m/z), C (651.40-m/z), D (635.41-m/z) were signatures that led to differential clusters and all stresses induced on plants caused a significant decline in sutherlandins and sutherlandiosides. Using MS<sup>E</sup> fragmentation patterns, 51 compounds were tentatively identified. Derivatives of sutherlandiosides, soyasaponins and kaempferols were rich in *Sutherlandia* extracts. We further conducted a comparative shotgun proteomic analysis to determine differentially expressed proteins. Stress regulated the expression of heat shock proteins, photosynthesis proteins, glycolysis, TCA cycle enzymes, N-assimilation and proteins involved in secondary metabolism. Regulation of these proteins and enzymes validated metabolite changes that were evident in sugars, amino acids and flavonoids. The metabolome-proteome responses to salt and nutrient stresses may partly explain the variable phytochemical patterns that are inherent to wild *S. frutescens*.

**411 - The last step in 3,5-dicaffeoyl quinic acid biosynthesis is catalyzed by a GDSL lipase-like in Ipomoea batatas**

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The synthesis of 3,5-dicaffeoylquinic acid (3,5-DiCQA) has attracted the interest of many researchers for more than 30 years. Recently, enzymes belonging to the BAHD acyltransferase family were shown to mediate its synthesis, albeit with notably low efficiency. In this study, a new enzyme belonging to the GDSL lipase-like family was identified and proven to be able to transform chlorogenic acid (5-O-caffeoylquinic acid, 5-CQA) in 3,5-DiCQA with a conversion rate of more than 60%. The enzyme has been produced in different expression systems but has only been shown to be active when transiently synthesized in *Nicotiana benthamiana* or stably expressed in *Pichia pastoris*. The synthesis of the molecule could be performed in vitro but also by a bioconversion approach beginning from pure 5-CQA or from green coffee bean extract, thereby paving the road for producing it on an industrial scale.

#### **448 - Turning a green alga red: engineering astaxanthin biosynthesis by intragenic pseudogene revival in *Chlamydomonas reinhardtii***

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Ketocarotenoids, among them astaxanthin, are powerful natural antioxidant with promising applications in human health and nutrition. The key enzyme to synthesize ketocarotenoids is  $\beta$ -carotene ketolase that convert carotenoids  $\beta$ -carotene and zeaxanthin into canthaxanthin and astaxanthin respectively. The gene for this enzyme can be found in *Chlamydomonas reinhardtii* but it is poorly expressed and the green alga do not synthesize astaxanthin. Using synthetic redesign of endogenous pseudogene to enable its constitutive overexpression, we are able to accumulate ketocarotenoids in *C. reinhardtii* causing noticeable changes from green to reddish-brown in the alga colour. Besides, in order to further increase the amount of ketocarotenoids accumulated, genome editing technology was used to alter the carotenoids biosynthetic pathway and obtain *C. reinhardtii* strains containing zeaxanthin as only  $\beta$ - $\beta$ -xanthophyll (npq2) or zeaxanthin and  $\beta$ -carotene as only carotenoids (dzt). Up to 50% of native carotenoids could be converted into astaxanthin and more than 70% into ketocarotenoids by robust ketolase overexpression. Under different growth conditions, the best performing overexpression strain was found to reach ketocarotenoid productivities up to 4.3 mg/L/day that might be competitive with that reported for *Haematococcus Pluvialis* which is currently the main organism cultivated for industrial astaxanthin production. In addition, the extractability and bio-accessibility of these pigments were much higher than the resting cysts of *H. Pluvialis*. Engineered *C. reinhardtii* strains could thus be a promising alternative to natural astaxanthin producing algal strains and may open the possibility of other tailor-made pigments from this host.

**453 - Cell suspension cultures elicitation for enhanced metabolite production in tamarillo (*Solanum betaceum* Cav.)**

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*Solanum betaceum* Cav. (tamarillo) is a Solanaceae tree cultivated worldwide due to the edible fruits. Plants can be easily micropropagated and callus and cell suspension cultures can be induced from different explants. These callus/cell suspension cultures have a strong biotechnological potential, namely for the analysis of cellular and molecular processes, metabolite synthesis and differentiation. This ability of tamarillo cell cultures has been described for other plants and is on the basis of the development of technologies for the sustainable and large-scale production of several classes of metabolites relevant in pharmaceuticals, cosmetics and food industries. Within the range of approaches used to increase the production of metabolites by plant cells, one of the most recurrent is the application of elicitors capable of enhancing and diversifying metabolite production through the stimulation of cell defense mechanisms. In this context, the main objective of this work was to characterize and optimise the growth of tamarillo cell lines and metabolite production under in vitro controlled conditions. The role of sucrose and the effects of two sets of biotic elicitors, one directed for cell growth and protein production (casein hydrolysate and yeast extract) and the other for the promotion of plant defense mechanisms (chitosan), were analysed for two cell lines. The results determined optimal growth conditions for the cell lines analysed and showed that 3% (w/v) sucrose concentration in the liquid medium affected the production of different proteins. The addition of casein hydrolysed at 500 and 1500 mg/l promoted protein production, whereas yeast extract (500 mg/l) promoted the production of glycosidases. Meanwhile, chitosan at 50 and 100 mg/l enhanced glycosidases, alkaline phosphates, and proteases production.

These results set the basis for establishing and optimizing tamarillo cell suspension cultures as a promising system for plant-derived metabolites production in scaled-up approaches, such as bioreactors.

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#### 460 - Gene networks underlying shikonin regulation and biosynthesis in *Lithospermum officinale*

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Shikonin and their derivatives, known as 1,4-Naphthoquinones, are commercially interesting plant secondary metabolites which are specifically produced in the roots of different species of the Boraginaceae family. Specifically, red/purple colour extracts from the roots of *Alkanna* spp., *Echium* spp. and *Lithospermum* spp. were traditionally used as dyes and in herbal preparations in both Europe and Asia for several centuries. To date, only two enzymatic (LePGT1 and AeGHQH) and one regulatory gene (LeMYB1) have been functionally characterized in the shikonin biosynthesis pathway. In this study, we performed comparative transcriptomic and targeted metabolite (shikonin and phytohormones) analysis to elucidate candidate genes involved in shikonin biosynthesis and regulation in *Lithospermum officinale*. Leveraging on exclusivity of shikonin production in response to methyl jasmonate (MeJA) but not salicylic acid (SA), we compared root transcriptional and metabolic profiles between MeJA and SA treated, and non-treated plants. Our co-expression analysis identified a gene module which was positively and significantly correlated with the presence of shikonin and harboured previously characterized genes (PGT, GHQH and MYB1). This module also contained additional potential candidate genes up- and downstream of PGT and GHQH. Gene ontology (GO) and KEGG enrichment analysis suggest that the genes in the candidate module may be involved in isoprenoid and quinone biosynthesis, which is in full agreement with the proposed biosynthesis of shikonin. Overall, this study lays the groundwork for developing a better understanding of shikonin biosynthesis and regulation in Boraginaceae. The herein identified candidate genes may be further exploited for their functional characterisation.

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**468 - Single gene bioengineering to produce a new generation of super crops with enhanced photosynthetic efficiency, stress tolerance, plant yield, and nutritional content**

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Climate change affects soil properties and the atmospheric conditions of our planet, causing dryness and salinity in the soil, and ultimately affecting crops and food production. Together with the concomitant increasing world population, this will have a direct impact on food demand and require a sustainable increase in crop productivity. Here, we describe a single gene manipulation in a carotenoid biosynthetic gene that enhances pro-vitamin A content, photosynthesis, photoprotection, abiotic stress tolerance (high light, salt, drought), biomass, and yield in the model plant tobacco. Because carotenoid pathway is present in all plants, our findings serve as a solid ground to design the next generation of super crops able to cope with climate change-related environmental issues, enhanced nutritional content (pro-vitamin A), and crop productivity.

## 511 - Composition, biosynthesis and effect of environmental factors on cuticular wax in bilberry

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The outer surface of plants is covered by cuticular wax, which plays a role in non-stomatal water loss, protection from UV rays and plant defense. We have found differences in chemical composition and morphology while characterizing the cuticular wax in bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), bog bilberry (*Vaccinium uliginosum*) and crowberry (*Empetrum nigrum*) fruits using GC-MS and SEM analysis. Triterpenoids were found to be dominant compounds in bilberry and lingonberry cuticular wax while fatty acids and alkanes dominated in bog bilberry and crowberry, respectively. All studied berry waxes showed high in vitro Sun protection factors (SPFs) depicting high UV-B absorbing capacities. Developmental and environmental factors are known to play an important role in cuticular wax biosynthesis. Therefore, we have characterized cuticular wax of glossy mutants of bilberry along with wildtype bilberry through developmental stages. The wax load between the mutant and wildtype bilberry was found to be almost similar, however the proportion of triterpenoids was higher; fatty acids and ketones, lower in mutant wax as compared to wildtype bilberry. Based on morphology and compositional analysis, we propose a correlation of glaucousness and rod like structures with ketones and fatty acids in bilberry. Peel specific expression of CER26-like, FAR2, CER3-like, LTP, MIXTA, and BAS genes indicates their role in wax biosynthesis in bilberry. In studying the effect of environmental factors, we observed that the proportion of triterpenoids increases in bilberry cuticular wax as we move from northern latitudes to south. Phytotron study revealed the effect of temperature on the chemical composition of bilberry cuticular wax, especially triterpenoids. Our studies bring new information on the biosynthesis and effect of environmental factors on composition, morphology of cuticular wax layer in bilberry.

[1] Trivedi P et al. 2019. Developmental and environmental regulation of cuticular wax biosynthesis in fleshy fruits. *Frontiers in Plant Science*, 10, 431

[2] Trivedi P, et al. 2019. Compositional and morphological analyses of wax in wild northern berry species. *Food Chemistry*, 295: 441-448

[3] Analysis of composition and biosynthesis of cuticular wax among wild type bilberry (*Vaccinium myrtillus*) and its glossy mutant (manuscript in revision; *Food Chemistry*)

[4] Effect of geographical location and climatic conditions on chemical composition of cuticular wax of bilberry fruit (manuscript in preparation)

## 524 - Metabolic engineering of BAHD acyltransferases involved in the production of an unusual chemical signature in Asteraceae pollen coat

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Asteraceae are a widely spread family in the plant kingdom. Their evolutionary success story can be correlated to traits of different levels (phenotypic, genetic, molecular), including the emergence of new metabolic pathways. Specialized metabolites are a great example considering their great diversity and their involvement in a vast array of plant responses and acclimations to their environment. Our work is centered on Eudicots pollen coat composition in specialized metabolites, and more precisely the accumulation of fully substituted phenolamides. Phenolamides are polyamines conjugated with phenolic acids, and have been widely studied for their roles in development, reproduction or even defense. The roles of fully substituted phenolamide conjugates however are poorly understood. Their presence is restricted to the pollen coat of Eudicots with tri-substituted spermidine conjugates being the common marker to all. In light of the key participation of the pollen grain during the colonization of the terrestrial world by Angiosperms, the conservation of this molecular marker suggests its major importance in the evolution of the green lineage.

<sup>[1]</sup><sup>[SEP]</sup> The biosynthesis of substituted spermidine was elucidated in Eudicots models such as *Arabidopsis thaliana* and *Malus domestica* and attributed to SHT enzymes (Spermidine Hydroxycinnamoyl Transferase). These SHTs belong to the BAHD family that catalyze acyl-CoA dependent acylation. They were up to now the only two characterized representatives of enzymes with ability to catalyze acylation of secondary amino-group of an aliphatic polyamine. Yet, in our regional model *Cichorium intybus* L. we uncovered the presence of a novel pollen coat marker, a phenolamide with a tetra amine backbone : tetra-coumaroyl spermine, whose biosynthesis is led by two SHT enzymes, an Asteraceae specificity. We characterized both genes and now aim to understand their functional discrepancies. Indeed, despite a very high sequence identity percentage, chicory SHTs display distinct activity types and substrate preferences. We were able to demonstrate their sequential action on spermine conjugates through heterologous expression assays. A structural analysis is led to identify the amino acids involved in these differences by the meaning of chimeric protein creation, site-directed mutagenesis as well as homology modelling.<sup>[1]</sup><sup>[SEP]</sup> Such knowledge of chicory SHT proteic structures are essential to enhance the production of our metabolite of interest and define its activitie(s). This will provide further insights into the role(s) of pollen coat phenolamides in regard to the metabolic diversification observed in the Asteraceae family and their evolutionary success.

### 536 - Metabolome and transcriptional changes in ripening grapes in relation to berry tissue-specific photosynthesis

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The metabolite composition of grape berry tissues is key to the quality of grape derived-products and co-products, and depends on various factors including ripening stage and received sunlight. Previously, we investigated the photosynthetic activity of grape berry tissues from a white variety (cv. Alvarinho) and showed that both the exocarp (skin) and the seed integuments were photosynthetically competent. By comparing grapes growing in two contrasting light microclimates of the plant canopy (LL - low light berry clusters, mostly shaded, at the inner parts of canopy; and HL - high light berry clusters, mostly exposed to direct sunlight) at three developmental stages (green, véraison and mature), we also showed that this berry photosynthesis was dependent on both developmental stage and microclimate. However, the contribution of this fruit-specific photosynthesis to the overall grape metabolome and especially to the biosynthesis of compounds key to grape quality, as well as its effect on the transcription of associated pathway genes, is still unknown. To investigate this role of berry photosynthesis, we applied both untargeted comprehensive metabolomics and directed transcriptional analysis to exocarp and seed samples from LL and HL-grown berries and from three developmental stages, and subsequently linked the metabolome and transcriptome data. In both photosynthetic tissues, the genes involved in Calvin-Benson cycle showed an increase in their transcript levels by HL microclimate as compared to LL, suggesting an active role of grape berry photosynthesis in determining the metabolite composition. In exocarp the HL microclimate resulted in an increase in flavonols and an up-regulation of associated genes and enzymes, while in seed of mature berries the stilbene pathway was down-regulated by HL microclimate, as compared to the LL microclimate. Overall, this study provides new insights for the role of berry tissue-specific photosynthesis in the metabolism of berries and in the final composition of grape products.

## 584 - Physiological changes in the shoots of *Spinacia oleracea* and *Pteris cretica* by arsenic toxicity

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In this study, we evaluated the As effect influencing the growth, nutrient dynamics and free amino acids (AAs) metabolism in the shoots of two plants with different ability in As accumulation - *Spinacia oleracea* and *Pteris cretica*. Plants were cultivated under arsenate treatment (0, 20 and 100 mg As/kg of soil) in a pot experiment with haplic chernozem. *S. oleracea* and *P. cretica* were harvested after 40 and 91 days of treatments, respectively. Nutrients were determined by ICP-OES and AAs by GC-MS. In both plants, stress tolerance index for growth showed stimulation of 20 mg As/kg treatment, while it was decreased by 100 mg As/kg treatment. Results of As bioaccumulation factor ( $BF_{As}$ ) showed a difference between plants. In *S. oleracea* shoots,  $BF_{As}$  decreased with increasing As treatment by 80 and 94% compared to control. In contrast, in *P. cretica* shoots,  $BF_{As}$  increased with increasing As treatment by 185 and 117% compared to control. Results showed difference between plants in nutrient dynamics and its change by As treatment. Compared to control, significant effect on Mg, Mn, and Zn showed 100 mg As/kg treatment, however, nutrients were affected differently in *S. oleracea* (decrease) and *P. cretica* (increase). N and S contents increased with same treatment in *S. oleracea* (by 11 and 30%, respectively) and *P. cretica* (by 16 and 83%, respectively). In change of other nutrients (Ca, Cu, Fe, K, and P) was not observed clear trend of As treatment. Furthermore, different response to As toxicity between plants showed total content of AAs, which increased with increasing As treatment in *S. oleracea* (by 30 and 35% compared to control) and decreased in *P. cretica* (by 43 and 53% compared to control). Total AAs content in plants confirms that the synthesis of AAs is affected by the level of As in the soil.

### 603 - Supercritical CO<sub>2</sub> extraction of squalene from amaranth seeds and in vitro antioxidant activities

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The extraction of high-added value bioactive compounds from different matrices of plant origin plays a key role in the field of phyto-pharmaceutical research. To solubilize these compounds is essential an appropriate solvent, with similar chemical properties. For non-polar compounds there are several valid solvents, but often dangerous for the environment and the operators.

Supercritical fluids (SFs) can be alternative solvents and, in particular, supercritical CO<sub>2</sub> is one of the elective SF for its temperature and pressure values suitable to reach the supercritical status. This kind of extraction shows several advantages compared to the classical techniques, because it is more eco-friendly and safer, but also useful for thermolabile compounds and not interested by oxidation reactions.

A chemical compound of high interest to the scientific community is squalene. This triterpene is finding several applications in fields such as cosmetics and nutraceuticals, for its strong antioxidant activity. High amounts of squalene are found in amaranth seeds. Amaranth (*Amaranthus* spp.) is a plant native of South America, cultivated for food production. It's a gluten-free cereal and almost 80% of its lipid fraction is composed by mono or polyunsaturated fatty acids.

The aim of this project was to evaluate and compare the cytotoxicity and antioxidant activity of both squalene and squalene-rich oleolite, extracted by supercritical CO<sub>2</sub>. The analysis performed by HPLC revealed 5% w/w squalene content in the amaranth crude oil. For in vivo tests, bovine aortic endothelial cells (BAE-1) were used as a model and increasing concentrations of pure squalene were used for the different treatments. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was used as positive control. At 24h, cell viability treated with squalene was higher than the one of the cells treated with 1mM H<sub>2</sub>O<sub>2</sub>. Co-treatment with squalene, hydrogen peroxide, and amaranth seeds extract are underway.

## **647 - Biological activities and accumulation patterns of fluorescently labelled auxins in *Arabidopsis thaliana***

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Many aspects of plant growth and development, including patterning and tropisms, are largely dependent on the asymmetric distribution of the plant signaling molecule auxin. Various approaches are used to either directly or indirectly monitor auxin distribution in vivo to elucidate the basis of its regulation. Fluorescently labelled auxins provide a great opportunity to study and visualize auxin transport and localization sites without the need of specific marker lines.

Herein, we report novel fluorescently labeled auxin derivatives, assessment of their biological activities and fluorescence properties. These compounds do not possess auxin activity but on the contrary, they inhibit auxin-induced effects, such as primary root growth inhibition and auxin reporter DR5::GUS expression in a dose-dependent manner. The most promising compound was further investigated for its capacity to mark subcellular and tissue-specific auxin localization by confocal microscopy.

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## **648 - Dried leaves of post-fire regenerated *Eucalyptus globulus* reveal great pre-emergent herbicidal potential**

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Due to its fire-adaptation traits, besides promoting a fire-prone environment, *Eucalyptus globulus* Labill. has a great post-fire regeneration capacity, mainly by bud resprouting, which ends up hampering the post-fire management efforts by locals. Therefore, it becomes urgent to develop new strategies for better management of eucalyptus populations, outside plantations. As *E. globulus* can inhibit the growth of neighboring plants, this allelopathic property can be targeted for weed control. Thus, this study aimed at assessing the pre-emergent herbicidal potential of eucalyptus leaves regenerated in a post-fire context in *Portulaca oleracea* L, a common weed species. For this purpose, fresh (FL) and oven-dried leaves (DL) were incorporated at different concentrations [0, 1, 5 and 10% (w/w)] into an artificial soil. After 2 weeks of stabilization, purslane seeds were sown. As a positive control, the pre- and post-emergent herbicide s-metolachlor was used. After five weeks of treatments, the results revealed that, although the FL incorporation at 5 and 10% (w/w) considerably affected purslane growth, it did not present any herbicidal effect since no seed germination inhibition or death of any plant was observed. However, the incorporation of DL at 10% (w/w) successfully inhibited seed germination by 63% comparing with the negative control (0% w/w), and the few resulting seedlings presented an interrupted development. Additionally, it was possible to detect that while the DL incorporation at 10 % (w/w) impaired the seed germination, the s-metolachlor only affected the seedling growth. Overall, this study showed that dried leaves of young eucalyptus trees have powerful potential to become a new biocide, capable of reducing the application of synthetic herbicides in the control of weeds. To unravel the mode-of-action of this biocide, ultrastructural and biochemical analyses are being carried out in purslane plants at the seedling stage.



**658 - Identification and characterization of a putative geranylgeranyl diphosphate reductase (TkGGDR1) from the natural rubber producing species *Taraxacum koksaghyz***

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The Russian dandelion *Taraxacum koksaghyz* produces high amounts of the isoprenoid-derived biopolymer natural rubber (NR) in its roots. In order to establish *T. koksaghyz* as commercially feasible crop however, further elucidation of NR biosynthesis for the breeding of rubber-enriched varieties is required. The main component of NR is poly(cis-1,4-isoprene) that is composed of 300-70,000 isopentenyl diphosphate (C<sub>5</sub>) units. Its biosynthesis is catalyzed by a complex of cis-prenyltransferases and rubber transferase activators at the surface of rubber particles in the cytosol of specialized cells called laticifers (latex). In a proteomics approach of latex, we identified a protein (TkGGDR1) with similarities to geranylgeranyl diphosphate reductases, which reduce the C<sub>20</sub> isoprenoid geranylgeranyl diphosphate to phytyl diphosphate. To elucidate the role of TkGGDR1 in isoprenoid metabolism in latex, we characterized the enzyme in homologous as well as heterologous organisms. TkGGDR1 is predominantly expressed in latex and on the subcellular level the corresponding protein locates to plastoglobules inside chloroplasts when expressed in *Nicotiana benthamiana*. The affinity of TkGGDR1 to lipid structures could further be shown upon heterologous expression in the yeast *Saccharomyces cerevisiae*, where it associates with lipid droplets. Further we performed comprehensive analyses of transgenic lines of *T. brevicorniculatum*, a close, apomictic relative of *T. koksaghyz*, with either enhanced or declined levels of latex-specific TkGGDR1/TbGGDR1 expression. TkGGDR1 overexpression resulted in decreased NR and triterpene contents, whereas TbGGDR1 silencing did not affect metabolite concentrations. Our findings indicate an impact of TkGGDR1 on isoprenoid-derived root and latex metabolites in dandelion. Further analyses will gain a deeper insight into the contribution of this enzyme to the complex network of isoprenoid metabolism in latex and its modulation within breeding approaches.

**684 - Glycomic and phytochemical profile of olive oil vegetative waters after membrane-based filtration to recover bioactive compounds.**

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Olive (*Olea europaea* L.) belongs to the Oleaceae family and it is mainly cultivated to produce olive oil, which is one of the cornerstones of the Mediterranean diet. The production of olive oil consists in several steps that produce several byproducts. In modern mill oil extraction processes (multiphase decanter), a by-product consisting in hydrated pulp with a high humidity (75-80%), called "paté", is produced. After 'paté' centrifugation, the vegetative water (VW) can be recovered as supernatant. The VW, usually discarded as a toxic fraction for both natural beneficial and pathogenic microorganisms, could be a source rich of bioactive molecules such as phenols, secoiridoids and oligosaccharides possibly effective in plant protection against pathogens.

In the present research, the isolated VW have been fractionated by various stages of membrane filtration (MF) (i.e microfiltration, ultrafiltration and nanofiltration) to identify and quantify bioactive compounds useful in agriculture as phytopesticides and/or agents in crop protection against pathogens.

The various MF fractions were analyzed by high-resolution NMR spectroscopy. Both the hydro and lipid-soluble fractions indicated the presence of tyrosol, hydroxytyrosol, oleuropein and lingstroside with a known antifungal and antimicrobial activity. Carbohydrate profile has been obtained by HPAE-PAD. Cell wall-derived pectin and oligogalacturonides (OGs) were identified. OGs are pectic fragments, elicitors of plant immune responses. Biological tests were performed to identify bioactive elicitors. Elicitation of cytosolic calcium transients, an early plant defense response to elicitors were observed in Arabidopsis plants expressing a GFP-based calcium biosensor (GCaMP3). In addition, a dose-dependent reduction of root growth in Arabidopsis seedlings, a well-known physiological response induced by elicitors, was observed in specific MFT fractions. This finding highlights the potential of these by-products as bio stimulant of plant defense responses.

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**694 - Two bi-functional cytochrome P450 CYP72 enzymes from olive (*Olea europaea*) catalyze the oxidative C-C bond cleavage in the biosynthesis of secoxy-iridoids - flavor and quality determinants in olive oil**

**ORNELLA CALDERINI - Carlos E Rodríguez-López - Benke Hong - Christian Paetz - Yoko Nakamura - Konstantinos Koudounas - Valentina Passeri - Luciana Baldoni - Fiammetta Alagna - Sarah E O'Connor**

Olive (*Olea europaea*) is an important crop in Europe, with a particular agricultural and economic relevance in the Mediterranean area. The nutritional significance of olive fruits and oil is widely recognized. Olive oil flavor and quality depend on phenolic secoiridoids, whose biosynthetic pathway is just beginning to be understood.

We report the characterization of two bifunctional cytochrome P450 enzymes, catalyzing the rare oxidative C-C bond cleavage of 7-epi-loganin to produce oleoside methyl ester (gene *OeOMES*) and secoxyloganin (gene *OeSXS*), both through a ketologanin intermediary. The reported enzymes are homologous to the known *Catharanthus roseus* secologanin synthase (*CrSLS*) but their substrate and product profiles differ. Biochemical assays and model-guided mutations allowed insights into the molecular basis and mechanism of the reactions.

Notably the results suggest that, in contrast to published hypotheses, in planta production of secoxy-iridoids is secologanin-independent. Genomic analysis of cultivated and wild olives suggest a relation between domestication and *OeOMES* expression, this relevant result has the potential to enable development of genetic markers to guide next-generation breeding programs.

#### 697 - Unravelling the biosynthetic pathway genes of olive (*Olea europaea*, L.) triterpenoids

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Olive triterpenoids (*Olea europaea*, L.), such as oleanolic and maslinic acids, are metabolites exerting numerous valuable properties. They contribute to the healthy effects of olive oil and can be used as bioactive components of pharmaceuticals and cosmetics. Despite olive is among the species accumulating the highest amount of these compounds, only a few genes underpinning their biosynthesis have been identified.

We searched for terpene synthases and cytochromes P450 involved in the biosynthesis of olive triterpenoids. Candidate genes have been cloned and functionally characterized in yeast (*Saccharomyces cerevisiae*) and/or plant (*Nicotiana benthamiana*) host systems. Putative  $\beta$ -amyrin synthase, a multifunctional  $\alpha$ -amyrin synthase and two candidate CYP450s were co-expressed in *N. benthamiana* leaves with a feedback-insensitive version of the upstream mevalonate pathway gene 3-hydroxy,3-methylglutaryl-CoA reductase (tHMGR). GC-MS assays have been performed on plant extracts. The analysis of the enzymatic products allowed to functionally characterize the target genes defining their role in triterpenoid biosynthesis. Our results will be applied for the development of molecular markers for health-related traits and to produce high amount of valuable compounds by metabolic engineering.

## **702 - Changes in the secretome of *Vitis vinifera* cv. Monastrell cell cultures treated with cyclodextrins and methyl jasmonate**

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Elicitors induce defense responses that resemble those triggered by pathogen attack, such as the synthesis of phytoalexins and pathogen-related proteins which are accumulated in the extracellular space. In suspension cultured cells of *V. vinifera* cv. Monastrell, the extracellular medium is vital for cell life because it is considered a dynamic cell wall compartment which plays a key role in both defense responses and nutrition. In this work, the study of differentially expressed proteins in the secretome of *V. vinifera* cv. Monastrell suspension cultured cells, which refers to secreted proteome obtained from cell cultures, in response to treatment with cyclodextrins and methyl jasmonate, separately or in combination using label free quantitative approaches have been analyzed. Among all the proteins found, thirty-three did not show significant differences among the different treatments carried out indicating that these proteins were expressed in a constitutive way in both control and elicited-grapevine cell cultures. These proteins include pathogenesis-related proteins 4 and 5, class III peroxidases, NtPRp-27, chitinases and class IV endochitinases, among others. Moreover, eleven proteins were differentially expressed in presence of cyclodextrins and/or methyl jasmonate: three different peroxidases, two pathogenesis related protein 1, LysM domain-containing GPI-anchored protein 1, glycerophosphoryl diester phosphodiesterase, reticulic oxidase, heparanase,  $\beta$ -1,3-glucanase and xyloglucan endotransglycosylase. The treatment with cyclodextrins reinforces the defensive arsenal and induces the accumulation of peroxidase V and xyloglucan endotransglycosylase. However, the elicitation with methyl jasmonate decreased the levels of several proteins such as pathogenesis related protein 1, LysM domain-containing GPI-anchored protein 1, cationic peroxidase, and a glycerophosphoryl diester phosphodiesterase whereas this signal molecule increased the levels of new gene products such as heparanase,  $\beta$ -1,3 glucanase, reticulic oxidase, and peroxidase IV, which could be used as potential biomarkers in the grapevine defense responses.

## TOPIC:

### Plant nutrition

#### **Keynote Lecture**

**Getting to the Root of Plant Mineral Nutrition: System genetics to study how plants make sense of various nutrient signals**

**Hatem Rouached** <sup>(1)</sup>

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Modern plant breeding especially in the last 50 years has been oriented to provide crops with high intrinsic growth rates and yields, under the condition that mineral nutrition is not limiting. Such conditions are obtained by massive use of fertilizers. The future of agriculture will undoubtedly require the use of uncultivated lands, some of them exhibiting unfavorable soil mineral composition, and reduction in the use of fertilizers in order to promote sustainable production. In a context of lower-input agriculture, new cultivated genotypes will need to be selected for improved nutrient use efficiency. Reaching this goal can be accomplished using a knowledge-based approach rooted in understanding how plants sense and signal changes in the availability of nutrients.

In nature, plants often cope with simultaneous deficiencies of more than one nutrient. Decades of research have led to a reductionist view of mineral nutrient homeostasis regulation in which each nutrient's level is controlled by its own mechanisms and signaling pathways. Indeed, to date, research in the field of plant mineral nutrition, has studied each nutrient individually. Although this is an important way to address the question, it can only give an incomplete view of the situation. Recent, yet limited, studies suggest however that there is complex coordination between the homeostasis of various mineral nutrients required for plant growth and that under combined nutrient stress, emergent properties arise that are distinct from individual nutrient stresses. I will present recent progress on our understanding on how plants co-regulate the homeostasis of different essential nutrients, which is required for devising future strategies to improve crop yield.

## TOPIC:

### Plant nutrition

#### Oral Communications

##### **187 - F-bZIPs – the Sensors and Molecular Switches for Plant Zinc Acquisition**

**Grmay H. Lilay<sup>(1)</sup> - Daniel P. Persson<sup>(1)</sup> - Pedro Humberto Castro<sup>(2)</sup> - Feixue Liao<sup>(1)</sup> - Ross D. Alexander<sup>(3)</sup> - Mark G.M. Aarts<sup>(3)</sup> - Ana G.L. Assunção<sup>(1)</sup>**

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Zinc is an essential micronutrient for all organisms, which plays key structural and regulatory roles in many proteins. Zn deficiency is widespread in agricultural soils, adversely affecting the yield and nutritional quality of crops. As a result, billions of people suffer from Zn malnutrition that causes/leads to serious mental and physical health problems. Since plants are at the base of the food chain, Zn biofortification of crops is a high priority in the fight against malnutrition. Plants maintain adequate Zn levels through tightly regulated Zn homeostasis mechanisms, involving proper Zn uptake, distribution and storage. Many of the genes involved in Zn homeostasis are induced transcriptionally under Zn deficiency. That is, plants respond to Zn deficiency by upregulating the expression of Zn transporters and chelators thereby enhancing their capacity for Zn uptake and distribution. In Arabidopsis, bZIP19 and bZIP23, members of the group-F bZIP (F-bZIP) transcription factors (TF) family, are the central regulators of these genes involved in the response to Zn deficiency. Their activity regulates the plant zinc levels through Zn-dependent changes in the expression of their target genes. The mechanisms by which these TFs sense cellular zinc levels and/or how the activity of these TFs is modulated by cellular zinc status remained unknown. Here, I will present results that show that the Arabidopsis F-bZIP TFs, bZIP19 and bZIP23, not only control the transcriptional response to Zn deficiency but also act as Zn sensors by binding Zn<sup>2+</sup> ions to their conserved Histidine/Cysteine-rich Zn sensor motif (ZSM). Deletion or modification of this ZSM disrupts Zn binding and induces a constitutive Zn deficiency response, which prompt plants to over-accumulate Zn. The identification of the first plant Zn-sensors will boost new strategies to improve the Zn nutritional quality of plant-derived food and feed and, thus, help tackle the global challenge of Zn malnutrition.

**483 - ITPK1-dependent generation of inositol pyrophosphates is required for systemic regulation of phosphorus homeostasis**

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Phosphorus (P) is an essential element, and often limits crop growth as only a minor fraction of soil P is plant available. Because worldwide P-deposits that can be exploited to produce P-fertilizer are limited and because P is a strong, global pollutant of open water bodies, there is a high interest to increase crop P-efficiency. Furthermore, a large portion of P is present as phytic acid in plant seeds that represents an antinutrient for humans and non-ruminant animals as it renders essential micronutrients such as iron and zinc unavailable. In plants, phosphate (P<sub>i</sub>) homeostasis is regulated by the crosstalk of P<sub>i</sub> starvation response transcription factors (PHRs) with stand-alone SPX proteins, which act as sensors for inositol pyrophosphates (PP-InsPs), raising the importance of these molecules for P<sub>i</sub> signaling. Arabidopsis thaliana ITPK1 was shown recently to generate 5-InsP<sub>7</sub> from InsP<sub>6</sub> in vitro. We will present evidence that disruption of ITPK1 impairs P<sub>i</sub>-dependent InsP<sub>7</sub> and InsP<sub>8</sub> synthesis in planta and leads to uncontrolled P<sub>i</sub> accumulation. In addition, we will show how nuclear magnetic resonance assays and PAGE analyses with recombinant protein reveal that besides InsP<sub>6</sub> pyrophosphorylation, ITPK1 also functions as ATP synthetase using 5-InsP<sub>7</sub> but not any other InsP<sub>7</sub> isomer as a P-donor when ATP is low. Furthermore, we will show that the dynamic changes in InsP<sub>7</sub> and InsP<sub>8</sub> to cellular P<sub>i</sub> are conserved from land plant species to human cells, suggesting that P<sub>i</sub>-dependent PP-InsP synthesis is a common component of P<sub>i</sub> signaling across kingdoms. Together, our study demonstrates how P<sub>i</sub>-dependent changes in nutritional and energetic states modulate ITPK1 activities to fine-tune the synthesis of PP-InsPs. To potential use the knowledge to breed more P-use efficient and healthy plants with reduced phytic acid content will be discussed.



## TOPIC:

### Plant nutrition

#### **Extended Elevator Pitches**

**150 - Effect of different biostimulants on tomato crop performance grown under combined nutrient and water stress**

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Drought stress is considered one of the most important abiotic factors limiting plant growth. On the other hand, plants can be exposed to nutrient stress in the future, as the use of fertilizers in agriculture tends to decrease due to constraints imposed via legislation to minimize the negative environmental impact of their residues. Tomato is a water and nutrient demanding plant and yield can be severely restricted by both water and nutrient stress. In view of the above background, three different biostimulants were tested as means to minimize combined water and nutrient stress in tomato cultivated in an experimental greenhouse of the Laboratory of Vegetable Production at the Agricultural University of Athens. More specifically, tomato plants 'Nostymi F1' grown hydroponically on rockwool were exposed to combined nutrient and water stress by reducing water and nutrient (N, P) supply to 60% of that applied in the non-stress treatments. Three different biostimulants were applied to the plants: a) "StrigoLab's biostimulant", a novel biostimulant with Strigolactones, b) "Coupe Regeneracion Plus" in combination with "Procuaje Radicular" which contain regenerating proteins, soil extracts of plant origin and precursors of fertilization, respectively and c) "Maxicrop", an organic biostimulant based on seaweed extracts. Plant vegetative growth, leaf nutrient concentrations, total fruit production and fruit quality characteristics (acidity, total soluble solids) were determined. The results showed that combined water and nutrient stress decreased plant growth and total fruit yield. Moreover, application of StrigoLab's biostimulant increased the fruit production while the reverse was the case when either Coupe Regeneracion Plus combined with Procuaje Radicular, or Maxicrop were applied. Fruit quality was generally not affected by combined stress or biostimulant application. In conclusion, StrigoLab biostimulant can be considered a promising biostimulant that might reduce the negative impact of water and/or nutrient stress on tomato growth and production.

## **160 - PGPR isolated from the rhizosphere of plants grown under harsh environments enhance tomato seedling performance under abiotic stress**

**Katerina Karamanoli**<sup>(1)</sup> - **Ifigeneia Mellidou**<sup>(2)</sup> - **Anastasia Papadopoulou**<sup>(1)</sup> - **Kleopatra Leontidou**<sup>(1)</sup> - **Savvas Genitsaris**<sup>(3)</sup> - **Nathalie Kamou**<sup>(1)</sup>

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Plant rhizosphere is considered as a hot spot of microbial activity and harbors a wide range of bacteria, many of them acting in concert with the host plant and exerting positive effects on their performance. These plant-growth-promoting rhizobacteria (PGPR) aggressively colonize plant roots of many plant species, and biotic interactions with beneficial associations for both partners are established. Among them, resilience against abiotic stresses is of major importance since these stresses consist serious constraints to plant growth and crop production worldwide. Under this scope, we isolated a wide range of bacterial strains from the rhizosphere of plants grown under severe saline conditions, and evaluated their potential PGP properties selecting those with the most desirable combination of characteristics. As a further step, we studied their ability to colonize tomato plants as well as their in vitro impact on tomato seedlings under stress conditions. In particular, the effect of selected PGPR inoculum on tomato seedlings against salinity stress was evaluated by measuring agronomic characteristics (plant height and biomass), physiological parameters (chlorophyll content index, quantum yield, net photosynthesis, relative water content), biochemical traits (H<sub>2</sub>O<sub>2</sub>, MDA content and antioxidant capacity), as well as their metabolite profiles by GC-MS analysis. According to our results, several bacterial strains were successfully established on tomato seedlings, which showed higher germination rate than the controls. Among these strains, two found very efficient in augmenting salinity tolerance of tomato seedlings. Based on whole genome sequencing on Illumina MiSeq platform, these strains were grouped in *Pseudomonas oryzae* family. Our research on both strains is ongoing in order to evaluate their impact on plant metabolomics and also their performance under field conditions.

**399 - Systemic responses in two hazelnut genotypes to the colonization by the black truffle *Tuber melanosporum***

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It is well known that the hazelnut, in addition to being an economically relevant fruit plant, is also a host plant for fungi belonging to the genus *Tuber* that, as symbiotic fungi, can offer nutritional advantages to the plant and also represent themselves a priced food product (truffles). The main objective of this work was to evaluate the impact of the colonization with *Tuber melanosporum* on two different hazelnut genotypes: the wild, which is naturally colonized by *T. melanosporum*, and the Tonda Gentile delle Langhe (TGL). The transcriptomics profiles in the leaves of the two genotypes colonized and not colonized with *Tuber melanosporum* have been evaluated through an RNAseq experiment, and the polyphenolic compounds have been analyzed by HPLC-DAD. Comparison between leaf transcriptomes of the two genotypes by RNAseq allowed to identify differentially expressed genes (DEG) associated to their phenotypic differences, including differential regulation of several genes involved in flavonoid biosynthesis. Results of biochemical analysis showed that the wild genotype has a higher content of flavonols, in particular of myricetin and quercetin derivatives, compared to TGL genotype. In inoculated plants, foliar large-scale transcriptomics revealed a significant expression repatterning of both hazelnut genotypes after fungal colonization. The two genotypes shared several DEG putatively involved in systemic response to *Tuber melanosporum* root colonization. In addition to this common set, each genotype regulated specific set of genes. From a biochemical point of view, the colonization with *Tuber melanosporum* significantly increases the content of flavonols in both genotypes, whereas it does not affect the content of other polyphenols (caffeic acid and gallic acid derivatives) found in hazelnut leaves. Overall, the data obtained will provide novel information about the impact of a symbiotic fungus on hazelnut metabolism at systemic level and will be useful for the development of hazelnut cultivation associated with truffle production.

## TOPIC:

### Plant nutrition

#### Posters

##### **561 - Influence of magnesium on photosynthesis and photoprotection in barley (*Hordeum vulgare* L.)**

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Under Mg deficiency, the photosynthetic capacity is limited and the balance between the production of reactive oxygen species (ROS) -which are the byproducts of photosynthesis- and their quenching activity is destroyed. Hence, the photoprotective mechanism non-photochemical-quenching (NPQ) increases in order to protect the plants from oxidative damage. To investigate the impacts of low Mg on different photosynthetic processes, four different Mg supply levels (1 (control), 0.05, 0.025, 0.015 mM) were applied on hydroponically cultivated barley plants. After 17 days under Mg deficiency (DuD), CO<sub>2</sub> assimilation, photosynthetic efficiency, NPQ, and electron transfer rate (ETR) were measured on the youngest fully expanded leaves. At the 21 DuD, samples for gene expression of ROS scavenging enzymes and xanthophyll cycle pigments quantification were harvested. Results showed that lowest Mg supply (0.015 mM) reduced assimilation rates by 78% of the control at DuD. NPQ increased within all treatments in comparison with the control. At the lowest Mg level, photosynthetic efficiency and ETR was decreased by 17% and 43%, respectively. At 17 DuD, the assimilation at 0.015 mM was decreased to 21.3% of the control. The gene expression of ROS scavenging enzymes such as Cu/Zn-SOD, CAT1 and GR was upregulated. Xanthophyll pigments quantifications revealed that at 0.025 and 0.015 mM Mg treatments, the zeaxanthin (Zx) concentration was increased, which is one of the main elements in NPQ. In this experiment, as the plants developed, the need for more Mg was observed while a decline in different photosynthetic parameters and assimilation rate was measured. Simultaneously, with very low Mg supply, an increase in the VAZ-pool (violaxanthin+ antheraxanthin+ zeaxanthin) revealed involvement of xanthophyll cycle in mitigation of oxidative stress. Considering Mg as an essential element in photosynthetic machinery, scarcity in its supply has a pronounced effect on photosynthetic efficiency and photoprotective mechanisms.

## 609 - Intercropping with *Poa pratensis* alleviates Fe chlorosis in *Brassica oleracea*

Teresa Saavedra <sup>(1)</sup> - João Costa <sup>(1)</sup> - José Paulo Da Silva <sup>(2)</sup> - Maribela Pestana <sup>(1)</sup> - Pedro José Correia <sup>(1)</sup>

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Iron (Fe) chlorosis is very common in plants cultivated in calcareous soils of the Mediterranean basin. When faced with Fe chlorosis, plants upregulate several mechanisms that may be grouped into two distinct strategies for iron acquisition: Strategy I or reducing strategy, and Strategy II or complexant strategy. In this case, phytosiderophores (PS) are released by the roots, producing stable Fe-PS complexes.

To study if horticultural plants may benefit from Fe-PS complexes, we established an intercropping experiment using *Brassica oleracea* L. (Strategy I) and *Poa pratensis* L. (Strategy II) in hydroponics. Young *Brassica* plants were grown alone (monocropping-MC) under three Fe levels in nutrient solution: without Fe (Fe0), with 5 mM of Fe as Fe-EDDHA (control, Fe5) and with a low concentration of Fe (Fe1). Additionally, one *Brassica* plant with 10 cm were grown together with 30 g of fresh *Poa* plants (intercropping-IC) in the same pot submerged in the nutrient solution previously described for MC. At least six *Brassica* plants with or without *Poa* plants were used per treatment. All plants were monitored three times a week for chlorophyll (Chl) using a SPAD apparatus, and the activity of root ferric chelate – reductase (FC-R; EC 1.16.1.17) was measured at the end of the experiment. Thirteen days after Fe deprivation (Fe0 and Fe1), *Brassica* plants, when growing alone, became chlorotic, as expected. However, in the IC system with no Fe (Fe0), a temporary greening of the young leaves of *Brassica* was observed together with an increase of FC-R activity. The results probably suggest that the Fe-PS complexes released by grasses were available to the dicot, improving their Fe root uptake. This positive effect makes IC an attractive practice and may replace, at least partially, addition of synthetic Fe chelates to crops in calcareous soil conditions.

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## **616 - Identification of *Medicago truncatula* F-bZIP transcription factors and their role in the zinc deficiency response**

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Plant F-bZIP transcription factors play a central role in the transcriptional regulation of the Zn deficiency response, with evidence of evolutionarily conservation of this regulatory network in land plants. Fundamental knowledge on the Zn deficiency response and Zn homeostasis regulation in plant crop species will contribute to developing crops with improved Zn nutritional content (biofortified) and improved resilience to Zn deficient soils.

Zinc-deficient soils are widespread globally and human Zn malnutrition affects about one-third of the world's population with a negative impact on growth, immune system and cognitive functioning, being more prevalent in populations that rely on cereal grains as staple food. However, micronutrient deficiencies are also becoming an issue in developed countries. Legumes are, in general, protein-rich crops, resilient in nutrient-poor soils, used worldwide as part of traditional diets and as animal forage, being therefore an attractive target for micronutrient biofortification.

Our recent work revealed that the *Arabidopsis* F-bZIPs, bZIP19 and bZIP23, the central regulators of the Zn deficiency response, also function as Zn sensors, through a direct binding of Zn<sup>2+</sup> ions to their Zn-sensor motif (ZSM), i.e. a cysteine/histidine-rich motif at the protein N terminus. These results indicated that it is plausible that the activity of F-bZIP homologues from crop species can be modulated, through modifications in the ZSM, to improve Zn content in plants.

Here, we present the identification of the F-bZIP homologs in the model legume *Medicago truncatula*. We identified two MtF-bZIP proteins and investigated their function in the Zn deficiency response. Our results suggest conservation of this regulatory network in *M. truncatula*. In addition, we performed a phylogenetic analysis with F-bZIP homologs from representative legume species. Fundamental knowledge on the Zn deficiency response in *M. truncatula* and legume crops will contribute to develop plant-based strategies to address the problems of Zn deficiency in soils, crops, and human diets.

## **622 - The Application of a Plant Biostimulant Based on Seaweed and Yeast Extract Improved Tomato Fruit Development and Quality**

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In the last decades, the world population has been constantly growing and its complete feeding is actually considered a hard challenge. In the recent years, with the aim to find new areas to use as agricultural land, agrochemicals and organic fertilizers were successfully used to allow the growth of plants also under adverse environmental conditions. However, these agricultural practices have resulted not only in a well-awaited intensification of food production, but also in serious environmental pollution. In this context, plant biostimulants are under investigation as innovative products to improve plant production and fruit quality, without resulting in environmental and food contaminations.

In this work, the effects derived from the application of a biostimulant based on seaweed and yeast extract, on plant productivity, fruit ripening times, and fruit quality of *Solanum lycopersicum* var. Micro-Tom were evaluated. After biostimulant treatment, a two-week reduction of ripening times and a concomitant enhancement of the production percentage during the earliest ripening times (fruit yield: +110%; fruit size: +85%) were observed. Concerning fruit quality, proximate analysis showed that tomatoes treated with the biostimulant had better nutritional composition compared to untreated samples, since both the quality of unsaturated fatty acids (C16:3 $\omega$ 3: +328%; C18:2 $\omega$ 6: -23%) and micronutrients essential for human health (Fe: +14%; Cu: +21%; Zn: +24%) were increased. From a nutraceutical point of view, despite strong changes in bioactive compound profile were not observed, an increase of the antioxidant properties was recorded in fruits harvested by plants treated with the biostimulant (ABTS: +38%; DPPH: +11%). In conclusion, the biostimulant application was able to reduce the ripening times, increase fruit yield, size and slightly nutritional and nutraceutical values, leading to more marketable tomato fruits.

## **625 - Phenotypic and Ionomic Characterization of Arabidopsis Zinc-Sensor Motif Mutant Lines**

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Arabidopsis F-bZIP transcription factors, bZIP19 and bZIP23, play a central role in the transcriptional regulation of the Zn deficiency response. Under Zn deficiency, they bind to Zinc Deficiency Response Elements (ZDRE) in the promoters of their target genes that are transcriptionally activated. Target genes include Zn homeostasis genes involved in Zn transport and distribution, which are upregulated and constitute the primary response to Zn deficiency.

Our recent work revealed that the Arabidopsis bZIP19 and bZIP23, in addition to being the regulators of the Zn deficiency response, also function as Zn sensors, through a direct binding of Zn<sup>2+</sup> ions to their Zn-sensor motif (ZSM), i.e. a cysteine/histidine-rich motif at the protein N terminus. Under Zn sufficient conditions, the Zn-protein binding at the ZSM represses the bZIP19 and bZIP23 transcriptional activity. Because deletions or modifications of the ZSM likely disrupt Zn-protein binding, such modifications in the F-bZIP protein disrupt its Zn-sensor function. Analysis of Arabidopsis lines with bZIP19 ZSM variants showed a Zn-insensitive and constitutive transcriptional activation of the bZIP19/23 target genes, causing a significant increase in plant and seed Zn accumulation. Here, we further investigate how modifications in the ZSM of the Arabidopsis bZIP19 transcription factor affect plant Zn homeostasis and Zn accumulation traits. We analyzed plant performance parameters and phenotypes in different Arabidopsis ZSM mutant lines grown with different levels of Zn supply.

Zinc-deficient soils are widespread globally and human Zn malnutrition affects about one-third of the world's population. Fundamental knowledge on the Zn deficiency response anchored at the F-bZIP transcription factors in model Arabidopsis and crop species, will contribute to developing crops with improved Zn nutritional content (biofortified) and improved resilience to Zn deficient soils.



## **6 - PROXIMATE ANALYSIS AND MINERAL COMPOSITIONS OF ANDROPOGON TECTORUM-ANDROPOGON GAYANUS COMPLEX IN SOUTHWESTERN, NIGERIA**

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Andropogon gayanus- Andropogon tectorum complex from Southwestern Nigeria were evaluated for their nutritional composition using standard methods. Field trips for plant collection covered agro-ecological zones of the following states in Nigeria: Osun, Ondo, Ogun, Oyo and Ekiti. Whole plants of Andropogon gayanus- Andropogon tectorum were collected from different locations. Accession numbers were given to the specimens. Seeds were also collected for planting and preservation. Garden populations were raised from the vegetative parts of some accessions and the hybrids were also maintained in Botanical Garden of the Obafemi Awolowo University, Ile- Ife, Osun State, Nigeria. The accessions were nurtured to maturity and used for these studies. The parts of the plants that were used were leaves and young stems from each of the species studied which were analyzed for proximate and mineral contents.

The result of the analysis showed that the moisture contents were moderate in all plant parts used (45.56%-71.46%). The highest moisture contents was recorded stem of *A. gayanus*. The percentage Fat content and Nitrogen contents were low in all the accessions studied (0.20-0.38%) and (0.60-0.90%) respectively. The lowest value of crude fibre content was recorded in the leaves and stems of *A. gayanus*. The lowest value of dry matter content was recorded in the stem of *A. gayanus*. The lowest value of ash content was recorded in the stem of *A. gayanus*. The lowest and the highest values of Lignin contents were recorded in the leaves and stem of *A. gayanus*. *A. gayanus* was the lowest in the source of protein (3.75-3.78%). The carbohydrate contents ranged between 19.92 and 42.02%.

The lowest and the highest values of mineral nutrients were recorded in the leaves and stems of *A. gayanus*. Calcium, Magnesium and Potassium were the most abundant minerals in the accessions studied. Leaf of Transition zone has the highest value of Sodium.

These results indicated that the highest value of Moisture content in the stem of *A. gayanus* explained the reason for the lodging of the plant when transplanted in the Botanical garden. It also revealed the potentials of the complex as fodder, bioremediator and industrial raw materials.

Key words: *A. gayanus*, *A. tectorum*, Accessions, Mineral composition.

### **30 - A fungal endophyte increases plant resilience to abiotic stress and disease/pest resistance: molecular dissection of beneficial plant-fungal interactions**

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An endophytic *Fusarium solani* strain (FsK) was isolated from the roots of tomato plants grown on a suppressive compost. The fungus is capable of protecting the plants against root pathogens and to elicit induced systemic resistance in tomato plants. The protective ability of strain FsK prerequisites plant responses is mediated by the ethylene and ABA signalling pathways. Moreover, root colonization by FsK affected the plant response to spider mites and enhanced indirect tomato defense as FsK-colonized plants attracted more predators than un-colonized plants. The fungus is capable of promoting plant growth under nutrient deficiency conditions and alleviates water stress. Colonization can be established in other plant species. In the FsK-Lotus japonicus association, fungal adaptations to endophytic growth as well as plant cellular responses to first encounter and intracellular accommodation of the fungus resemble typical characteristics, common among symbionts and pathogens. In addition, the Common Symbiotic Signalling Pathway that is triggered for the accommodation of both rhizobial and arbuscular mycorrhizal symbionts in legumes, is also required for the FsK-legume interaction. This novel plant-endophytic interaction delineates the continuum that appears to be present in the host-microbe interactions and further extends the quest of the molecular means that are used by the plant to discriminate responses to microbes in the complex rhizosphere environment.

## 59 - Aluminum detoxification in Vochysiaceae species

**Marcelo Claro de Souza** <sup>(1)</sup> - **Simone Pádua Teixeira** <sup>(1)</sup> - **Thomas Christopher Rhys Williams** <sup>(2)</sup> - **Charlotte Poschenrieder** <sup>(3)</sup> - **Steven Jansen** <sup>(4)</sup> - **David Burslem** <sup>(5)</sup> - **Marcelo Henrique Ongaro Pinheiro** <sup>(6)</sup> - **Itânia Pinheiro Soares** <sup>(7)</sup> - **Tiago Venâncio** <sup>(8)</sup> - **Augusto Cesar Franco** <sup>(2)</sup>

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Al-accumulating species can accrue high concentrations of Al in their tissues without any apparent damage. Aiming to understand the mechanisms used by Vochysiaceae species from the Brazilian Cerrado to deal with Al toxicity we investigated: (1) the forms of Al storage in leaves; (2) the leaf Al, Ca and Fe concentrations; (3) the leaf concentration of organic acids and phenolic compounds. Leaf samples were collected in the field and the species were previously classified according to their natural distribution: species restricted to acidic soils (*Vochysia* sp.); species restricted to calcareous soils (*Callisthene* sp.); and species that occur on both (*Qualea* sp.). Using Al-27 NMR analysis we identified that all Vochysiaceae species bound Al with oxalate in leaves. However, *Callisthene* sp. was the only Vochysiaceae species that combined oxalate forms and catechin to detoxify Al in its leaves. All species showed leaf Al concentration above 5000 mg.kg<sup>-1</sup>. The leaf Al and Ca concentrations varied among the species. As expected, *Callisthene* sp. showed the highest concentrations of Ca and the lowest concentrations of Al in the leaves, while the contrary was observed for *Vochysia* species. All species showed similar leaf Fe concentrations. A significant correlation was observed between leaf Al and Fe concentrations, but no correlations were observed neither between leaf Al and Ca, nor between leaf Ca and Fe concentrations. The *Callisthene* species showed the highest leaf concentrations of citrate, malate and catechin, and the lowest oxalate concentrations. The opposite profiles of organic acids and catechin were observed for the *Vochysia* species, and an intermediate profile was observed for *Qualea* species. This investigation clarified that oxalate is the main organic acid used by the Vochysiaceae family to deal with Al-toxicity. In addition, these results suggest that Al may be involved in the prevention of Fe oxidative stress in these species.

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## 81 - Host cell cycle reactivation for fungal accommodation in arbuscular mycorrhizal symbiosis

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Arbuscular mycorrhizas (AM) are widespread symbioses between plants and soil fungi with a major role in soil nutrient uptake. In this study we investigated cell cycle reactivation in the root cortex, associated with AM fungal accommodation by combining targeted sampling of early root colonization sites, detailed confocal imaging, flow cytometry and gene expression analyses.

Protein-localization studies, by means of fluorescence microscopy, reported the recruitment of a GFP fusion with TPLATE, a cell-plate-associated endocytic marker, during the construction of the symbiotic interface. In particular, TPLATE-GFP labelling was evident at sites of cell-to-cell hyphal passage between adjacent cortical cells, where the perifungal membrane fuses with the plasmalemma. This is evocative of the co-optation of cell division-related membrane dynamics in symbiotic responses.

Moreover, promoter activation analyses showed that TPLATE expression is induced in cortical root cells colonized by AM fungi, alongside with the upregulation of other cell division transcripts. In the same area we also observed the occurrence of ectopic anticlinal cell division events, generating cells that were half the size of the surrounding cortical cells and persisted throughout root colonization.

Interestingly, recursive events of endoreduplication were also documented in the vicinity of intraradical hyphae, leading to a local increase in ploidy levels and the activation of endocycle specific markers. Cell division and endoreduplication anticipated the progression of fungal colonization and were limited to cells preparing for fungal accommodation in the cortical tissue. Both responses were absent in non-mycorrhizal *M. truncatula* mutants for symbiotic signalling pathway genes.

On this basis, we propose cell cycle reactivation, leading to both ectopic division and endoreduplication, as part of the host cell prepenetration responses that anticipate AM fungal accommodation in the root cortex. We speculate that this mechanism is part of a conserved strategy derived from the 400-Myr-old AM symbiosis and co-opted by more recent biotrophic partners.

### **83 - A systematic review and meta-analysis from 60 years of research on the essential plant nutrient magnesium**

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Magnesium (Mg) as an essential plant nutrient was intensively researched in numerous studies during the last decades. Although these single studies addressed the effect of Mg deficiency on biomass, CO<sub>2</sub> assimilation and production of ROS (reactive oxygen species), a summary evaluating the effect of Mg supply on plant growth and photosynthesis is so far missing. Hence, a systematic review and meta-analysis was performed. Data of 80 reports on the impact of Mg nutrition on plant growth and photosynthesis and 22 reports on its impact on photo-oxidative stress response were combined. Further 28 reports were used for calculating species-specific critical leaf concentrations. The objectives were to provide estimates of the magnitude of Mg fertilization on biomass formation, CO<sub>2</sub> assimilation, and anti-oxidative enzyme activities; and to calculate leaf Mg concentrations critical for net CO<sub>2</sub> assimilation under consideration of all relevant scientifically published data.

Averaged over all reviewed studies, the positive effect of Mg supply on root biomass (77% increase compared to Mg deficient) was greater than on shoot biomass (59%). Biomass partitioning between shoot and root varied due to different cultivation techniques. If plants were raised under adequate Mg supply during initial growth stages before exposing them to Mg deficiency, the shoot-root ratio was not affected. Otherwise, the shoot-root ratio significantly decreased in contrast to Mg deficient control plants. Critical Mg concentrations for assimilation were highly species-specific and were mostly higher than for biomass production. Concentration of ROS decreased under adequate Mg supply by 31 % compared to Mg deficient plants, resulting in decreased activities of most antioxidant enzymes and metabolites under adequate Mg supply. In conclusion, our evaluation shows the specific requirement of Mg for various crop species for maintaining physiologically important processes and thereby may help to optimize fertilization strategies.

## 84 - Influence of magnesium on photosynthesis and photoprotection in barley (*Hordeum vulgare* L.)

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Under Mg deficiency, the photosynthetic capacity is limited and the balance between the production of reactive oxygen species (ROS) -which are the byproducts of photosynthesis- and their quenching activity is destructed. Hence, the photoprotective mechanism non-photochemical-quenching (NPQ) increases in order to protect the plants from oxidative damage. To investigate the impacts of low Mg on different photosynthetic processes, five different Mg supply levels (1 (control), 0.05, 0.025, 0.015, 0.01 mM) were applied on hydroponically cultivated barley plants. At 10 and 17 days under Mg deficiency (DuD), CO<sub>2</sub> assimilation, photosynthetic efficiency, NPQ, and electron transfer rate (ETR) were measured on the youngest fully expanded leaves. At the 21 DuD, samples for gene expression of ROS scavenging enzymes were harvested. Results showed that lowest Mg supply (0.01 mM) reduced assimilation rates by 76% at DuD. NPQ increased within all treatments in comparison with the control, and was highest with 36% at lowest Mg supply. At this Mg level, photosynthetic efficiency and ETR was decreased by 7.9% and 31%, respectively. At 17 DuD, the assimilation was 88.4% decreased at lowest Mg supply. At the same Mg level, NPQ decreased 11.3%, while increasing in other treatments. At lowest Mg supply, the photosynthetic efficiency, was reduced the most (24%). The reduction in ETR was observed in all treatments. In this experiment, as the plants developed, the need for more Mg was observed while a decline in different photosynthetic parameters and assimilation rate was measured. Simultaneously, with very low Mg supply, a complete decline in photoprotective mechanisms (NPQ in this experiment) was recognized. Considering Mg as an essential element in photosynthetic machinery, scarcity in its supply has a pronounced effect on photosynthetic efficiency and photoprotective mechanisms. ROS scavenging enzymes gene expression results will be included in the presentation.

## 172 - Purification of fungal chito-oligosaccharides as promoters of arbuscular mycorrhizal symbiosis

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Arbuscular mycorrhizal (AM) fungi are widespread root symbionts that improve nutrition and stress resistance in the majority of plants, including most crops. AM fungi are known to release short-chain chito-oligomers (Myc-COs), soluble chitin-related molecules that activate in the host plant a partially characterized signalling pathway, which includes the induction of repeated oscillations in nuclear  $\text{Ca}^{2+}$  concentration and the regulation of AM-related gene expression and root colonization by the symbiotic fungi.

This role of Myc-COs as symbiotic signals opens the way to their possible applications as stimulants of AM establishment in crops.

Commercially available short-chain COs analogous to Myc-COs are currently obtained through expensive and environmentally risky chemical processing of shrimp fishing industry wastes.

The aim of this work is to extract low-cost Myc-COs from the biomass of three fast-growing fungal species (*Pleurotus ostreatus*, *Cunninghamella bertholletiae* and *Tricoderma viride*), characterize such fungal-derived Myc-COs and test their biological activity on AM development.

Following chitin extraction and hydrolysis, NMR analyses were used to characterize the resulting Myc-COs, which resulted to be more acetylated than the commercial COs. We then compared the effectiveness of fungal- and shrimp-derived Myc-COs in 1) triggering nuclear  $\text{Ca}^{2+}$  spiking in epidermal cells of *Medicago truncatula* root cultures and 2) stimulate AM establishment in *M. truncatula* pot cultures inoculated with the AM fungus *Funnelliformis mosseae*.

Our results indicate that the purification protocol efficiently isolates functional Myc-COs from all three fungi and their bio-activity is stronger compared to commercial shrimp-derived COs. Production scale-up, the choice of cheaper substrates for fungal culture and an optimization of the extraction protocol are expected to reduce costs, making the use of Myc-COs in agricultural context an achievable goal in the next future.

**196 - Effects of different levels of P nutrition on tomato plants inoculated with the AM fungus *Funnelliformis mosseae* and grown in controlled conditions**

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Arbuscular mycorrhizal (AM) fungi are obligate biotrophs, depending on the host plant to carry out their life cycle. The symbiosis is mutualistic: plant provides photosynthates to the fungus and, in return, the fungus facilitates plant mineral nutrition (especially the phosphatic one) and water supply, improving plant growth and photosynthesis. The ability of AM fungi to colonize plant root systems not only depends on the specificity of plant-fungus interaction, but it is also strongly affected by the phosphorus (P) concentration in the soil. The aim of this work was to evaluate the effects of different levels of P fertilization (32, 96 and 288  $\mu\text{M}$ ) on AM colonization and on growth, nutrition and photosynthesis of tomato plants grown under controlled conditions.

At harvest the following parameters were recorded: fresh and dry weight of different plant organs; AM colonization (M%) in the root system; shoots and roots mineral (P, Mg, Mn, Fe, K and Ca) concentration; chlorophyll a, b and carotenoid concentrations in leaves and the expression of different PSII proteins. Moreover, to supplement the analysis on plant photosynthetic performance, the chlorophyll a fluorescence was measured in vivo on dark adapted leaves in order to assess the activity of the two photosystems (PSI and PSII). All the obtained data were subjected to ANOVA followed by Fisher's post hoc test.

Results showed that a high level of P in the growth medium on one side negatively affected AM colonization in tomato roots, while on the other side it enhanced plant biomass production. P concentration and AM inoculation differently affected the uptake of each micro/macronutrient considered. Chlorophyll fluorescence and PSII protein analyses suggested that P and AMF differently affected NPQ and electron transport efficiency, probably modulating the xanthophyll cycle.



**256 - Uptake and synthesis of organic iodine compounds in lettuce plants – role of vanadium and transcriptome analysis of iodine-enriched plants**

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The effect of vanadium (V) on iodine (I) uptake and metabolism in crop plants have not yet been described. Similarly, there is a lack of knowledge regarding the potential synthesis and role of iodosalicylates (ISAs), iodobenzoates (IBeAs) and plant-derived thyroid hormone analogs (PDTHAs) in plants. Within a three-year research project, seven independent experiments were carried out with lettuce cultivation in pots or a hydroponic system. A synergistic effect of V was noted on the uptake of trace amounts of iodine present in the rhizosphere. A transcriptome analysis of lettuce fertilized with iodine and vanadium was also performed. The activity of vanadium-dependent haloperoxidases was linked to the process of iodine uptake. The exogenous ISA and IBeA were taken up by roots and transported to the leaves. It has been determined that lettuce plants can synthesize 5-iodosalicylic acid (5-ISA), 3,5-diiodosalicylic acid (3,5-diISA), 2-iodobenzoic acid, 4-iodobenzoic acid, 2,3,5-triiodobenzoic acid, iodotyrosine as well as PDTHAs such as: triiodothyronine (T3) and thyroxine (T4). It has been found that exogenous 5-ISA and 3,5-diISA as well as inorganic KIO<sub>3</sub> can stimulate PDTHA synthesis in lettuce plants. Depending on the application of KIO<sub>3</sub>, 5-ISA and 3,5-diISA (separately and together with V), differentiated expression of selected genes in lettuce roots and leaves was noted including those responsible for the synthesis of: CBL-interacting serine/threonine-protein kinase 6 (which is most likely a PDTHA receptor), peroxidase 12, peroxidase 64 as well as S-adenosyl-L-methionine-dependent methyltransferase MAB\_3787. It should be mentioned that in agricultural practice, iodine biofortification of plants increases the content of organic iodine compounds in plants. This can have a positive effect on the consumer's organism. This research was financed by the NCN, Poland (grant UMO-2017/25/B/NZ9/00312).

## 291 - SEARCHING FOR ALTERNATIVE SOURCES FOR STARCH AND FLOUR PRODUCTION

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Starch is the most plentiful carbohydrate reserved in plants and one of the most important polymers which have been used extensively in food and non-food application daily. The aim is to produce commercial flour and starch from the modified stem of *Anchomanes difformis*, *Ipomoea trichantha*, *Colocasia esculenta*. The tubers were peeled, cut into small cubes, sun-dried and milled to obtain flour. This was homogenized with distilled water in a Waring commercial blender and starch was extracted by passing the slurry obtained through a double-layered cheesecloth. Proximate analysis and mineral composition of the flour, and physicochemical characterization and micromorphology of the starch were determined. The ranges of proximate analyses were: dry matter (92.64-93.07%), moisture content (6.93-7.36%), crude protein (5.01-5.99%), crude fat (2.68-3.72%), crude fibre (1.10-1.85%), total ash (1.92-4.27%) and carbohydrate (75.27-82.06%) in the three plants. Mineral composition ranges were: Na (4.00-4.17 mg/L), K (3.57-6.60 mg/L), Ca (1.00-1.63 mg/L), Mg (5.32-5.57 mg/L), Fe (0.05-0.08 mg/L), Zn (0.03-0.07 mg/L). Physicochemical characterization of the starch showed the range of bulk density (0.75-0.88), gelatinization temperature (60.23-70.33°C), pH (6.38-7.09), amylose content (33.15-34.94), pasting temperature (50.35-86.55°C), peak time (1.20-4.80 min), peak viscosity (471.00-2433.00 cp), trough viscosity (132.00-1663.00 cp), final viscosity (415.00-2924.00 cp), break down viscosity (339.00-770.0 cp) and setback viscosity (283.00-1244.00 cp). The micromorphology of starch granules was observed with the scanning electron microscope; the sizes and shapes of the granules were appropriate for industrial-based starch. The results from the studied plants compared favourably with the conventional flour and starches of cassava and corn, and are, therefore, potential candidates for industrial applications.

### **312 - Characterization of the ionic signatures of plants submitted to nutritional deficiencies and some potentially involved mechanism**

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It is suggested the ionome composition (i.e. macro, micro and beneficial elements) of plant tissues, resulting from multiple interactions between nutrients, can reveal plant physiological status. This study aims to identify elemental interactions and hence ionic signatures, resulting from different mineral deficiencies in *Brassica napus* and *Triticum aestivum*. Therefore, plants were submitted to 17 individual nutritional deficiencies (N, Mg, P, S, K, Ca, B, Cl, Mn, Fe, Ni, Cu, Zn, Mo, Na, Co and Se) and harvested before reduction of growth. The main objectives were to i) analyze plant tissue ionic signatures and compare the response of the both species, ii) identify some subjacent metabolic pathways, iii) and quantify the net remobilization of each nutrients.

In both species, the main results revealed elemental interactions already described that could be the consequence of the up-regulation of nonspecific root transporters, hence increasing the uptake of other elements available in nutrient solutions. For examples, a strong increase of divalent cation uptake was found under Fe deficiency, as it was also the case for Mo under S deficiency. An original interaction concerned a stimulation of vanadium uptake in plants submitted to S deficiency, probably a result of an over-expression of root sulfate transporters confirmed notably by ionome analysis of *Sultr1;1* and *Sultr1;2* knock-out *Arabidopsis* lines. Our study showed also another original (but negative) interaction between N and Na that was also supported using *Arabidopsis* lines knock-out for genes encoding nitrate transporters. Finally, in rapeseed roots deficient in macronutrients, specific and mutual physiological and molecular processes were identified using transcriptomic and metabolomic approaches.

**315 - Enterobacter sp. SA187 mediates plant thermotolerance by chromatin modification of heat stress memory genes**

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Global warming has become a critical challenge to food safety, causing severe yield losses of major crops worldwide. Here, we report that the endophytic bacterium *Enterobacter* sp. SA187 induces thermotolerance in *Arabidopsis thaliana* by reprogramming the plant transcriptome. Heat acclimation induces priming of heat stress memory genes such as APX2 and HSP18.2 via the transcription factors HSFA1A, B, D, and E and the downstream master regulator HSFA2. Similar to heat acclimation, SA187 colonized plants completely lose their priming capacity in *hsfa2* mutant but not in *hsfa1q*. However, SA187 induced priming via HSFA2 is also dependent on ethylene signaling that is completely compromised in *ein2-1* or *ein3-1* mutants. While heat acclimation transiently modifies H3K4me3 levels at heat stress memory gene loci, SA187 induces the constitutive priming of these loci. In summary, we demonstrate the molecular mechanism by which SA187 induces thermotolerance in *Arabidopsis*, suggesting the use of beneficial microbes to enhance crop production under global warming conditions.

### **385 - Improvement of Tomato Fruit Quality by using Arbuscular Mycorrhiza Fungi as Soil Additive in the Greenhouse**

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Arbuscular mycorrhiza (AM) describes a 400 million years old symbiosis between a soil fungus from the Glomeromycota family and the plant root. Thereby the plant benefits mainly from an improved phosphate supply and a higher tolerance against abiotic and biotic stresses. Additional profits for crop plants, such as a positive effect on tomato fruit quality, were reported more recently. In this respect, our cooperative project between research institutes and companies aims to determine and apply positive effects of AM on tomato fruit quality under commercial production conditions in the greenhouse. Therefore, several challenges needed to be addressed: i) search for an appropriate growth substrate, ii) finding fertilizer conditions suitable for commercial purposes and AM formation, and iii) determination of universal effects regarding different tomato cultivars and AM fungi. After several tests, a substrate containing a low coco percentage, the commercially used tomato cultivar Picolino and the commercially produced AM fungus *Rhizophagus irregularis* were selected. To detect AM effects on gene expression, green and red fruits of mycorrhizal and non-mycorrhizal plants were harvested to perform transcriptomics using an RNAseq approach. The results revealed the highest effect of AM presence on the gene expression of green fruits. Among 80 differentially expressed genes between fruits from mycorrhizal and non-mycorrhizal plants, genes encoding water- and lipid transport and cell wall related proteins as well as transcription factors could be identified. Metabolic profiling of red fruits mainly focusing on organic acids and amino acids as well as carotenoid contents will provide insights for putative direct positive effects of AM on the production of taste and health promoting substances in tomato fruits.

#### **428 - Proteomics and hormonomics to unravel the molecular bases underlying tomato-beneficial fungus interaction**

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During plant life, roots support beneficial associations with soil inhabiting microbes, mainly fungi and bacteria. Increasing evidence suggests that plant-associated microorganisms can promote plant growth and expand immune functions of the plant host. Some beneficial soil fungi are known to act as insect control agents, either through a plant-mediated response or by exerting a direct insecticidal effect. Among them, the entomopathogenic fungus *Beauveria bassiana* can colonize plant tissues in an asymptomatic way, triggering poorly characterized plant metabolic changes, which negatively affect both pest insects and plant pathogens. *B. bassiana* has an extremely broad host spectrum, including tomato (*Solanum lycopersicum* L.), a species of great economic importance which is widely cultivated all over the world. In order to obtain a great overall snapshot of molecular events regulating tomato-*B. bassiana* interaction, plant proteome and hormone changes induced over the time by the fungus have been in-depth analyzed by using a combination of high throughput profiling techniques and bioinformatics tools. Hormonomics data showed an interesting up-regulation of diverse hormones, among which are gibberellin precursors and active forms. The proteomics data highlighted interesting molecular pathways affected by *B. bassiana* related to primary and secondary metabolism and plant growth. Additionally, downregulation of a member of the endochitinase family and upregulation of calcium channel and transporter proteins suggested well-established plant-fungus symbiosis. Finally, the molecular pathways linked to protein/amino acids turn-over and to the biosynthesis of energy compounds shed some light on the strategies exploited by the plant to get the most out of the beneficial interaction in improving growth and development.

**509 - A novel experimental approach to characterize non-electrogenic vacuolar NHX proton/potassium antiporters: inhibition by phosphoinositides**

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In this work, we combined the patch-clamp technique with ratiometric fluorescence imaging using the proton-responsive BCECF dye as a luminal probe. Upon application of a steep cytosol-directed K<sup>+</sup> gradient in isolated Arabidopsis mesophyll vacuoles, a strong and reversible acidification of the vacuolar lumen was detected, while no associated electrical currents were observed, in agreement with electroneutral cation/H<sup>+</sup> exchange. Our data show that this acidification was generated by NHX antiport activity, since i) it did not distinguish between potassium and sodium ions, ii) it was sensitive to the NHX inhibitor benzamil, and iii) it was completely absent in vacuoles isolated from nhx1 nhx2 double knockout plants. Our data further show that NHX activity could be reversed, was voltage independent and specifically impaired by the low-abundance signaling lipid phosphatidylinositol-3,5-bisphosphate (PI(3,5)P<sub>2</sub>). Together with previous studies demonstrating that PI(3,5)P<sub>2</sub> inhibits the vacuolar anion/H<sup>+</sup> exchanger CLC-a, these data suggest that PI(3,5)P<sub>2</sub> may contribute to efficiently regulate salt accumulation in plants by acting as a common messenger to coordinately shut down secondary active carriers, which mediate cation and anion uptake inside the vacuolar lumen. Finally, we developed a theory based on thermodynamics, which supports the data obtained by our novel experimental approach. This work, therefore, represents a proof of principle that can be applied to the study of plant and animal proton-dependent exchangers, which are barely detectable using conventional approaches.

## **600 - RNAi silencing of SbPPC3, a non-photosynthetic PEPC, influences sorghum responses to phosphate deficiency**

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Phosphoenolpyruvate carboxylase (PEPC) (EC 4.1.1.31) is responsible for the initial fixation of atmospheric CO<sub>2</sub> in C<sub>4</sub> and CAM plants. In addition, it has a central function coordinating carbon and nitrogen metabolisms in different physiological contexts. Six different isoforms of PEPC (SbPPC1-6) exist in sorghum (*Sorghum bicolor* L.). This study is focused on SbPPC3, a C<sub>3</sub>-type enzyme. This isozyme plays key roles on seeds and roots, and also in responses to abiotic stresses. We generated modified sorghum plants by silencing the SbPPC3 gene by RNA interference (RNAi) (Ppc3 plants) and analysed the consequences on the responses to phosphate deficiency. In the absence of phosphate (-P), growth decreased, but the treatment with insoluble phosphate in form of calcium phosphate (PCa) partly recovered these values in WT and Ppc3 lines. The same results were observed for photosynthetic parameters. PCa treatment induced the phosphorylation of PEPC and the accumulation of anthocyanin in leaves from Ppc3 but not from WT plants. In leaves, PCa treatment, and particularly -P treatment, increased the expression of SbPPC2 in WT and transgenic lines and SbPPC3 only in WT, also SbPPCK1-3 expression increased in all plants. These last results were correlated with an increase in kinase activity measured in vitro in Ppc3 leaves. In roots, PEPC activity was increased by both treatments in WT roots, but not in Ppc3 lines, this correlated with the levels of SbPPC3 expression detected. Also, SbPPC2 expression was enhanced in WT and transgenic roots. Finally, total phosphate content in tissues was quantified, PCa treatment allowed to accumulate phosphate in roots to similar levels found in control conditions, but not in leaves. Altogether, these results suggest that SbPPC3 contributes to some of the responses to phosphate deficiency.

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## 629 - POTASSIUM METABOLISM IN ARABIDOPSIS PLANTS GROWN UNDER AMMONIUM NUTRITION

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Nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) are major nitrogen (N) sources for plants. Ammonium could be the preferred form, because its reduced state makes it less energetically expensive to assimilate. Surprisingly, when supplied as the sole nitrogen source  $\text{NH}_4^+$  leads to severe growth retardation and other toxicity symptoms commonly referred to as ammonium syndrome. Mechanisms of  $\text{NH}_4^+$  toxicity still remain unclear. Our research indicated that one of the factors responsible for ammonium syndrome in *Arabidopsis thaliana* is the increased formation of a toxic glycolytic byproduct, methylglyoxal (MG) or intermediates of its catabolic pathway, D-lactate or S-D-lactoylglutathione (SLG). MG degradation is catalysed via the two-step reduced glutathione-dependent glyoxalase pathway comprising glyoxalase I (GLXI) and glyoxalase II (GLXII). Our previous research showed that glycolytic flux is upregulated, which leads to enhanced MG formation indicated by increased triosephosphate isomerase (TPI) activity and higher MG concentration despite an efficient detoxification system. Published experimental results have suggested that MG metabolism may influence on potassium ( $\text{K}^+$ ) homeostasis. Potassium is one of the major nutrients, essential for plant growth and development. It is required in vital processes of primary metabolism, controlling cell guard movement, and maintaining cell turgor pressure. Our results suggest that a higher concentration of MG leads to change in  $\text{K}^+$  uptake and distribution causing potassium deficiency. Indeed, our research confirmed that long-term  $\text{NH}_4^+$  nutrition results in significantly lower (by 20%) potassium content in leaf tissue. Transcript levels were mostly downregulated for some genes encoding potassium channels and transporters (e.g. AKT1, KC1, TPK1, SKOR). Moreover our analyses suggest changes in  $\text{K}^+$  localization in plant tissue (confocal methods). In conclusion, ammonium nutrition can modify potassium homeostasis and reinforce ammonium syndrome development. This work was partially funded by grant 2014/14/E/NZ3/00155 from the National Science Centre (Poland) given to Bożena Szal.

## 660 - Changes in auxin metabolism caused by ammonium nutrition

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Plants can use  $\text{NO}_3^-$  or  $\text{NH}_4^+$  as a nitrogen source. When  $\text{NH}_4^+$  is a sole source of nitrogen, it causes many visible changes as decreased biomass and diameter of rosettes as well as shorter roots with more lateral roots. One of the substances responsible for the regulation of plant growth is phytohormone auxin (IAA). We discovered that long-term ammonium nutrition of *A. thaliana* in hydroponic culture changes transcript level of genes involved in IAA synthesis – lower in leaves and higher in roots compared to control. Also, a higher concentration of free auxin, oxidized auxin, and auxin conjugates as well as higher transcript level of genes involved in conjugation pathways was found in ammonium grown plants. Auxin response was examined using DR5::GUS and DR5::GFP lines. We concluded that altered metabolism of auxin during ammonium nutrition may contribute to growth inhibition and affect root morphology and therefore be responsible for development of characteristic symptoms for the ammonium syndrome.

Founded by Polish National Science Centre, grant nr 2018/29/B/NZ3/02687

## TOPIC:

### PlantHUB consortium

#### Posters

##### **354 - Starch biosynthesis in developing barley endosperm**

**Anton Hochmuth** <sup>(1)</sup> - Mercedes Thieme <sup>(1)</sup> - Monica Jane Emerson <sup>(2)</sup> - Jose Antonio Cuesta-Seijo <sup>(3)</sup> - Ilka Braumann <sup>(4)</sup> - Sam C. Zeeman <sup>(5)</sup>

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Plants polymerise simple sugars into starch and store it in form of water insoluble semi-crystalline starch granules until its degradation to provide the plant with energy. This starch is highly relevant for human nutrition, as it depicts the most important source of carbohydrates for feeding livestock and in human diets. Further, it has innumerable industrial applications, like in papermaking and brewing. The various industrial sectors utilise starch from a broad variety of plants to cover their specific requirements linked to certain properties of starch granules. These granules differ in shape, size and chemical composition among plant species and tissues. The barley grain stores starch in the endosperm so that sugar can be made available during later germination. These sugars are as well used in the brewing industry for the fermentation. Although the degradation of starch is well described, the formation of that in the barley grain is not yet sufficiently described to explain the bi-modal size distribution of starch granules. That distribution pattern is based on the existence of large A-granules and small B-granules. It is known that they form at different time points in the endosperm development. These events lack a detailed genetic description to understand the underlying mechanism leading to the specific starch morphology. Our transcriptomic analysis reveals differences within the endosperm across several developmental time points. In combination with strong visual descriptive 3D-methods like X-ray microtomography, we will show results with high spatial-temporal resolution and contribute to the understanding of the starch biosynthesis in barley grains.

### **365 - Reaching natural growth: Effect of asynchronous light and temperature fluctuations in indoor growth facilities**

**Camilo Chiang**<sup>(1)</sup> - **Daniel Bänkestad**<sup>(2)</sup> - **Guenter Hoch**<sup>(3)</sup>

**PhD Student, University of Basel, Basel, Switzerland**<sup>(1)</sup> - **Department of research and development, Heliospectra, Goteborg, Sweden**<sup>(2)</sup> - **Professor, University of Basel, Basel, Switzerland**<sup>(3)</sup>

Several studies have recommended the incorporation of environmental fluctuations in indoor experiments if closer-to-nature results in plant experiments is desired. However, evidence suggested that applying such fluctuations unsynchronized among environmental factors, leads to stressful conditions for plants, since plants have evolved to cope with synchronic environmental fluctuations. Following a series of experiments in indoor conditions, the present study aims to identify the effect of the disparity in the fluctuation of two important environmental variables, light quantity and temperature, on the growth of 7 plant species from different functional plant types. A factorial combination of light and temperature under either fixed or variable conditions in phytotrons was compared with field-grown plants of the same species. In all phytotron scenarios, the average of the environmental variables was the same as in the previous field trial. Productivity-, gas exchange- and leaf pigment-traits were recorded in all species at the end of the experiments. As in our previous studies, plant trait responses were highly dependent on species and treatment, but some general trends were observed. The addition of light fluctuations was responsible for an increase in specific leaf area (SLA), Chlorophyll A concentration, and reductions in total plant dry weight and chlorophyll A:B ratio. When light conditions were fixed but with variable temperature, several light-related plant traits were affected, with lower  $F_v/F_m$  values,  $A_{max}$  and  $CO_2$  yield. Under fixed light conditions and variable temperatures  $F_v/F_m$  increased compared with totally fixed or variable temperature and light conditions. Overall, our results highlight the necessity to incorporating light and temperature fluctuations in synchrony in order to reach more natural-like plant performance in indoor growth facilities.

### 377 - Large-scale isolation and sequencing of full chromosomes

**Diop Seydina Issa** <sup>(1)</sup>

**Department of Systematic and Evolutionary Botany, University of Zurich, Zurich, Switzerland** <sup>(1)</sup>

Land plants evolved from an charophycean algae over 400 Ma and shaped formation of first complex biosystems on earth. To investigate in the earliest evolutionary events that multicellular organisms have been exposed, bryophytes have been used in a different area of cell and developmental biology and evolutionary ecology to better understand plant environment and genetic footprint. The basal position occupied by the embryophytes in the tree of life gives a unique chance to investigate in the earliest events that multicellular organisms have been exposed during the first evolutionary timescales.

In this study, we used the liverwort model organism *Marchantia polymorpha*, to investigate autosomal and sex-specific gene patterns. First, we established a mapping population and assessed eight linkage groups corresponding to the eight autosomes of *M. polymorpha*. We put these groups into scaffolds to improve the genome assembly of *M. polymorpha* at a chromosomal level and polished our new genome assembly using long reads sequencing. This first study showed a rate of recombination with low variability across the *M. polymorpha* genome.

Further, we took part in the *M. polymorpha* re-sequencing project, in which around 100 different accessions have been sequenced (using Illumina Novaseq). We built up an improved reference genome of *M. polymorpha*, by combining the new assembly genome including eight autosomes plus sex-specific scaffolds previously identify in the v3.1 draft assembly.

Next, we selected a subset of 16 males and 16 females individuals to uncover the genetic variability of the autosomes versus sex chromosomes. Our first observations suggest the autosomal genes are more polymorph than sex-specific genes. We also noticed that variants identified on the autosomes had deeper sequence coverage than those on the sex chromosomes. Overall, our data showed that a large part, of the variants identified, is located in intergenic regions.

### **386 - Predicting models – next important step to check the origin of agricultural product in the market**

**Markus Dr. Boner** <sup>(1)</sup>

**Agroisolab GmbH, Agroisolab GmbH, Juelich, Germany** <sup>(1)</sup>

Up to now the stable isotope method is one of the leading scientific tools to check the origin of agricultural products. An enormous advantage of that method is the fact, that hydrogen and oxygen in water show different pattern in the region and countries. As the plants are more or less reflecting that water pattern, the stable isotopic method offers an effective opportunity to track the origin.

Therefore various databases have been developed as the European wine database, the German asparagus or the Finish strawberry database.

Any developed stable isotopic database is normally using defined reference samples of defined locations and defined time. Unfortunately the information quality of the origin check is significantly reduced if the reference data are from different harvest periods or different locations. That is due to the fact, that plants are fractionating the water signatures depending on climate and environmental conditions. Both are changeable in time. Furthermore that mentioned fraction effect in the plants is not universal, it is more or less depending on its structural building and water balance.

Therefore the various databases can only be used for the defined species, a transfer to another plant species is hardly possible. Those issues are restricting the use of stable isotopic databases.

In consequence a cooperation has been started with the University of Basel and Agroisolab GmbH to create models for a universal prediction of the origin only based on physical parameters such as the relative humidity.

That prediction model needs on one hand an opportunity of experiments in climate-controlled growth chambers and on the other hand a verification of the experimental data with big reference databases from e.g. European regions. Therefore the cooperation between a university and a private company could be very effective as the Agroisolab GmbH delivered the relevant databases and the university the experimental opportunities. Now a first prediction model is existing which proved the mandatory reference sample as no longer essential.

## TOPIC:

### Plants in extreme environments

#### **Keynote Lecture**

**KEYNOTE PRESENTATION: The life-history spectrum in natural *Arabidopsis thaliana* along wide environmental gradients**

**Xavier Picó** <sup>(1)</sup>

**Consejo Superior de Investigaciones Científicas, Estación Biológica de Doñana, Sevilla, Spain** <sup>(1)</sup>

For a given organism, extreme environments are those that delimit its distribution range. Beyond particular environmental thresholds (e.g. temperature, water availability), populations cannot remain viable over time. In addition, extreme environments also impose severe selective pressures, which may enhance strong adaptation and speciation events right below the environmental thresholds determining population absence. Such edge populations occur at the geographic and environmental periphery of the distribution range, and the comparison between edge and core populations has long fueled a solid theoretical background to understand the distribution of life on this planet. Interestingly, this is not a static process, but highly dynamic (e.g. glacial and pre-glacial cycles because of warming and cooling periods), meaning that organisms are in a continuous struggle for existence. However, many of them have the means to cope with contrasting environments because they long survived in regions that experienced dramatic environmental changes or because they occur along wide environmental gradients spanning from benign to quasi-extreme conditions. Clearly, acquiring an in-depth knowledge of the ecological and genetic basis underlying adaptive variation to contrasting environments is of paramount importance to understand how evolution works. Here, I will present ecological, genetic and evolutionary evidence of adaptive variation in natural populations of the annual plant *Arabidopsis thaliana*, which occurs from seaside to alpine locations. I will focus on our long-term research conducted on *A. thaliana* across SW Mediterranean Basin, the region of the species' distribution range with the highest genomic and environmental diversity. I will show how *A. thaliana* can thrive in a wide array of environments in which abundance give us clues about the severity of the environments and the effects that increasing environmental severity has on *A. thaliana*. Finally, I will also present data on the potential response of *A. thaliana* in the challenging scenario of today's rapid global warming.

## TOPIC:

### Plants in extreme environments

#### Oral Communications

##### 166 - Global warming: friend or foe for the survival of the *C. quitensis* antarctic ecotype?

Laura Bertini <sup>(1)</sup> - Silvia Proietti <sup>(1)</sup> - Gaia Salvatore Falconieri <sup>(1)</sup> - Ilaria Iacobucci <sup>(2)</sup> - Angela Amoresano <sup>(2)</sup> - Maria Monti <sup>(2)</sup> - Piero Pucci <sup>(2)</sup> - Carla Caruso <sup>(1)</sup>

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Antarctica is one of the last pristine environments in which endemic organisms have progressively specialized to deal with very harsh weather conditions, living often at their physiological limit. Only two vascular plants, *Colobanthus quitensis* and *Deschampsia antarctica*, have been able to establish and survive in Antarctica, more precisely in the Peninsula, and their colonization has been increasing in the last five decades as a consequence of the rise in temperature due to the recent global warming. In this scenario, a new target in modern cell physiology is the study of the genetic and molecular traits of their adaptation to the rapidly changing environmental conditions which may permit the disclosure of molecular biomarkers for efficient climate change monitoring. To this, we performed differential proteomic and metabolomic analyses on *Colobanthus* plants grown in natural conditions (OUT samples) compared to plants grown for one year inside small greenhouses open on the top (OTC samples) which determine an increase of about 4 °C during midday, mimicking the effect of global warming. Interestingly, we found an important role of RUBISCO oxygenase in reducing ROS-mediated photodamage which is enhanced in plants grown at warmer temperature. On the other hand, plant resistance to abiotic and biotic stresses seems to be strongly favored by the interaction with rhizosphere microorganisms. Recently, we performed a metatranscriptomic analysis on *Colobanthus* leaves revealing the presence of many microbial species (fungi, bacteria, algae and viruses) associated to the aerial part of the plant too. Their role in the adaptation of *Colobanthus* to rapidly changing environmental conditions has been also investigated, comparing the metatranscriptomic data of OUT and OTC samples. Interestingly, most of the microbial differentially expressed genes were found to be up-regulated in plants grown in warmer conditions and many of them were involved in biosynthetic process of compounds functional to plant growth.



**436 - Do Ni-hyperaccumulators manage the high amount of metal in their shoots without affecting the photosynthetic activity?**

**Andrea Scartazza <sup>(1)</sup> - Daniela Di Baccio <sup>(1)</sup> - Lorenzo Mariotti <sup>(2)</sup> - Isabella Bettarini <sup>(3)</sup> - Federico Selvi <sup>(4)</sup> - Luigia Pazzagli <sup>(5)</sup> - Ilaria Colzi <sup>(3)</sup> - Cristina Gonnelli <sup>(3)</sup>**

**National Research Council, Institute of Research on Terrestrial Ecosystems, Pisa, Italy <sup>(1)</sup> - University of Pisa, Department of Agriculture, Food and Environment, Pisa, Italy <sup>(2)</sup> - University of Florence, Department of Biology, Firenze, Italy <sup>(3)</sup> - University of Florence, Department of Agriculture, Food, Environment and Forest Sciences, Firenze, Italy <sup>(4)</sup> - University of Florence, Department of Biomedical Experimental and Clinical Sciences, Firenze, Italy <sup>(5)</sup>**

Nickel-induced changes in photosynthetic activity were investigated in three *Odontarrhena* species with different Ni hyperaccumulation capacity. Plantlets were grown in hydroponics and exposed to NiSO<sub>4</sub> treatments (0.25 and 1 mM) for one week. Nickel effects on growth, accumulation, photosynthesis and nitrogen (N) allocation to components of the photosynthetic apparatus were measured. The three species showed different degrees of Ni tolerance and accumulation, with the increasing order: *O. muralis*, *O. moravensis* and *O. chalcidica*. The former showed an extremely reduced growth in the presence of Ni at the higher dose, along with specific changes in biomass partitioning, tissue hydration status and leaf traits that underlined its higher sensitivity to this element. *Odontarrhena chalcidica*, at both the metal concentrations, and *O. moravensis*, at the lowest dose, showed more efficient photosynthesis and N-use in presence of Ni in respect to *O. muralis*. Unexpectedly, Ni treatments in *O. chalcidica* increased not only the photochemical efficiency of PSII and the CO<sub>2</sub> assimilation rate, but also the CO<sub>2</sub> diffusion from the atmosphere to the carboxylation sites. Moreover, this species and *O. moravensis* displayed a specific increase and/or re-arrangement of photosynthetic pigments in the photosystems and a higher leaf N allocation to the photosynthetic components in presence of the metal. The increased efficiency in photosynthetic activity and N-use under high Ni levels were more striking in *O. chalcidica*. On the other hand, *O. muralis* displayed a decrease in the photosynthetic performance, already at the lower concentration used, due to a combination of both stomatal and non-stomatal factors. Our data represent the first complete report on the Ni effects on photosynthetic machinery in Ni-hyperaccumulating plants and clearly indicated a positive metal effect on the photosynthetic performance in the species with the highest hyperaccumulation capacity.

**499 - Exploring differential sensitivity to gamma radiation in plants: A systematic approach using growth studies, histology, and molecular biology tools.**

**Payel Bhattacharjee** <sup>(1)</sup> - **Dajana Blagojevic** <sup>(1)</sup> - **YeonKyeong Lee** <sup>(1)</sup> - **Gareth Benjamin Gillard** <sup>(2)</sup> - **Lars Grønvold** <sup>(3)</sup> - **Torgeir Hvidsten** <sup>(2)</sup> - **Simen Rød Sandve** <sup>(3)</sup> - **Ole Christian Lind** <sup>(4)</sup> - **Brit Salbu** <sup>(5)</sup> - **Dag Anders Brede** <sup>(4)</sup> - **Jorunn Elisabeth Olsen** <sup>(1)</sup>

**Norwegian University of Life Sciences, Department of Plant Sciences, Faculty of Biosciences (BIOVIT), Ås, Norway** <sup>(1)</sup> - **Norwegian University of Life Sciences, Faculty of Chemistry, Biotechnology, and Food Science, Ås, Norway** <sup>(2)</sup> - **Norwegian University of Life Sciences, Centre for Integrative Genetics (CIGENE), Department of Animal Science, Faculty of Biosciences, Ås, Norway** <sup>(3)</sup> - **Norwegian University of Life Sciences, Faculty of Environmental Sciences and Natural Resource Management, Ås, Norway** <sup>(4)</sup> - **Norwegian University of Life Sciences, Centre of Environmental Radioactivity (CERAD), Ås, Norway** <sup>(5)</sup>

Ionizing radiation (IR) in the natural environment includes cosmic radiation and radiation from soils, sediments, and bedrocks containing radioactive elements, as well as anthropogenic sources such as nuclear weapon tests/use, nuclear power-plant accidents and radioactive waste disposal. IR exposure of plants leads to oxidative stress resulting in reduced photosynthesis, growth inhibition, damage and lethality. Radiosensitivity is highly variable amongst plant species, however, information about the underlying molecular basis is scarce. In this study, a systematic approach was taken to elucidate the molecular basis of differential radiosensitivity between the radioresistant *Arabidopsis thaliana* and the radiosensitive conifer Norway spruce (*Picea abies*).

Gamma-exposure above dose rate 40 mGy h<sup>-1</sup> for 144 h significantly inhibited root and shoot growth, resulting in lethality in the conifers. Whereas, no lethality and only slight transient delays in lateral root growth, flower-bud development and inflorescence elongation were observed in *Arabidopsis* above 400 mGy h<sup>-1</sup>. The shoot apical meristems were damaged from 100 mGy h<sup>-1</sup> in Norway spruce, but not in *Arabidopsis*. Damage in cellular organs were observed in Norway spruce. However, persistent dose-rate dependent DNA-damage was observed in both species. Comparative RNA-sequencing revealed significant regulation of DNA damage repair genes, and many other biological pathways, from 1 mGy h<sup>-1</sup> in *Arabidopsis*, while in Norway Spruce mostly  $\geq 40$  mGy h<sup>-1</sup>. Antioxidants and hormone biosynthesis, and endoreduplication were upregulated in *Arabidopsis*. In conclusion, conifers are much less perceptive towards lower dose of gamma-induced DNA damage in terms of induction of DNA repair, while *Arabidopsis* is sensitive and efficient in engaging DNA damage repair even in the lowest gamma dose-rates. *Arabidopsis* activates other protective mechanisms in much lower dose-rates than Norway spruce, which could be advantageous, leading to higher radioresistance. Moreover, in higher doses *Arabidopsis* fortifies its cell wall and induces hormonal regulation and oxidative stress-related gene expression to provide further defense.

## TOPIC:

### Plants in extreme environments

#### **Extended Elevator Pitches**

##### **197 - Role of bacterial endosymbionts in the stress tolerance of lichens**

**Amina Daminova**<sup>(1)</sup> - Yuri Ignatiev<sup>(2)</sup> - Ilya Lexin<sup>(2)</sup> - Svetlana Gorina<sup>(3)</sup> - Farida Minibayeva<sup>(1)</sup>

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The lichen thallus is a symbiotic system, comprising two major components - a mycobiont and a photobiont. Recently, it was discovered that lichens have a more complex structure that can include a range of symbiotic bacteria. Lichens can survive in harsh environments and can tolerate severe abiotic stresses including desiccation, high and low temperatures, and oxidative stress. We suggest that bacteria may contribute to the stress tolerance of lichens. To confirm this, we cultured bacterial endosymbionts from five lichen species: *Leptogium furfuraceum*, *Lobaria retigera*, *Sticta limbata*, *Lobaria pulmonaria* and *Parmelia perlata*, and identified them by sequencing their 16s rRNA. Phylogenetic analysis of sequences showed that the bacteria are predominantly belong to the genera *Paenibacillus* and *Bacillus*. Using electron microscopy, we analyzed the morphology and ultrastructure of endosymbionts. In microbiological tests we studied the response of bacteria to heat and oxidative stress. Following exposure to 70°C the bacterial titer decreased by several orders of magnitude during first few hours, but later the bacterial number gradually increased, demonstrating the heat tolerance. Interestingly, two different responses were discovered to the treatment of bacteria with H<sub>2</sub>O<sub>2</sub>. Some bacterial species were not detected on the medium, while others only decreased the CFU titer but survived. This corresponded to the ability of bacteria to detoxify H<sub>2</sub>O<sub>2</sub>. The expression of several stress tolerance genes in lichen endosymbionts were analyzed by real-time PCR. Results obtained suggest that endosymbiotic bacteria can be an important part of lichen symbiosis and contribute to stress tolerance of lichens.

This work was partly supported by RSF grant № 18-14-00198.

**225 - Auxin is required for the long coleoptile trait in rice germination under submergence.**

**Kack Nhu Nghi <sup>(1)</sup> - Lorenzo Mariotti <sup>(2)</sup> - Daan A. Weits <sup>(3)</sup> - Andrea Tagliani <sup>(3)</sup> - Pierdomenico Perata <sup>(1)</sup> - Chiara Pucciariello <sup>(1)</sup>**

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Successful rice germination under low oxygen is due to its ability to mobilise starch to sugars so that the endosperm provides the growing seedling with an adequate sugar supply. This mechanism is unique to rice, since other cereals fail to degrade starch under low oxygen. Rice coleoptile elongation under submergence guarantees fast seedling establishment in the field. The role of auxin in the elongation of the coleoptile in air is well established, but not under submergence. We provide experimental evidence that supports the role of auxin in promoting coleoptile elongation during the germination of submerged rice seeds. Our experimental results indicated that increased auxin availability and transport promote low oxygen coleoptile elongation in varieties characterised by a long coleoptile. The development of long-coleoptile varieties, which can be directly sown in submerged paddies or can better tolerate unexpected flooding events, is a trait of considerable agronomic importance.

## TOPIC:

### Plants in extreme environments

#### Posters

599 - Short term copper and lead stress induces RNA oxidation in soybean seedlings (Glycine max L.).

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Metal stress is associated with the production of reactive oxygen species (ROS). These highly reactive molecules mediate oxidation of various cellular compounds including membrane lipids, proteins and nucleic acids. On one hand oxidation of biomolecules might lead to their damage resulting in alerting plants functioning. On the other hand, some molecules formed in the process of ROS-dependent oxidation can act as signaling molecules.

The aim of the present study is the assessment of the impact of Cu and Pb on the oxidative status of soybean seedlings, in particular in relation to RNA oxidation. Soybean seedlings were treated with Cu at concentrations 10 and 25 mg/l and Pb at concentrations 300 and 600 mg/l, where the lower and the higher concentrations reflected mild and severe stress conditions, respectively.

The metals were applied for short treatment times: 1, 3 and 24 h. Thereafter, metal uptake, superoxide anion level, protein carbonylation intensity and RNA oxidation reflected by the amount of 8-hydroxyguanosine (8-OHG) have been assessed.

The results show that both metals are readily taken up by soybean seedlings. Oxidative response of the seedlings depended on the applied metal and treatment duration. Both, Cu and Pb, induced RNA oxidation reflected by elevated levels of 8-OHG. This response has been observed only after 1 h of treatment. Longer treatment times (24 h) resulted in intensified lipid peroxidation induced by both metals. In addition, exposure to lead resulted in superoxide anion over-production observed in all treatment times.

The research is financed by National Science Center, Poland, in the frame of the projects number 2014/13/D/NZ9/04812 and 2019/33/B/NZ9/00058 and the grant of Plenipotentiary Representative of Poland in the Joint Institute of Nuclear Research (JINR) in Dubna, Russia (topic 03-4-1128-2017/2019).

#### **604 - Oxidative stress induced by glyphosate herbicide in *Amaranthus palmeri* sensitive and resistant populations**

**Mikel Vicente Eceiza** <sup>(1)</sup> - **María Barco-Antoñanzas** <sup>(1)</sup> - **Miriam Gil-Monreal** <sup>(1)</sup> - **Ana Zabalza** <sup>(1)</sup> - **Mercedes Royuela** <sup>(1)</sup>

**Institute for Multidisciplinary Research in Applied Biology (IMAB), Department of Science. Public University of Navarre, Pamplona, Spain** <sup>(1)</sup>

The herbicide glyphosate inhibits the biosynthesis of aromatic amino acids by the specific inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). It is currently the most widely used herbicide in the world and its massive usage has led to the development of resistant weeds like some populations of *Amaranthus palmeri*, whose most important mechanism of resistance is the amplification of the EPSPS gene. Glyphosate has been described to cause some oxidative stress on susceptible plants, but whether it also happens on resistant individuals is not known. This study, then, was performed to compare the response to glyphosate of a sensitive (S) and a resistant (R) population of *A. palmeri* in terms of diverse oxidative stress markers: accumulation of the reactive oxygen species (ROS)  $H_2O_2$  and  $O_2^-$ , oxidative damage to protein (protein carbonylation) and antioxidant status (peroxidases, ascorbate).

The measured parameters were similar between untreated S and R plants. Accumulation of  $H_2O_2$  and  $O_2^-$  was very meaningful in treated S plants. Nevertheless, there were no changes between treated and untreated S plants in terms of protein carbonylation. Peroxidase activity showed a dose response increase and dehydroascorbate was accumulated with the highest glyphosate dose. None of the measured parameters showed significant variation between treated and untreated R plants.

In light of this, oxidative stress seems a secondary effect of glyphosate detected in the S population, where the great ROS content did not cause protein carbonylation-probably due to an enhanced antioxidant activity. R plants were unaffected suggesting that oxidative stress is a direct consequence of the EPSPS inhibition.

Fellowship: MVE: Gobierno Vasco-Basque Government.

#### **634 - Leaf age-dependent variation in volatile organic compound emissions from wounded foliage of the aromatic plant *Cinna latifolia***

**Bin Liu** <sup>(1)</sup> - **Hassan Yusuf Sulaiman** <sup>(1)</sup> - **Tana Wuyun** <sup>(1)</sup> - **Ülo Niinemets** <sup>(1)</sup>

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Plants in nature frequently confront wounding stresses caused by biotic or abiotic stressors. Wounding stress typically triggers a burst of emissions of volatile organic compounds (VOCs) that can expel herbivores, trigger self-defence or signal surrounding plants. In fast growing plants, the upper canopy leaves are inevitably younger than lower canopy leaves, but there is limited understanding of within-canopy variation in leaf age affects wounding-dependent VOC emissions.

*Cinna latifolia* is a forest understory grass that is characterized by high content of secondary metabolites. Both immediate releases of stored and de novo synthesized volatiles upon wounding can depend on plant age, but their contribution to total emissions and age-dependent modifications have not been characterized. We applied a novel leaf-cutting device integrated into a portable gas-exchange chamber and a PTR-TOF-MS to investigate the immediate responses of net assimilation rate (A) and VOC emission from intact *Cinna* leaves of different age (L1 to L4; youngest/uppermost to oldest/bottommost leaves) upon the wounding stress. Leaf photosynthetic capacity decreased with leaf age both in intact and cut leaves. Leaf cutting resulted in a 16% decrease of A in L4 but less than 7% in other leaves, indicating that wounding stress was severer for older leaves. The cutting elicited immediate releases of lipoxygenase (LOX) pathway volatiles. Hexanal ([81<sup>+</sup>] and [99<sup>+</sup>]), the most abundant induced-LOX compounds, had similar emission maxima in L1, L3 and L4. And integrated hexanal emission rate in L2 was 7%-43% lower than other leaves. Wounded L1 had the highest emission maxima and integrated emission rates of another C5-LOX volatiles (pentenol/pentenone fragments, [69<sup>+</sup>]), while the older leaves had similar emissions. Wounding had no effects on methanol [33<sup>+</sup>] emission in any cases. These results demonstrate that despite differences in age, morphological traits and photosynthesis activity, leaf age has surprisingly limited effect on *Cinna* leaf volatile emissions.

**657 - Intraspecific genetic variation of *Rhizophagus irregularis* differentially affects drought response strategies in *Manihot esculenta*.**

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Arbuscular mycorrhizal fungi (AMF) form obligate mutualistic interactions with many plant species including globally important crops such as cassava. *Rhizophagus irregularis* is a model species of AMF with characterized intraspecific genetic variation. AMF fungi have recently been implicated in aiding plants during certain abiotic stressors such as drought. We hypothesized that the genetic background of certain *R. irregularis* isolates may be better suited to aiding cassava with stress-related responses during drought exposure and during recovery. Due to climate change, many cropping systems are facing erratic weather patterns with increased susceptibility to extreme drought. Cassava is one of few crops that is well suited to drought prone areas and marginal soils. Here, we test whether intraspecific variation of two *R. irregularis* isolates differentially affect cassava drought response and recovery in a controlled glasshouse experiment. Cassava stakes of the CM-4574-4 variety were harvested from a field experiment in Colombia and used to initiate a pot experiment. After emergence of vegetation, pots were inoculated with 1 gram of inert powder containing ~1000 spores of one of two AMF lines or 1 gram of inert powder with no spores. After plants were observed to be colonized, drought was started by water withholding and watering at 10% of the holding capacity. Following two weeks, droughted pots were rewatered and allowed to recovery for one week. Root RNA samples and physiological measurements of photosynthetic activity were recorded at specific intervals to represent early and late drought stress and early and late drought recovery. Differential expression analyses of RNAseq data reveal that genetically different *R. irregularis* isolates alter key stress-related genes during drought and recovery. Additionally, symbiosis related genes associated with lipid metabolism and nutrient transport are also differentially regulated by the two isolates during drought and recovery.



## **18 - Ethylene mitigates metals stress by modulating defence mechanisms in Indian mustard**

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**Jamia Hamdard, Botany, New Delhi, India** <sup>(1)</sup> - **Aligarh Muslim University, Botany, Aligarh, India** <sup>(2)</sup>

The addition of heavy metals (HMs) to agricultural soil due to the rapid industrialization is a potential threat to agricultural land and crops. Contamination of agricultural land by HMs especially in developing countries has invited attention in global research due to their high accumulation in living things through the food web. Ethylene is a gaseous plant hormone that have potentially influences growth and developmental processes of plants under optimal and stressful conditions. The role of ethylene (through application of ethephon) in the regulation of metal stresses such as nickel (Ni) and zinc (Zn) stress tolerance has been investigated. Ethephon at concentration of 200  $\mu\text{l l}^{-1}$  was applied to mustard (*Brassica juncea* L.) plants grown without and with 200  $\text{mg kg}^{-1}$  soil Ni and Zn to study the increased the growth, photosynthetic efficiency, nitrogen-sulphur assimilation, nutrients content and defence mechanisms (activities of antioxidants enzymes, glyoxalase systems and proline metabolism). In the absence of ethephon, HMs increased oxidative stress with a concomitant decrease in photosynthesis, growth and nutrients content. However, application of ethephon positively increased growth traits, photosynthetic parameters, nutrients content and also elevated the generation of antioxidants enzymes and glyoxalase systems, proline production to combat oxidative stress. Plants water relations and cellular homeostasis were maintained through increased in photosynthetic efficiency and proline production. This signifies role of ethylene in mediating Ni and Zn tolerance via regulating proline production and photosynthetic capacity. Ethephon can be used as an exogenous supplement on plants to confer HM tolerance. The results can be exploited to develop tolerance in plants via gene editing technology encoding enzymes responsible for proline synthesis, antioxidant defence, glyoxalase systems and photosynthetic effectiveness.

#### **49 - Morphophysiologic Traits of Spruce Trees in Conditions of Izhevsk (Udmurt Republic, Russia)**

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Data on the condition of Picea genus (spruce) representatives in an urban environment have been analyzed. The viability under different environmental conditions and stand types is evaluated. The relative viability of forest stands is evaluated. Morphogenic traits of conifers are examined for the annual increment development. The photosynthetic pigments dynamics is tracked for two coniferous plants across various forest types, including park forests, roadside hedgerows, and plantings in the residential area. The specific responses of pigment system to the urban environment have been revealed for the two coniferous plant species. We have found an increased concentration of carotinoids and higher resilience of blue spruce (Picea pungens Engelm.) in an urban environment.

The reported study was funded by RFBR, project number 19-34-60003/19

**169 - Exploring differential sensitivity to gamma radiation in plants: A systematic approach using growth studies, histology, and molecular biology tools.**

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Ionizing radiation (IR) in the natural environment includes cosmic radiation and radiation from soils, sediments, and bedrocks containing radioactive elements as well as anthropogenic sources such as nuclear weapon tests/use, nuclear power plant accidents and radioactive waste disposal. IR exposure of plants can lead to oxidative stress resulting in reduced photosynthesis, growth inhibition, severe damage and lethality. Although the radiosensitivity is highly variable amongst plant species, information about the underlying molecular basis is very limited. In this study, a systematic approach was taken to elucidate the molecular basis of differential radiosensitivity between the radioresistant *Arabidopsis thaliana* and the radiosensitive conifers Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*).

In gamma-exposure experiments using a <sup>60</sup>Co-source, dose rates  $\geq 40 \text{ mGy h}^{-1}$  for 144 h significantly inhibited root and shoot growth and resulted in lethality in the conifers. In comparison, no lethality and only smaller transient delays in lateral root growth, flower bud development and inflorescence elongation were observed in *Arabidopsis*-plants at  $\geq 400 \text{ mGy h}^{-1}$ . The shoot apical meristems were damaged from  $100 \text{ mGy h}^{-1}$  in the conifers, but not in *Arabidopsis*. However, persistent dose-rate dependent DNA-damage was observed in all species, indicating genomic instability. Comparative RNA-sequencing analysis revealed significant regulation of genes from  $1 \text{ mGy h}^{-1}$  in *Arabidopsis*, while Norway Spruce started regulation mostly at  $40 \text{ mGy h}^{-1}$ . Production of antioxidants, hormone biosynthesis and endoreduplication showed rapid and significant upregulation in *Arabidopsis*. Interestingly, the induction of genes involved in DNA-repair, in both *Arabidopsis* and Norway spruce, indicated the significance of DNA damage-repair in both species. Hence, it could be concluded that conifers are much less tolerant of persistent DNA-damage compared to *Arabidopsis*, making them much more sensitive to radiation. Additionally, *Arabidopsis* can activate its protective mechanisms in much lower dose-rates than Norway spruce, which could be an added advantage leading to higher radioresistance.

### **307 - Alleviation of selected Polycyclic Aromatic hydrocarbons (PAHs) by Lolium Multiflorum Lam (Rye Grass) from Diesel Spiked Soil**

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in environment produced by anthropogenic activities such as combustion of fossil fuels as well as natural processes. There are many routes for PAHs to enter in the soil environment, which interfere with the soil system and the plants grown in soil. The main objectives of the study were to determine potential of Lolium multiflorum Lam (Rye Grass) to remove selected PAHs from the diesel contaminated soil and subsequent uptake of selected PAHs by Lolium multiflorum Lam (Rye Grass). Soil was spiked with varying concentration of diesel and compost amendments. Soil PAH analysis was done after the 15 days of spiking and at the end 120days of experiments, analyzed by GC-MS. Plant growth revealed that diesel contamination negatively influenced Rye grass. The shoot height of Rye grass was reduced 76% in treatment of soil with 1% of diesel spiked as compared to control. Root biomass of rye grass in treatment with 1% diesel was reduced 50%. Uptake of naphthalene, Flouranthene and acenaphthalene by rye grass is significantly higher in shoots as compared to roots while pyrene concentration observed high in root part of the grass. GC-MS analysis for soil PAHs indicated that rye grass remediate 76% naphthalene concentration in soil contaminated with high diesel concentration and amended with compost. 83% Pyrene and 70 % Flouranthene remediated by rye grass in the soil in which soil was spiked with 1% diesel. This study clearly showed that the Lolium multiflorum Lam (Rye Grass) is the viable choice for the remediation of PAHs from contaminated soil.

**Key words:** PAHs, Rye grass, Pyrene, Flouranthene

### 369 - Effectiveness of RNeasy® Solution for RNA Preservation of bilberry and strawberry samples.

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Preserving plant material for gene expression analyses requires that the tissue is prepared and stored in a manner that maintains the integrity of RNA. Proper tissue preservation is required for successful plant RNA extraction for gene expression studies. Flash freezing of samples in liquid nitrogen is not always practical or possible. RNeasy®, a concentrated solution of ammonium and cesium sulfates, has become a standard preservative in the absence of liquid nitrogen. The liquid preservative RNeasy® provides an effective alternative to conventional freezing strategies, which are limited or unavailable in current spaceflight experiment scenarios. Here, we demonstrate the effectiveness of RNeasy in preserving bilberry (*Vaccinium myrtillus* L.) and strawberry (*Fragaria x ananassa*) tissues for the gene expression study analyses. Samples were stored in RNeasy® solution for 1 week and for 4 weeks at +4°C, +18°C and +28°C temperature conditions. For assessment of gene expression level of bilberry samples were selected following genes: GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and Actin. Following qPCR amplification, the cycle quantification (Cq) values were used to assess the expression level of each candidate gene, which indicate the cycle at which the fluorescence signal is significantly different from background. The quality of the bilberry samples was also verified in Bioanalyzer/Experion run. The results show that the yield and the quality of RNA in berry samples was still good after 4 weeks of sample storage in RNeasy®, in +4°C and even in +18°C. However, the berry samples stored in +28°C showed signs of degradation already after one week. Therefore, we can conclude that RNeasy® can be used even for longer period storage, up to 4 weeks, of bilberry and strawberry samples in cooler temperature conditions.

### **383 - NtSLAH8, a homologue of the slow type anion channel SLAC1, is involved in Cl<sup>-</sup> accumulation in *Nicotiana tabacum***

**Hui Zhang** <sup>(1)</sup> - **Chen Wang** <sup>(1)</sup> - **Guoyun Xu** <sup>(1)</sup> - **Zefeng Li** <sup>(1)</sup> - **Lifeng Jin** <sup>(1)</sup> - **Niu Zhai** <sup>(1)</sup> - **Qingxia Zheng** <sup>(1)</sup> - **Qiansi Chen** <sup>(1)</sup> - **Pingping Liu** <sup>(1)</sup> - **Huina Zhou** <sup>(1)</sup>

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<sup>(1)</sup>

#### **Background**

Chloride (Cl<sup>-</sup>) as an important micronutrient in plant, regulates a series of cellular events such as enzyme activity, photosynthesis and membrane potential. Tobacco is an economically important plant throughout the world. The concentration of Cl<sup>-</sup> in tobacco leaves has an essential influence on tobacco quality. However, little research has been conducted about chloride transport in tobacco.

#### **Results**

Four homologs of SLAC1, the slow-type anion channel-associated homologues (SLAH1 to 4) have been identified and predicted to be involved in anion transport. In this study, a tobaccoSLAH1-like gene named NtSLAH8 was identified in the genome of *Nicotiana tabacum*. The NtSLAH8: GFP fusion protein was localized in endoplasmic reticulum. qRT-PCR results showed that NtSLAH8 was highly expressed in root. Expression of NtSLAH8 in an *Arabidopsis* *slah1* knockout mutant abolished the mutant's stunted growth phenotypes under salt stress. Further validation was achieved through silencing of NtSLAH8 through virus-induced gene silencing (VIGS) that rendered a reduction of Cl<sup>-</sup> concentration in leaves, contrary to the wild-type. The CRISPR-Cas9 mediated knock-out of NtSLAH8 resulted a significantly decreased chloride contents comparable to wild-type plants, which confirmed that NtSLAH8 participated in Cl<sup>-</sup> transport in tobacco.

#### **Conclusions**

These findings indicate that NtSLAH8 plays an important role in Cl<sup>-</sup> accumulation in tobacco, and provided a solid basement for broadening the research of SLAC family members in Solanaceae.

**500 - Characterization of *Arabidopsis thaliana* and *Brassica napus* L. seedling growth under randomizing of plant position relative to the gravity vector**

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Gravity is the most important polarized external factor for plants. Plants can precisely “evaluate” their position in space and correct it by polar growth, thus demonstrating gravitropism. An effective way to study the role of gravity in the plant polarity can be achieved by randomizing the position of plants in space by their rotation around one or several axes (clinorotation). Here we studied the development of *A. thaliana* and *B. napus* L. seedlings under 3D-clinorotation. Seedlings demonstrated disoriented growth with roots skewing and waving. Hydrogen peroxide was accumulated in seedlings as the first response to clinorotation. Using GFP-fABD2, TUA6-GFP, and MAP4-GFP transgenic lines of *A. thaliana*, we have shown that the proportion of transverse and oblique cytoskeletal elements increased at the expense of longitudinal elements. The orientation spectrum of microtubules and actin microfilaments increased as well. Remarkably, the first static gravistimulation of continuously clinorotated seedlings for as short as 30 min converted microfilament organization to a longitudinal one in roots. Therefore the ‘scattered’ microfilament organization can serve as a frame structure for the rapid conversion of cytoskeleton to a ‘longitudinal’ structure under the static action of the gravity vector. The LC-MS-based bottom-up proteomics revealed 97 and 39 proteins the content of which increased and decreased, respectively, in rape seedlings under clinorotation. The level of proteins involved in energy and protein metabolism changed to the greatest extent. GC-MS based metabolomics revealed essential alterations in patterns of sugars and sugar phosphates (specifically glucose-6-phosphate), methionine and glycerol. Clinorotation has also resulted in increased number of proteins with non-enzymatic oxidative and glyco-oxidative modifications. The work was supported by grant 20-04-01041 from Russian Foundation for Basic Research with using the equipment of Research Park of Saint Petersburg State University.

**506 - PGPR isolated from the rhizosphere of plants grown under harsh environments enhance tomato seedling performance under abiotic stress**

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Plant rhizosphere is considered as a hot spot of microbial activity and harbors a wide range of bacteria, many of them acting in concert with the host plant and exerting positive effects on their performance. These plant-growth-promoting rhizobacteria (PGPR) aggressively colonize plant roots of many plant species, and biotic interactions with beneficial associations for both partners are established. Among them, resilience against abiotic stresses is of major importance since these stresses consist serious constraints to plant growth and crop production worldwide. Under this scope, we isolated a wide range of bacterial strains from the rhizosphere of plants grown under severe saline conditions, and evaluated their potential PGP properties selecting those with the most desirable combination of characteristics. As a further step, we studied their ability to successfully colonize tomato rhizosphere as well as their in vivo impact on tomato seedlings under stress conditions. In particular, the effect of a promising PGPR inoculum, identified as *Pseudomonas oryzae* based on whole genome next generation sequencing, on tomato seedlings against salinity stress was evaluated. In particular, a broad number of agronomic characteristics, physiological parameters, biochemical traits, as well as their metabolite profiles by GC-MS analysis, were evaluated, indicating strain's efficiency to augment tolerance of tomato seedlings against salt stress. As a further step, comparative transcriptome analysis revealed that the presence of the strain enhanced tomato seedling salt tolerance by up-regulating genes related to primary metabolism, antioxidant defense system, hormone signaling, and synthesis of osmoprotectants. Our study highlights the importance of application of halotolerant bacteria species isolated from severe saline soils to improve seedling performance under salt stress, and further provides intriguing insights into the underlying mechanisms of plant defense.



## **601 - Effect of ionizing radiation on growth and nutritional value of Brassica rapa L. microgreens**

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In the sight of long-term space exploration or the establishment of greenhouses on Moon and Mars, one of the main challenges is to make future space crews self-sufficient, also in terms of food supplies. In this context, studies on space farming have increased, since plants, apart from their role in resource regeneration (oxygen production, carbon dioxide removal, water recovery and wastes recycling) also represent a valuable source of fresh food.

However, outside the Earth's orbit, environmental factors like ionizing radiation can represent a significant source of abiotic stress for plants, with critical effects on growth and production performances.

The aim of this study was to analyze the effect of different doses of X-rays (0-control, 0.3, 1, 10, 20, and 30 Gy), on morpho-anatomical and nutritional traits of microgreens of *Brassica rapa* L. subsp. *sylvestris* var. *esculenta*.

Microgreens were irradiated at two different developmental stages, dry and germinated seeds. After the irradiation treatment, both dry and germinated seeds were cultivated in a growth chamber under controlled environmental conditions. Microgreens growth was monitored and quantified at harvest by measuring stem elongation, fresh and dry biomass, and total leaf area. Leaf morphogenesis was analyzed through light and epi-fluorescence microscopy by quantifying anatomical traits (e. g. lamina thickness, localization of phenolics, stomatal frequency). The nutritional value was evaluated by analyzing biochemical parameters with a specific focus on antioxidants content. The overall results showed that the outcomes of radiation are dose-specific and dependent on the target stage at the time of irradiation. This should be taken into account in the choice of the species to be cultivated in the Bioregenerative Life Support Systems in Space and in the definition of the shielding requirements for Space cultivation chambers.

## 619 - Acclimatisation of guard cell metabolism to long-term salinity

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Stomatal movements are enabled by changes in guard cell turgor facilitated via transient accumulation of inorganic and organic ions imported from the apoplast or biosynthesized within guard cells. Under salinity, excess salt ions accumulate within plant tissues resulting in osmotic and ionic stress. To elucidate whether (a) Na<sup>+</sup> and Cl<sup>-</sup> concentrations increase in guard cells in response to long-term NaCl exposure and how (b) guard cell metabolism acclimates to the anticipated stress, we profiled the ions and primary metabolites of leaves, the apoplast and isolated guard cells at darkness and during light, that is, closed and fully opened stomata. In contrast to leaves, the primary metabolism of guard cell preparations remained predominantly unaffected by increased salt ion concentrations. Orchestrated reductions of stomatal aperture and guard cell osmolyte synthesis were found, but unlike in leaves, no increases of stress responsive metabolites or compatible solutes occurred. Diverging regulation of guard cell metabolism might be a prerequisite to facilitate the constant adjustment of turgor that affects aperture. Moreover, the photoperiod-dependent sucrose accumulation in the apoplast and guard cells changed to a permanently replete condition under NaCl, indicating that stress-related photosynthate accumulation in leaves contributes to the permanent closing response of stomata under stress.

### 635 - Protective role of titanium dioxide nanoparticles against excessive light

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Thermal imaging was used to study the temporary response of nano-TiO<sub>2</sub>-treated *Arabidopsis thaliana* plants to excessive light. Plants were exposed to different levels of TiO<sub>2</sub> nanoparticles concentrations (0, 250, 500, and 1000 µg/ml nano-TiO<sub>2</sub>). Initial response of the plant was monitored by time-dependent thermograms, which showed that the temperature distribution over the leaf rosettes decreased in the nano-TiO<sub>2</sub> concentration-related manner. The maximum quantum yield of photosystem II and photosynthetic pigment content decreased only in the case of plants exposed to 1000 µg/ml nano-TiO<sub>2</sub>. A significant decline in the maximal quantum yield of photosystem II under excessive light was noticeable for all plants treated with nano-TiO<sub>2</sub>. High light caused [the](#) increased [lipid peroxide](#) content for all examined plants. This study shows that nano-TiO<sub>2</sub> may protect the plants against the incident light energy, even if lower concentrations (<1000 µg/ml nano-TiO<sub>2</sub>) are used.

**TOPIC:**

**Priming and memory of stress - from model to crop**

**Keynote Lecture**

***Molecular changes induced by stress and developmental reprogramming in plants***

**Jose Gutierrez-Marcos**

**Warwick University, United Kingdom**

Epigenetic modifications such as cytosine methylation and histone modifications influence phenotype without permanent modification of the DNA sequence. I will discuss how newly acquired epigenetic imprints can be inherited over many generations and how these modifications can provide adaptive values to offspring

## TOPIC:

### Priming and memory of stress - from model to crop

#### **Oral Communications**

**48 - The Arabidopsis thaliana LysM-containing receptor-like kinase AtLYK2 is required for elicitor-induced priming of defenses against fungal infection independently of chitin perception.**

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Treatments of plants with microbial Pathogen-Associated Molecular Patterns (PAMPs), such as bacterial flagellin or fungal chitin, or with host-derived Damage-Associated Molecular Patterns (DAMPs), like oligogalacturonides (OGs), results in an increased resistance against a wide range of pathogens, named PAMP-Triggered Immunity (PTI). Enhanced resistance induced by PAMPs and DAMPs (also called biological elicitors) can last several days, even though many responses activated during PTI are transient, and likely occurs through a mechanism that involves priming of defense mechanisms. However, the exact mechanisms linking elicitor recognition to enhanced resistance is not well understood. In Arabidopsis thaliana, perception of chitin from fungal cell walls is mediated by three LysM-containing receptor-like kinases (LYKs): AtCERK1, which is absolutely required for chitin perception, and AtLYK4 and AtLYK5, which act redundantly in chitin recognition. The role of a fourth member of this protein family, AtLYK2, is not known. We have evaluated basal and elicitor-induced resistance to the fungal pathogen Botrytis cinerea, and chitin-triggered activation of early and long term responses in Arabidopsis mutants for each AtLYK gene. Loss of either AtCERK1, AtLYK2, AtLYK4 or AtLYK5 but impairs chitin-induced resistance to B. cinerea, without affecting basal susceptibility to this pathogen. Notably, AtLYK2, but not other AtLYKs, is also necessary for resistance to B. cinerea induced by other elicitors, such as OGs and flagellin. AtLYK2 interacts with AtLYK5 and modulates late responses to chitin, such as callose deposition and priming of defense genes, though it has a limited role in chitin perception and early signaling. Moreover, AtLYK2 can promote priming of specific defenses independently of chitin perception. Elucidation of the mechanisms underlying the AtLYK2-dependent protection triggered by different elicitors will provide clues in the molecular machinery modulating plant immunity and help improve the ability of crops to cope with microbial diseases.

## 147 - Genotoxic stress induces DDR to prime defence genes expression in Arabidopsis

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Priming comprises the process in which a plant becomes physiologically prepared to respond to an upcoming stress by application of a previous moderate treatment of the same stress. Seed priming has long been the favored method for inducing tolerance to biotic stress, but priming at the seedling stage has also been shown to have beneficial impacts on productivity. DNA damage response (DDR) has often been reported in plants submitted to biotic stress. Pathogen recognition by nucleotide-binding leucine-rich repeat (NLR) immune receptors can lead to the activation of programmed cell death (PCD) as a part of the plant hypersensitive response. Moreover, phosphorylation of the histone H2 variant ( $\gamma$ -H2AX), a marker of DNA damage, is evident in Arabidopsis after infection with the bacterial pathogen *Pseudomonas*. Furthermore, infection with the oomycete pathogen *Hyaloperonospora* (Hpa) is attenuated by previous defence priming with UV-C radiation, with increases in DNA lesions associated with improved resistance. Similarly, simultaneous treatment with the dsDNA break-inducing bleomycin and a salicylic acid (SA) analogue show a synergistic effect on the regulation of defence genes. Here we show that priming with bleomycin enhances subsequent upregulation of defence genes in response to *P. syringae*, which is also observed with DDR induction by radiation with different types of genotoxic stress. Based on transcript analysis of Arabidopsis DDR-related mutants, we propose that a primed state is established days after a period of genotoxic stress and show that bleomycin enhances the activation of PR1 and other defence-related pathways, such as poly ADP-ribosylation (PARylation), increasing resistance to biotic stress. Our work implies a direct connection between DDR and the subsequent establishment of a primed state in the Arabidopsis defence transcriptome.

#### **408 - From tomato to Arabidopsis and back: role of strigolactones in the stomatal memory of drought stress**

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Plants are frequently exposed to repeated cycles of similar stresses; responding differently to them, thus displaying “stress memory”, is an adaptive strategy to cope with fluctuating environmental conditions. The so-called “after-effect” of drought, for example, is a feature of stress memory seen at the stomatal level: an incomplete recovery of conductance after drought, even when water potential has fully recovered. Besides the likely dependence on abscisic acid (ABA), the molecular and physiological mechanisms driving the after-effect are not clear yet. So, we set to investigate whether the phytohormones strigolactones may contribute to it. Interestingly in fact, among other roles, strigolactones promote stomatal closure in both ABA-dependent and independent manners, and are needed for effective drought acclimation processes. We investigated their physiological and molecular contributions to the after-effect, by contrasting stomatal conductance and transcriptome of wt versus strigolactone biosynthetic/signalling mutants of Arabidopsis and tomato in repeated dehydration cycles. In both model plants, strigolactones were needed for a sustained after-effect during recovery from the first stress. They were also indispensable in tomato for a full after-effect during recovery in the second cycle, in which stress avoidance by stomatal closure was overall stronger. Differently, Arabidopsis seems to adopt a different drought tolerance strategy (e.g. osmotic adjustment) by avoiding the stomatal closure during the second stress and recovery cycle. Since in both models however, strigolactones had been proven necessary for the after effect from the first stress, we took a RNA-seq and promoter analysis approach to investigate the links between strigolactones and transcriptome changes in both species under recovery conditions. Results will be presented that highlight how strigolactone-dependent specific transcription factor categories may underlie stomatal memory of drought.

Our work has received funding from the European Union’s Horizon 2020 research and innovation programme under Grant Agreement n. 727929 - TOMRES.

## TOPIC:

### Priming and memory of stress - from model to crop

#### Posters

**253 - Priming effects of a new protein hydrolysate-based biostimulant in promoting relief from drought stress in *Capsicum annuum* L.**

**Chiara Agliassa** <sup>(1)</sup> - **Dario Molino** <sup>(1)</sup> - **Silvia Cavalletto** <sup>(1)</sup> - **Tiziano Strano** <sup>(1)</sup> - **Giuseppe Mannino** <sup>(2)</sup> - **Valeria Contartese** <sup>(3)</sup> - **Cinzia Margherita Berteà** <sup>(2)</sup> - **Francesca Secchi** <sup>(1)</sup>

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Recently, biostimulants have been used in sustainable agriculture to increase crop productivity and plant tolerance to abiotic stresses, especially because of their role in inducing a “priming memory” that can be recruited under stress conditions. In the present study, a priming treatment with GHI\_16\_VHL, a plant protein hydrolysate-based biostimulant, was tested for its capability to mitigate severe water stress effects on potted *Capsicum annuum* plants at their flowering stage, the most sensitive to drought stress. GHI\_16\_VHL was applied by fertigation twice with a 7-day interval before the drought treatment. In order to evaluate the biostimulant influence on plant physiological status under stress and recovery conditions, stem water potential, stomata conductance and plant growth were measured throughout the experiment. Plant osmoregulation and oxidative stress level during drought and recovery were monitored by quantifying free proline, total soluble sugars, ROS-scavenging activity and H<sub>2</sub>O<sub>2</sub> level in the leaves. During drought, biostimulant-primed plants showed a faster stem water potential decreasing trend with respect to untreated plants, whereas stomata conductance dynamics did not change. However, the priming treatment accelerated the stem water potential recovery to control values, probably by promoting higher leaf proline level during the early recovery phase along with higher leaf total soluble sugars accumulation during drought. Although the biostimulant treatments did not influence antioxidant enzyme activity, H<sub>2</sub>O<sub>2</sub> level was significantly lower during the stress and early recovery in biostimulant-primed plants, thus suggesting non-enzymatic scavenging of ROS species. Finally, the biostimulant priming increased leaf relative growth rate and final fruit yield. Our data suggest that the biostimulant priming treatment promotes a faster and more efficient plant recovery from drought either mediated by enhanced osmoregulation or by a higher plant final yield.



#### **412 - Photoperiod stress protects Arabidopsis plants against pathogen attack**

**Anne Cortleven** <sup>(1)</sup> - **Venja Röber** <sup>(1)</sup> - **Sylvia Illgen** <sup>(1)</sup> - **Vivien Lortzing** <sup>(2)</sup> - **Thomas Schmülling** <sup>(1)</sup>

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Plants have to adapt to the photoperiod which is the duration of light and darkness in a diurnal cycle of 24 hours. Prolongation of the light period causes photoperiod stress in short-day adapted *Arabidopsis thaliana*. This newly identified form of abiotic stress is characterized by the induction of stress and cell death marker genes and an oxidative burst during the night following the altered light period. The following day, stress-sensitive plants show a reduced photosynthetic capacity and lesion formation eventually resulting in cell death.

RNA-seq analysis revealed that the transcriptomic changes caused by photoperiod stress show a strong overlap with changes occurring in response to ozone stress and pathogen attack. Characteristic for these stresses is the occurrence of an oxidative burst in the apoplast as is also the case during photoperiod stress. In addition, a strong induction of salicylic acid (SA) biosynthesis and signalling genes was observed in response to photoperiod stress. Genetic analysis pointed to a central role for NPR1 in the occurrence of the stress syndrome as *npr1* mutants were insensitive to photoperiod stress.

Recently, it has been discovered that a first photoperiod stress event makes plants for a limited time insensitive to a subsequent photoperiod stress event indicating priming and memory. Interestingly, photoperiod stress also protects *Arabidopsis* against a later infection by *Pseudomonas syringae* DC3000. SA levels were induced and genes involved in systemic acquired resistance (SAR) were strongly upregulated in response to a prolonged light period resulting in an improved SAR. Genetic analysis indicated that several factors known to be involved in SAR are required for this increased plant immunity by photoperiod stress. Together, this study indicates that a prolongation of the light period which results in photoperiod stress, can protect plants against future pathogen infections.

#### 432 - Priming and memory in response to photoperiod stress in *Arabidopsis thaliana*

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Plants are exposed to regular diurnal rhythms of light and dark. Changes in the photoperiod by the prolongation of the light period causes in short day-adapted *Arabidopsis thaliana* a stress response which was named photoperiod stress. This recently identified form of abiotic stress induces the expression of stress marker genes and results in an oxidative burst in the night following a prolonged light period. In the leaves of strongly stressed plants, programmed cell death ensues.

The present research explores, for the first time, if *Arabidopsis* plants are able to remember previous prolongations of the light period and if a photoperiod stress event prepares them for future stresses. Experiments with 4-weeks-old short day-grown plants revealed that these memorize a first photoperiod stress stimulus (prolongation of the light period by four hours – priming stimulus) on the transcriptional, biochemical and physiological level as their response to a second similar second photoperiod stress event (triggering stimulus) was suppressed (cis-priming). The altered response to a triggering stress lasts for several days indicating priming and memory. Prolongation of the light period shorter than four hours did not result in a significant photoperiod stress response and did not cause priming suggesting that the priming stress needs to induce a significant response in order to be memorized. In addition, the primability of plants is dependent on their age. Furthermore, the current research also indicates that prolonged light periods as priming stimulus improve the resistance of *Arabidopsis* plants to subsequent biotic stress, such as infection by the pathogen *Pseudomonas syringae* pv. tomato DC3000 (trans-priming). Taken together, these results indicate that priming by photoperiod stress prepares plants against subsequent photoperiod stress (cis-priming) and pathogen infection (trans-priming).

**441 - Understanding the response to water stress in poplars: can the balance between non-structural carbohydrates metabolism and growth prime recovery after stress relief?**

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In trees, recovery from drought relies on the plant's ability to efficiently restore water transport. This capacity differs in species in relation to anatomical and physiological traits and pre-drought growth conditions. Among physiological traits, non-structural carbohydrates (NSC) have been intensively studied as main drivers in plant energy supply and osmotic adjustment processes. NSC are present in the plant as soluble sugars and starch (reserves), and these pools depend on the carbon balance between plant growth and NSC accumulation. In this work, we hypothesized that mild drought treatments can prime a faster recovery of plants experiencing a further severe water stress, as consequence of a tradeoff between NSC accumulation and plant growth. To test our hypothesis, one group of *Populus nigra* plants were exposed to two cycles of mild drought treatment followed by a severe water stress imposition, while a second group of plants underwent only severe drought. Plant growth, hydraulics parameters and NSC content were measured before and during drought, and after re-watering (recovery). Poplars exposed to mild drought priming showed a reduced growth and a higher NSC tissue content respect to not primed. When severe drought was imposed, both groups of plants displayed similar loss of hydraulic conductivity (PLC); however, during the period of stress relief, a delay in PLC recovery was observed in not primed plants. During drought, the NSC content in xylem sap was significant higher in primed than untreated poplars. Sugar content was still higher also 1 and 3 days after stress relief in treated plants, although stem water potential of both groups returned to pre-stress values. We thus suggest that, in the short-term, the reduction of plant growth leads to the formation of a pool of "ready-to-use" carbohydrates that can be mobilized for allowing a faster and more efficient recovery of xylem functionality.

**528 - Long-term effect of stress hormone abscisic acid on Populus shoots during their transition from in vitro to ex vitro conditions**

**Greta Striganavičiūtė<sup>(1)</sup> - Jonas Žiauka<sup>(1)</sup> - Iwona Szy-Borowska<sup>(2)</sup> - Marzena Niemczyk<sup>(2)</sup>**

**Lithuanian research centre for agriculture and forestry, Institute of Forestry, Kauno raj., Lithuania<sup>(1)</sup> - Forest Research Institute, Silviculture and Forest Tree Genetics, Raszyn, Poland<sup>(2)</sup>**

Populus genotypes, selected for their economic or ornamental value, are often propagated vegetatively via in vitro culture. One of the most critical moments, following in vitro propagation, is plant transition to ex vitro conditions. The transferred plants must cope with the stress, resulting from a radical change in environmental conditions, e.g., decreased humidity. The aim of this study was to test if a stress signal, simulated in vitro by the application of exogenous abscisic acid (ABA), has a lasting effect on the development of treated Populus shoots, including the stage of ex vitro adaptation. The experiments were conducted by culturing shoot segments of two Populus genotypes, a hybrid aspen (*Populus tremula* × *P. tremuloides*) and a Berlin poplar (*Populus* × *berolinensis*), on the medium, enriched by ABA (1 or 3  $\mu\text{mol l}^{-1}$ ), for three weeks and by observing plant development during subsequent culture stages. The immediate effect of ABA was suppressive for the growth of both studied Populus genotypes; however, the ABA-treated shoots showed a clear advantage over their control counterparts after being transferred to ex vitro conditions. For the Berlin poplar, the use of 3  $\mu\text{mol l}^{-1}$  of ABA during the penultimate in vitro culture stage resulted in increases in both shoot and root size of ex vitro-adapted plants (3.5-fold and 2.3-fold fresh mass differences from the control plants, respectively). For the hybrid aspen, the strongest positive effect on ex vitro-adapted plants was achieved by 1  $\mu\text{mol l}^{-1}$  of ABA; the fresh shoot and root mass of ABA-treated plants surpassed the untreated control by 5 and even 53 times, respectively. Hence, it was confirmed that chemical stress simulation in vitro by the application of ABA can have a decisive role for the viability of Populus during the stage of ex vitro adaptation.

## **570 - 'Sequential effects of high light and drought stress in *Arabidopsis thaliana*'**

**Soyanni Holness** <sup>(1)</sup> - **Giovanna Serino** <sup>(1)</sup> - **Paola Vittorioso** <sup>(1)</sup>

**Sapienza University of Rome, Biology and Biotechnology, Rome, Italy** <sup>(1)</sup>

Single treatments with abiotic stresses such as drought and light can trigger a molecular response that can last from several days to weeks and that can help plants to deal with reoccurring stresses. However, it is often not clear if this is due to acclimation or to epigenetic memory (priming), and what will occur when plants are subjected to subsequent stresses. We are interested in investigating the potential priming effects in plants subjected to subsequent stresses. Here we selected two specific abiotic stresses: high light and drought. *Arabidopsis* plants were subjected to a short-term high light stress, to a long-term drought stress or to the two stresses administered sequentially. The stress responses were assessed at the physiological and molecular level and compared. Our results provide an initial characterization of the molecular mechanisms through which one stress can influence the plant response to a subsequent stress.

**Keywords:** Drought, High light, abiotic stress, priming

**596 - Towards assessment of priming responses of *Petunia hybrida* to hydric stress and transmission of primed status through clonal propagation**

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Crop adaptation to stress-inducing environment is a key issue in the general context of climatic change but also to face the increasing demand for plants best adapted to urban adverse growth conditions (building shade, irregular watering or restricted water usage, heat, poor soils). Genetic selection of new varieties through crossing of parental lines is the classical way to improve plants, but is a long process. New methods for crop adaptation are therefore studied, among them priming. Priming is the memorization of a physiological state following exposure to a stimulus (eg. a stress) that allows a plant to respond quicker or more efficiently to a next stimulus. Priming can be associated to epigenetics marks, such as DNA methylation, induced by the stimulus. Priming has been studied only in few plant species and yet, little is known on the duration, intensity required for a stress to induce priming, or on the length of plant memory of such priming state. Concerning transgenerational inheritance, epigenetics marks seem best conserved through mitosis than through meiosis.

Priming horticultural plants to stress during the production process could contribute to their adaptation after plantation. Moreover, in clonally propagated plants, if priming state could be transmitted through vegetative propagation, growers could produce more robust young plants by applying priming treatment onto mother plants. Using *Petunia hybrida* and water stress, we thus address the following questions: 1) can primed stock plants be obtained through exposure of successive vegetative generations to water stress ?, 2) if so, how long would such priming state last ?, 3) would priming state of stock plants be stable enough to be transmitted clonally to cuttings and improve later their acclimation to water stress ? and 4) would such priming state be linked to a particular genome methylation pattern?

First results concerning phenotypical, physiological responses to water stress of successive clonal generations grown under comfort irrigation or hydric stress conditions will be presented.

This research was conducted in the framework of the regional programme "Objectif Végétal, Research, Education and Innovation in Pays de la Loire", supported by the French Region Pays de la Loire, Angers Loire Métropole and the European Regional Development Fund." This project contributes to the scientific program of UMT STRATège (ASTREDHOR-INRAE -Université d'Angers, Institut Agro Agrocampus Ouest, Beaucouzé, France).

## TOPIC:

### Seeds of tomorrow

#### **Keynote Lecture**

**How to make a protein body: a cell biologist's evolutionary view**

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The most common classes of seed storage proteins accumulate in protein storage vacuoles. Prolamins, our major food proteins, have recently evolved in grasses from vacuolar storage proteins of the 2S albumin class by insertions, deletions or fusions; this led to their accumulation within the endoplasmic reticulum (ER) as unique insoluble heteropolymeric structures termed protein bodies (PB). Using two maize prolamins (27kD  $\alpha$ -zein or 16kD  $\alpha$ -zein) as models, we have defined a fundamental role of inter-chain disulphide bonds in PB formation, and how 16kD  $\alpha$ -zein, very recently evolved from 27kD  $\alpha$ -zein, has acquired new solubility features and a new function in PB assembly. Ectopic expression of either  $\alpha$ -zein induces the ER unfolded protein response (UPR) without associated autophagy, with a weaker effect of 27kD  $\alpha$ -zein compared to 16kD  $\alpha$ -zein, correlating with the higher availability of the latter for binding by the major ER chaperone BiP. Conversely, expression of a model vacuolar storage protein or a mutated, soluble form of 27kD  $\alpha$ -zein that traffics along the secretory pathway have no UPR-inducing effects. Such wide UPR-inducing variability may have allowed the evolution of prolamins, which challenge the ER folding machinery but at the same time avoid their own disposal.

## TOPIC:

### Seeds of tomorrow

#### **Oral Communications**

**106 - A NOVEL ROLE FOR ATHB2/HAT4 AS A REGULATOR OF GERMINATION IN ARABIDOPSIS THALIANA SEEDS.**

**ROCÍO SOLEDAD TOGNACCA <sup>(1)</sup> - MONICA CARABELLI <sup>(2)</sup> - IDA RUBERTI <sup>(2)</sup> - JAVIER BOTTO <sup>(1)</sup>**

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Seed germination is a process of extreme relevance for a successful seedling establishment in the field. Processes that modulate dormancy depth and its alleviation define the germination timing of seed population and are consequently of the outmost adaptive importance. Light has been one of the most characterized factors regulating the promotion of seed germination. Phytochromes are the best-known photoreceptors perceiving red (R) and far-red (FR) light. Moreover, seed germination depends on changes in hormones levels, mainly those of gibberellin and abscisic acid (ABA). Transcription factors (TF) play a central role in the integration of endogenous and environmental signals for the adjustment of germination in the correct time and place. The *Arabidopsis thaliana* genome encodes around 1500 TF, 40 % of which are specific to plants. HD-Zip TF constitute a large family of 48 members classified in four classes. ATHB2, member of the HD-Zip II class, is expressed at low levels in high R:FR light but is rapidly and strongly induced by low R:FR, and this induction is mediated by the phytochrome system. Even though ATHB2 has been involved in many biological processes in seedlings and mature plants as well as during embryogenesis, its role in seed germination has not yet been explored, although its gene expression levels significantly change during light induced seed germination. Here, we provide new results showing that ATHB2 (1) acts as a negative regulator of the phyB-dependent germination, (2) promotes gene expression of negative regulators of seed germination, (3) increases ABA sensitivity, and (4) reduces IAA content of *Arabidopsis thaliana* seeds. The identification of key regulatory factors such as ATHB2 as a repressor of light-mediated seed germination can allow us to modify the timing of seed germination in order to change the competitiveness of crops and weeds during the establishment of seedlings in the field.



#### **402 - Seed physical state critically affects the influence of oxygen on seed deterioration.**

**Davide Gerna**<sup>(1)</sup> - **Daniel Ballesteros**<sup>(2)</sup> - **Wolfgang Stöggli**<sup>(1)</sup> - **Erwann Arc**<sup>(1)</sup> - **Charlotte E. Seal**<sup>(2)</sup> - **Chae Sun Na**<sup>(3)</sup> - **ilse Kranner**<sup>(1)</sup> - **Thomas Roach**<sup>(1)</sup>

**University of Innsbruck, Department of Botany and Center for Molecular Biosciences Innsbruck, Innsbruck, Austria**<sup>(1)</sup> - **Royal Botanic Gardens, Kew, Wellcome Trust Millenium Building, Department of Comparative Plant and Fungal Biology, Ardingly, Wakehurst Place, United Kingdom**<sup>(2)</sup> - **Baekdudaegan National Arboretum, Seed Conservation Research Division, Department of Seed Vault, Munsu-ro, Chunyang-myeon, Bonghwa-gun, Gyeongsangb, Korea, Republic Of**<sup>(3)</sup>

Seed quality is integral to agriculture, as well as safeguarding of plant genetic resources via long-term storage in seed banks. During seed desiccation, seed water contents equilibrate with environmental relative humidity (RH), and cytoplasm viscosity increases restricting molecular diffusion and mobility, minimising enzymatic reactions, and extending seed longevity. When storage temperatures and water contents are sufficiently low, seeds enter a so-called "glassy state" whereby the cytoplasm behaves like a solid. Through formation of reactive oxygen species, O<sub>2</sub> is believed to support reactions at the basis of seed ageing. Nonetheless, how O<sub>2</sub> contributes to ageing of seeds with different physical state remains unclear.

Here, we combined advanced biophysical and biochemical methods to elucidate the impact of O<sub>2</sub> on the ageing mechanisms of *Pinus densiflora* seeds, exposed to controlled deterioration (CD) at 45 °C and distinct RHs, resulting in a glassy (9 and 33% RH) or fluid cytoplasm (64 and 85% RH), as determined by dynamic mechanical analyses. Compared to normoxia (19.6% O<sub>2</sub>), hypoxia (0.4% O<sub>2</sub>) prevented seed deterioration only in the glassy state, limiting the consumption of antioxidants (glutathione and tocopherols) and unsaturated fatty acids (FAs). Differential scanning calorimetry revealed that this decline of FAs associated to both a drop in the enthalpy of lipid melt and an extensive production of aldehydes and reactive electrophile species (RES), indicative of lipid peroxidation. Conversely, seed deterioration with fluid cytoplasm was independent of O<sub>2</sub> availability, and metabolite changes indicated resumption of enzymatic activities attributed to glutathione metabolism and RES processing.

Furthermore, biochemical profiles of seeds stored for 20 years under dry/cold conditions were similar to those of seeds aged by CD under normoxia and with a glassy cytoplasm.

The relevance of these findings will be discussed considering new germplasm management practices based on dry/cold storage of glassy-state seeds under hypoxia.

### **535 - Multiscale imaging reveals novel trafficking routes and novel roles for the storage vacuole in maize endosperm**

**Elsa Arcalis** <sup>(1)</sup> - **Eva Stöger** <sup>(1)</sup>

**Department of Applied Genetic and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria** <sup>(1)</sup>

Cereal endosperm is solely devoted to the storage of proteins and starch that will be used by the embryo upon germination. The high degree of specialization of this tissue is reflected in its endomembrane system, in which ER derived protein bodies and protein storage vacuoles (PSVs) are of particular interest. In maize seeds, the main storage proteins are zeins, that form transport incompetent aggregates within the ER lumen and finally build protein bodies that bud from the ER. In contrast to the zeins, the maize globulins are not very abundant and the vacuolar storage compartment of maize endosperm is not fully described. In other cereals, including wheat and barley, the PSV is the only protein storage compartment, and prolamins are deposited in addition to globulins, following an ER to vacuole route bypassing the Golgi. In maize, a large number of small, globulin-containing PSVs have been identified within the cytoplasm. However, with data based on 2D microscopy it is difficult to decide whether these are indeed discrete, individual vacuoles or part of an interconnected protein storage vacuolar network, as has been described in other seeds. Similarly, it has remained an open question whether the abundant zein bodies in maize are also incorporated into the PSVs. We have therefore used various microscopy techniques, ranging from life-cell imaging to sophisticated 3D electron microscopy techniques (SBF-SEM and electron tomography) to present a multi-scale set of data that clarifies the role of the PSVs in maize endosperm cells.

## 106 - A NOVEL ROLE FOR ATHB2/HAT4 AS A REGULATOR OF GERMINATION IN ARABIDOPSIS THALIANA SEEDS.

**ROCÍO SOLEDAD TOGNACCA** <sup>(1)</sup> - **MONICA CARABELLI** <sup>(2)</sup> - **IDA RUBERTI** <sup>(2)</sup> - **JAVIER BOTTO** <sup>(1)</sup>

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Seed germination is a process of extreme relevance for a successful seedling establishment in the field. Processes that modulate dormancy depth and its alleviation define the germination timing of seed population and are consequently of the outmost adaptive importance. Light has been one of the most characterized factors regulating the promotion of seed germination. Phytochromes are the best-known photoreceptors perceiving red (R) and far-red (FR) light. Moreover, seed germination depends on changes in hormones levels, mainly those of gibberellin and abscisic acid (ABA). Transcription factors (TF) play a central role in the integration of endogenous and environmental signals for the adjustment of germination in the correct time and place. The *Arabidopsis thaliana* genome encodes around 1500 TF, 40 % of which are specific to plants. HD-Zip TF constitute a large family of 48 members classified in four classes. ATHB2, member of the HD-Zip II class, is expressed at low levels in high R:FR light but is rapidly and strongly induced by low R:FR, and this induction is mediated by the phytochrome system. Even though ATHB2 has been involved in many biological processes in seedlings and mature plants as well as during embryogenesis, its role in seed germination has not yet been explored, although its gene expression levels significantly change during light induced seed germination. Here, we provide new results showing that ATHB2 (1) acts as a negative regulator of the phyB-dependent germination, (2) promotes gene expression of negative regulators of seed germination, (3) increases ABA sensitivity, and (4) reduces IAA content of *Arabidopsis thaliana* seeds. The identification of key regulatory factors such as ATHB2 as a repressor of light-mediated seed germination can allow us to modify the timing of seed germination in order to change the competitiveness of crops and weeds during the establishment of seedlings in the field.

## TOPIC:

### Seeds of tomorrow

#### Posters

**238 - Regulation of autophagic bodies degradation by asparagine in sugar-starved lupin embryo axes: a transcriptomic and proteomic approach**

**Szymon Stefaniak** <sup>(1)</sup> - **Łukasz Wojtyła** <sup>(1)</sup> - **Katarzyna Nuc** <sup>(2)</sup> - **Łukasz Wolko** <sup>(2)</sup> - **Małgorzata Pietrowska-Borek** <sup>(2)</sup> - **Sławomir Borek** <sup>(1)</sup>

**Adam Mickiewicz University, Department of Plant Physiology, Poznań, Poland** <sup>(1)</sup> - **Poznań University of Life Sciences, Department of Biochemistry and Biotechnology, Poznań, Poland** <sup>(2)</sup>

We focused on the role of asparagine (a central amino acid in lupin seed metabolism) in a course of autophagy in cells of lupin embryo axes. Previously we found that sugar starvation enhances autophagy in cells of lupin embryo axes, but the decomposition of autophagic bodies inside the vacuole (the final stage of autophagy) is remarkably inhibited by asparagine. Trying to describe the role of asparagine in autophagy, especially in a mechanism of the degradation of autophagic bodies, we performed transcriptomic analyses of lupin embryo axes. The experiments were performed on embryo axes isolated from imbibed seeds of white lupin (*Lupinus albus* L.) and Andean lupin (*Lupinus mutabilis* Sweet). Embryo axes were cultured in vitro for 96 h on a mineral medium supplemented with 60 mM sucrose, without the sugar, and on both the above-mentioned media enriched in 35 mM asparagine. The quality of the libraries was verified by Sanger sequencing method, and the large-scale transcriptomic sequencing using Illumina HiSeq Next Generation Sequencing technology (NGS) was performed. The obtained sequence reads were aligned to reference transcriptome and counted in the aim to find differentially expressed genes. As transcriptome modulation could be manifested in proteomic changes isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomics was performed to screen the differentially expressed proteins. Our goal was to analyze the effect of asparagine on the expression of genes and accumulations of proteins involved in autophagy. First of all, we focused on changes in the level of transcripts of genes coding for ATG proteins and genes coding for vacuolar lytic enzymes (e.g. proteases) as well as on the changes in the accumulation of appropriate proteins.

This work was financed by the National Science Centre, Poland (Grant no. 2016/23/B/NZ3/00735) and European Social Funds (Grant no. POWR.03.02.00-00-I006/17-00).

**333 - Calcium redistribution contributes to the hard-to-cook phenotype and increases PHA-L lectin thermal stability in common bean low phytic acid 1 (lpa1) mutant seeds**

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Phytic acid (PA), the main form of phosphorus storage present in seeds, is an antinutritional factor for its ability to chelate cations important for human nutrition. Plant breeders have spent many efforts to isolate and develop low phytic acid (lpa) mutants in different important crops.

We isolated different common bean (*Phaseolus vulgaris* L.) lpa mutants with reduction of PA content at different extent. The consumption of common bean seeds harboring the lpa1 mutation, affecting the PvMRP1 transporter and causing a reduction of 90% in PA content, improved iron status of volunteers in human trials, but caused adverse gastrointestinal effects, presumably due to the increased stability of lectin phytohemagglutinin L (PHA-L) in these seeds, compared to the wild type (wt) ones. A hard-to-cook (HTC) defect observed in the lpa1 seeds intensified the problem.

We confirmed and quantified the HTC phenotype of the lpa1 common bean seeds in three different genetic backgrounds, giving a genetic demonstration of the so-called “phytase-phytate- pectin” theory and found differences depending on the background. In one of them, we correlated the HTC defect to the redistribution of calcium, whose concentration in all parts of the seed and, particularly in the cell walls, was larger in the lpa1 compared to the wt. Furthermore, the lpa1 mutation, combined with the presence of different PHA alleles, affected the stability of the PHA-L lectin, due to an excess of free cations.

### 334 - Isolation and characterization of novel *Phaseolus vulgaris* low phytic acid mutants

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Phytic acid (PA) is the major phosphorus storage sink within the plant seed. It is one of the most highly electronegative molecules present in the cell and it chelates positively charged metal ions, such as  $Mg^{2+}$ ,  $Fe^{2+}$ ,  $Zn^{2+}$ ,  $Mn^{2+}$  and  $Ca^{2+}$ , important for human nutrition, reducing their bioavailability. For this reason, it is considered an antinutrient. Plant breeders have spent many efforts to isolate and develop low phytic acid (lpa) mutants in different important crops.

In *Phaseolus vulgaris*, two allelic low phytic acid (lpa1–lpa1<sup>2</sup>) mutants have been identified and characterized. These mutations are in the PvMRP1 gene coding for a putative tonoplastic phytic acid transporter. Other 29 putative mutants, showing altered PA content, have been recently identified. Three of these putative mutants have been initially characterized. Two showed reduced levels of PA and were thus considered as lpa mutants, the third one showed an increased free phosphate content, without a significant decrease in PA content, and was classified as a high inorganic phosphate (hip) mutant. These mutants, grown under standard conditions in a growth chamber, do not show any evident phenotypic alterations, compared to the BAT 93 common bean reference genotype plants. A candidate gene approach for these mutants did not reveal any mutation in known PA biosynthetic genes or in genes coding for PA transporters. A mapping approach is underway in order to identify the affected genes.

### **392 - Regulation of seed dormancy and seed vigor by the SCARECROW-like protein SCL15**

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**Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, Saskatoon, Canada** <sup>(1)</sup> - **State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, China** <sup>(2)</sup>

Seed dormancy, germination and seed vigor are important agronomical traits and extensively controlled by many factors including hormones and external stimuli. We have identified that SCARECROW-like transcription factor SCL15 is an HISTONE DEACETYLASE19 (HDA19)-interacting protein and functions as a key regulator for the control of seed-to-seedling phase transition in Arabidopsis. Seed dormancy and germination are two key events during the transition of seed to seedling. To further elucidate the biological function of SCL15 during seed-to-seedling transition, we identified homozygous *scl15-1* mutant, generated SCL15 overexpressing *Napin::SCL15* lines that are driven by the seed-specific *Napin* promoter and *35S::SCL15* lines. Effects of SCL15 transcript abundance on seed dormancy, germination speed and seed vigor were investigated. SCL15 mutation markedly led to reduction of germination rate, moderate late flowering and longer lifespan, significantly increased seed oil content, and changes in compositions of seed storage proteins and fatty acids. Germination and post-germination growth of *scl15-1* mutant is hypersensitive to ABA at concentration as low as 0.1 mM. SCL15 mutation increased germination sensitivity to the inhibition of endogenous auxin level, implying auxin transport or signaling is disrupted with the mutation of SCL15. SCL15 was found to act as a negative regulator of seed dormancy based on the germination response to hormones and dry storage. We also demonstrate that *scl15-1* seeds are significantly more sensitive to the Controlled Deterioration Treatment (CDT) and display reduced germination speed compared to wild type and the overexpressing lines. A subset of genes that are critical for seed vigor maintenance changes in *scl15-1* after CDT. These results further demonstrate the importance of SCL15 during seed-to-seedling; more specifically SCL15 acts as a negative regulator of primary seed dormancy and positively regulates seed vigor.

**425 - Designing an original system to evaluate how DNA Damage Response (DDR) affects seedlings development in the model legume *Medicago truncatula***

**Alma Balestrazzi** <sup>(1)</sup> - **Andrea Pagano** <sup>(1)</sup> - **Carla Gualtieri** <sup>(1)</sup> - **Maraeva Gianella** <sup>(1)</sup> - **Paola Pagano** <sup>(1)</sup> - **Giulia Folini** <sup>(1)</sup> - **Anca Macovei** <sup>(1)</sup>

**University of Pavia, Department of Biology and Biotechnology, Pavia, Italy** <sup>(1)</sup>

Accurate conservation and propagation of the genome in proliferating cells and from one generation to the next is essential for all living organisms. Cells continually undergo DNA damage and mutations due to reactive cell metabolites or environmental stress, compromising plant growth and development. Plants evolved complex mechanisms to adapt to these challenging conditions. Among them, the DNA Damage Response (DDR), a complex signal-transduction pathway, specifically aims to aid plants to cope with the detrimental effects of genotoxic stress. Several studies have recognized the crucial role that DDR plays during seed germination and early seedling development, but the complexity of these mechanism are still puzzling and hence worth a deeper investigation. To this purpose, in the current work, we developed an original approach to inhibit the DDR pathway using *Medicago truncatula* seed germination as a model working system. Specific treatments with camptothecin (CPT) and/or NSC120686 (NSC), targeting distinct components of DDR, namely topoisomerase I (Top1) and tyrosyl-DNA phosphodiesterase 1 (TDP1), were used. Phenotypic (germination percentage and speed, seedling growth) and molecular (cell death, DNA damage, gene expression profiles) analyses demonstrated that the imposed treatments affected DDR. Our results show that these treatments do not influence the germination process but rather inhibit seedling development, causing an increase in cell death and accumulation of DNA damage. Moreover, treatment-specific changes in the expression of SOG1 (Suppressor of gamma response 1), master-regulator of plant DDR, were observed. Additionally, the expression of multiple genes playing important roles in different DNA repair pathways and cell cycle regulation were differentially expressed in a treatment-specific manner. Moreover, specific changes in miRNA-target gene expression patterns were revealed, pointing at the presence of post-transcriptional regulatory mechanisms of DDR in plants.



#### 440 - Time to sleep or to germinate ? A case of legumes seed dormancy.

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**Palacky University, Department of Botany, Olomouc, Czech Republic** <sup>(1)</sup>

Timing of seed germination is one of the key steps in plant life. It determines when plants enter natural or agricultural ecosystems. Plants have evolved various mechanisms to control the entry of the quiescent seed protecting embryo into vulnerable environment. Understanding of the genetic basis of local adaptation has relevance to climate change, crop production as well as understanding of the speciation. Along with other traits, seed dormancy has been removed during domestication. Seed coats of wild legume species tend to be thicker, have different composition, and are less water permeable than their cultivated counterparts. We have used a comparative anatomy, metabolomics and transcriptome profiling of pea seed coats in order to identify changes and genes associated with loss of seed dormancy in relation to domestication. In parallel, we tested adaptation to environmental conditions influencing dormancy release and the timing of legume seed germination, using wild pea (*Pisum* sp.) with relevance to crop and *Medicago truncatula* models. Level of seed dormancy correlated with increased aridity, suggesting that plastic responses to external stimuli provide seeds with strong bet-hedging capacity and the potential to cope with high levels of environmental heterogeneity. Genome-wide association analysis of sequenced *Medicago* lines identified candidate genes associated with dormancy release related to secondary metabolites synthesis, hormone regulation and modification of the cell wall. Analysis of chemical composition of pea seed coat using mass spectrometry in various modes identified differences in the profile of proanthocyanidins, glycosylated flavonoids and fatty acids, related to impermeability for water. RNA sequencing identified several dozen differentially expressed genes between dormant and non-dormant pea seeds, and genome wide DARTseq approach applied to RIL mapping population yielded candidate loci regions. This analysis has been recently extended to chickpea and lentil crops, and has therefore applicability to other economically important legume species.

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**495 - Comparative analysis of the *Pisum sativum* L. embryonic axes with different tolerance to desiccation**

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At the late maturation stage, orthodox seeds develop desiccation tolerance, which allows maintaining seed viability after the loss of up to 95% of water. Desiccation tolerance allows long-term survival of dormant seeds under varying and often adverse environmental conditions. Interestingly, orthodox seeds are tolerant to desiccation not only during the period of dormancy, but also in the germination phase. Up to the moment of embryonic root initiation (radicle protrusion), the seeds can be dried without loss in viability and the metabolic processes can be resumed upon subsequent rehydration. The mechanisms behind this fascinating feature of germinating seeds are still not well understood. Here we compared physiological states of the embryonic axes isolated from germinating *Pisum sativum* L. seeds before and after radicle protrusion. The root growth initiation resulted in up to 2-fold increase in the level of lipid peroxidation, 4-fold rise in hydrogen peroxide, and 8-fold increase in ascorbic acid. Transcriptome analysis using the Illumina NovaSeq 6000 SP high-throughput genome-wide sequencing system identified 24789 genes with non-zero expression. The analysis of differential gene expression showed a decrease in the expression of 6415 genes and an increase in the expression of 7465 genes in the embryonic axes after radicle protrusion. Gas chromatography-mass spectrometry based metabolomics also revealed statistically significant differences. The relative contents of 42 and 32 metabolites demonstrated more than 1.5-fold decrease and increase, respectively. Many of the differentially regulated metabolites were amino acids, fatty and organic acids, aldonic and uronic acids, polyamines, mono-, di- and trisaccharides, sugar alcohols, mono- and disaccharophosphates, phosphoglycerols, phosphoinositols, lysolipids and sterols. This data might provide a new insight in the mechanisms behind the desiccation tolerance blockage during seed to seedling transition. The work was supported by grant no. 20-16-00086 from the Russian Science Foundation with using the equipment of Research Park of Saint Petersburg State University.

## TOPIC:

### Stress resilience in horticultural and fruit crops

#### **Keynote Lecture**

**A tale of plant hormones: how strigolactones cross-talk with ABA to set drought responses in tomato**

**Francesca Cardinale** <sup>(1)</sup> - **Ivan Visentin** <sup>(1)</sup> - **Claudio Lovisolo** <sup>(1)</sup> - **Andrea Schubert** <sup>(1)</sup>

**University of Turin, DISAFA - PlantStressLab, Grugliasco (TO), Italy** <sup>(1)</sup>

Strigolactones are carotenoid-derived metabolites with a multitude of functions, both in the plant and in the rhizosphere. They have a prominent role as hormones, influencing whole-plant morphology and development also in response to environmental stress. In tomato (*Solanum lycopersicum* L.) and other dicots experiencing drought, their synthesis partly shifts from roots to shoots. The drop in root-synthesised strigolactones seems to exert local effects on ABA levels and mobilisation within root tissues. However, it also acts as a systemic signal triggering strigolactone synthesis in the leaves and priming guard-cell sensitivity to ABA, thus governing a delayed recovery of stomatal conductance after stress (the so-called “after-effect”), and better stress avoidance during the next drought spell. The microRNA mir156, a highly conserved microRNA, was identified as a good candidate mediator of the ABA-dependent subset of drought responses controlled by strigolactones. This is based on the correlation with, and strict requirement for endogenous strigolactones in miR156 induction by drought; and on the ecophysiological and molecular effects of excess strigolactones vs miR156. Its systemic mobility pattern also defines miR156 as a strigolactone-related signal feeding back from the shoot to the root in response to stress, possibly to influence the increase in root water use efficiency under drought. A preliminary model will be presented, which captures organ-specific strigolactone action and dynamics under drought in tomato.

Our work has received funding from the European Union’s Horizon 2020 research and innovation programme under Grant Agreement n. 727929 - TOMRES

## TOPIC:

### Stress resilience in horticultural and fruit crops

#### Oral Communications

##### 539 - Characteristics of a salt stress resilient transcriptome – splice variants in tomato roots

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Salinity is an increasing problem for the cultivation of tomato plants. Roots are exposed first to salt stress condition in the soil and recently biostimulants have shown promise in overcoming salt stress in tomato. Interestingly, preliminary data suggested that genes related to alternative splicing are among the top genes affected by a biostimulant. Therefore, alternative splicing might be a key for plants to be resilient to salinity and it might as well be a molecular mechanism for a biostimulant to shift the tomato root transcriptome towards salt resilience. In order to understand the pathways and networks in tomato varieties exhibiting different levels of salt resilience and in tomato plants upon biostimulant treatment, we have to understand the effect of salinity on the transcriptome's splice variant composition.

Using PacBio full length cDNA long reads, we obtained a reference of splicing variants from salt treated and control *Solanum lycopersicum* roots grown in a greenhouse rockwool setup. Using this data set we determined the relative abundance of transcript variants with short read RNAseq data.

We use this reference to analyse the effects of the biostimulant on splicing variants, their abundance and generated full length cDNA data for the same samples to further elucidate how the biostimulant treatment translates to the diversity of the tomato root transcriptome and potentially confers resilience to salinity in tomato.

Finally, as the wild tomato relative *Solanum pennellii* is known to be salt stress tolerant we used our salt alternative splicing data set to compare the response of *Solanum pennellii* roots grown in the same setup as the reference.

## TOPIC:

### Stress resilience in horticultural and fruit crops

#### ***Extended Elevator Pitches***

**58 - Comparative transcriptomics between *Solanum lycopersicum* and *S. pennellii* sheds light into adaptation to arbuscular mycorrhizal symbiosis and combined stress resilience**

**Matteo Chialva<sup>(1)</sup> - Stefania Stelluti<sup>(1)</sup> - Mara Novero<sup>(1)</sup> - Paola Bonfante<sup>(1)</sup> - Luisa Lanfranco<sup>(1)</sup>**

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The root-associated microbiota can protect plants against biotic and abiotic stresses. A role in plant resilience to nutrients limitation and drought, major issues for crop productivity in modern agriculture, has been attributed to arbuscular mycorrhizal (AM) fungi that establish a mutualistic symbiosis with most land plants. Among Mediterranean crops, tomato (*Solanum lycopersicum*) will be one of the most disadvantaged, and innovative solutions, from genotypes selection to growing practices, are required. In this work we characterized, under controlled conditions, susceptibility and responsiveness to the AM fungus *Funneliformis mosseae* in the tomato 'M82' cultivar and the drought-tolerant wild species *Solanum pennellii*, which is largely exploited in tomato breeding. *S. pennellii* showed a reduced colonization, although arbuscule morphology was normal, and a negative growth response upon AM inoculation, differently from tomato. To elucidate the molecular mechanisms underpinning this phenotype an RNA-seq was performed considering both species and combined water/nutrient stress conditions. Among 20,162 orthologs identified, 1,822 genes were modulated by AM symbiosis across species and conditions. Under well-watered conditions, the number of AM-responsive genes was lower in *S. pennellii* compared to tomato, but under combined-stress numbers were similar. A different expression pattern was observed in symbiotic signaling pathway genes since the up-regulation detected in tomato under well-watered conditions was not observed in *S. pennellii*. Also genes involved in synthesis and metabolism of strigolactones, important signaling molecules during early AM interaction, showed a lower expression in *S. pennellii*. However, no difference was found in the expression of genes related to nutrient exchanges, in line with the occurrence in *S. pennellii* roots of few, but well-developed, arbuscules. This work will enlarge our knowledge on plant adaptation to the AM symbiosis and on the effect of the symbiosis on plant responses to water and nutrients limiting conditions, possibly highlighting relevant alleles involved in plant resilience.

**442 - Contrasting responses of two grapevine cultivar with different hydraulic behaviour to drought: the role of non-structural carbohydrates in xylem embolism**

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Xylem embolism is one of the effects triggered by the increase in xylem tension when plants face drought. Some species have been shown to recover from embolism and recent studies have highlighted the role of non-structural carbohydrates (NSC) in the generation of osmotic gradients required for the refilling of the embolized vessels. In the frame of the classification of plants according to their isohydric/anisohydric strategies, this study aims to evaluate embolism recovery in two grapevine cultivars considered at the opposite edges of the isohydric/anisohydric continuum. In particular, the connection with non-structural carbohydrates (NSC) was investigated. One-year grafted plants of Grenache (isohydric) and Barbera (anisohydric) were subjected to an intense water stress followed by a resumption of irrigation. The degree of embolism and its recovery at the stem level were quantified in vivo by micro-CT analysis and followed by xylem anatomical evaluation and NSC analysis. Both cultivars recovered the embolism after a resumption of irrigation even if Barbera plants were constitutively more prone to embolism. Modulation of soluble carbohydrates pattern during water stress and recovery at the expenses of starch was observed in both cultivars. However, only in Grenache plants a direct relationship between the soluble carbohydrate content and the degree of embolism was observed, evidencing its direct involvement in the embolism recovery process. Our findings showed that the two cultivars used different strategies in response to drought, suggesting an NSC-mediated recovery only for the isohydric Grenache.

## TOPIC:

### Stress resilience in horticultural and fruit crops

#### Posters

**581 - Promising PGPR isolated from drought-tolerant *Solanum lycopersicum* rhizosphere enhance drought tolerance of non-tolerant tomato plants**

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Plant growth promoting rhizobacteria (PGPR) play an important role by improving growth of the host plant and enhancing its tolerance under stress, such as drought. In these lines, a promising strain (SAESo11) was isolated from tomato (*Solanum lycopersicum*) rhizosphere cultivated in a naturally drought-stressed environment, identified as *Pseudomonas putida* based on whole genome sequencing, and tested for in vitro PGP traits. SAESo11 was found active in IAA and siderophore production, phosphate solubilization, and biofilm formation. To determine the ability of SAESo11 to confer drought tolerance, inoculated tomato seedlings were cultivated under drought stress and different agronomic and photosynthetic parameters were measured. Along with the evaluation of membrane lipid peroxidation, the activity of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX) and catalase (CAT), as well as the non-enzymatic total antioxidant activity and total phenolic content (TPC), was also determined. Moreover, primary metabolites of tomato leaf tissues were detected by Gas Chromatography-Mass Spectrometry analysis. According to the results, drought stress caused inhibition of plant growth, and photosynthesis. However, the negative effects of drought were mitigated in SAESo11 inoculated plants, resulting in significantly less reduction in the above-mentioned traits. Drought exposure led to an increase of MDA content. Surprisingly, MDA was also high at colonized non stressed tomato seedlings, indicating a possible PGPR mediated mechanism of priming. TPC was enhanced in non-inoculated drought-stressed seedlings, while, inoculation with SAESo11 did not further enhance it. Higher enzymatic activities were observed in drought-stressed plants, while inoculation led to reduced activity of the tested enzymes. The metabolomic profile of plant tissues was found to be altered in response to inoculation, indicating that bacterial colonization of tomato caused a shift in metabolic pathways at both stressed and non-stressed plants, compared to the uncolonized plants. Modulation in the metabolite accumulation under drought stress in response to SAESo11 application is possibly linked to the enhanced plant alertness to the imposed stress.

**687 - Exogenous MeJA application as a strategy to mitigate Al and Mn stress-induced in highbush blueberry (*Vaccinium corymbosum* L.) cultivars**

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Acidic soils ( $\text{pH} \leq 5$ ) occupy 30-40% of arable lands. Its acidic condition favors the Al and Mn availability, reaching toxic concentrations for plants. Several species are affected by these conditions, including highbush blueberry (*Vaccinium corymbosum* L.), which has great economic importance worldwide. Therefore, it is necessary to find agronomic strategies for mitigating the damage caused by Al and Mn toxicities, such as the use of exogenous phytohormones as Methyl Jasmonate (MeJA). Therefore, the aim was to evaluate the effect of exogenous MeJA application on the performance of *V. corymbosum* cultivars exposed to Al and Mn stress-induced. Star, Legacy, Camellia, and Cargo cultivars were exposed to Al ( $200\mu\text{M}$ ) and Mn ( $1000\mu\text{M}$ ) excess, with and without MeJA ( $10\mu\text{M}$ ), until 48h. Spectrophotometric analyzes were performed to determine lipid peroxidation (LP), total antioxidant activity (AA), total phenol content (TP), and auxin concentration in leaves; besides, the gene expression of the metal tolerance protein (MTP) was measured. Our results showed that the use of MeJA helps to decrease LP of all cultivars, indicating a decrease in oxidative damage in plant leaves up to 98%. The exogenous MeJA increased the TP up to 96%; however, this was not concomitant with the AA increase in all cases. On the other hand, a differential MTP transporter gene expression and the endogenous auxin concentration in the leaves varied depending on the cultivar and treatment. In conclusion, it is suggested that the MeJA application could be useful as an agricultural tool since it reduces the damages caused by Al and Mn toxicity in *V. corymbosum* cultivars.

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## 269 - A proteome analysis of lettuce plants exposed to pharmaceuticals.

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Pharmaceutical products are considered one group of emerging contaminants concerning water and soil pollution. These organic compounds have been increasing their presence in different ecosystems, leading to the exposure of different species, and consequently changes in their physiological and morphological properties. Pharmaceuticals may be present in rivers, groundwater and reach irrigation water, but the presence of these compounds may also be a result of fertilization with manure. Regardless of the sources, it is important to address how plants cope with these contaminants, especially with respect to accumulation in edible plants. The main objective of this work was to identify, via a proteomics approach, the main proteins involved in oxidative stress defence mechanisms induced by the presence of three pharmaceuticals in lettuce. Lettuce plants were developed during 8 days under contamination with 1 mg.L<sup>-1</sup> of carbamazepine, metformin and acetaminophen, separately, on hydroponic system, in parallel with a control experiment. Biomass parameters were evaluated followed by the proteomic analysis. Leaves and roots were harvested separately and a protein extraction followed by a LC-MS analysis were performed to identify proteins and observe the differences among conditions. A classification of these significant proteins using Gene Ontology terms was performed according their molecular function and biological processes, showing that different family proteins are associated with different studied conditions, for roots and leaves. According their molecular function, binding and catalytic activity showed to be the main groups characterized by the proteins with significant differences. Some proteins related with response to oxidative stress or to superoxide radicals were identified in contaminated leaves, such as thiosulfate sulfurtransferase, peptidylprolyl isomerase, fructose-bisphosphate aldolase, thioredoxin-disulfide reductase and glutathione reductase. Furthermore, glucose-6-phosphate dehydrogenase, peroxidases and glutathione S-conjugate-transporting ATPase 5 were some of the proteins found in contaminated roots. PCA was also obtained, indicating a clear difference between contaminated and control tissues.

### **387 - Adaptation of *Cynara cardunculus* plants and calli to saline stress constrain is associated to changes in non-enzymatic and enzymatic ROS scavenging mechanisms**

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Cardoon (*Cynara cardunculus* L.) is a multi-year food crop requiring low agronomical inputs and producing high biomass even in limiting growth conditions. Besides lignocellulosic biomass, cardoon produces oleaginous seeds with good fatty acid profile, roots rich in inulin and high amounts of bioactive molecules throughout the plant, and is therefore also being exploited as a feedstock for the bio-based industry. To understand key traits for sustainable plant cultivation, we characterized physiological, biochemical and molecular responses of the two cardoon genotypes “Bianco Avorio” and “Spagnolo” to short (2 days) and prolonged (21 days) 100 mM NaCl treatment in controlled hydroponic conditions. In our experimental system, prolonged stress did not constrain plant growth, and no symptoms of NaCl toxicity were detected. Looking at the metabolic investment, short saline imposition significantly depleted the amount of total chlorogenic acids only in “Bianco Avorio”. Prolonged stress strongly stimulated phenylpropanoid and proline accumulation in both genotypes, mirrored by higher non-enzymatic antioxidant capacity in terms of TEAC activity. The ROS scavenging and H<sub>2</sub>O<sub>2</sub> detoxifying enzymes SOD, CAT and APX were generally depleted under salt, except for the activities of APX, which was enhanced in “Spagnolo” under short saline stress, and CAT, which recovered upon prolonged saline stress. Overall, salt appears to impair the primary enzymatic antioxidant line of defense in “Spagnolo” less than in “Bianco Avorio”. However, the major contribution to H<sub>2</sub>O<sub>2</sub> scavenging and reduced lipid peroxidation, as estimated by malonyldialdehyde production, seems to be associated to phenylpropanoids and proline biosynthesis in both genotypes. To better understand the resistance mechanisms of “Spagnolo” to salt, we also investigated the cellular responses of this genotype to salt. NaCl-treated “Spagnolo” calli showed activation of non-enzymatic antioxidative defenses that paralleled those recruited in the whole plant, indicating that similar mechanisms operate also at the cell level.

## **582 - Sodium accumulation has minimal effect on the metabolite profile of onion bulbs**

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Onions (*Allium cepa* L.) were considered a salt sensitive crop. However, to date, there is very little evidence to that claim and information about the physiological and metabolomic effects of Na<sup>+</sup> accumulation in onion plants is lacking. The purpose of our research was to add knowledge and to assess changes in leaves and bulbs of three different onion cultivars after soil and foliar applications with moderate doses of chloride free Na<sub>2</sub>SO<sub>4</sub>. Additionally, the antioxidative defense mechanism in onion and the transport of Na<sup>+</sup> within the plant was also analyzed. Results demonstrated that Na<sup>+</sup> is mainly transported via xylem and therefore foliar application does not lead to Na<sup>+</sup> accumulation in the bulbs. Soil application with Na<sub>2</sub>SO<sub>4</sub> resulted in accumulation of Na<sup>+</sup> in leaves and bulbs but, with the exception of one onion variety, this did not alter the metabolite profile of onions significantly. Even K<sup>+</sup> concentration and organic solute levels were unchanged after application of Na<sup>+</sup>. But after Na<sub>2</sub>SO<sub>4</sub> treatment there was a moderate increase of the antioxidative defense system in onion bulbs. This study demonstrated that onion plants have the ability to exclude Na<sup>+</sup> at moderate Na<sub>2</sub>SO<sub>4</sub> treatment, and that the potential for quality onion production in saline soils with increased sodium concentration could be much higher than previously assumed.

### 583 - Suitability of abscisic acid and proline as markers for heat, drought, and combined stress in grapevines

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Heat and drought are among the most limiting abiotic factors in crop production. In view of changing climatic conditions, heat and drought stress will gain relevance in the coming years. Viticulture will be particularly affected by these changes, as above-average climatic changes are predicted for wine-growing regions. There are several reports on the responses of grapevines to simple heat or simple drought stress, but little is known so far about the effect of combined stress on grapevines. In studying heat, drought, and combined heat and drought stress, it is of particular interest to find traits that indicate stress before symptoms become apparent or yield declines. Therefore, we investigated whether the two commonly used traits proline and abscisic acid (ABA) biosynthesis are appropriate markers of heat, drought, or combined stress and whether gene expression of key ABA biosynthesis enzymes is regulated in leaves of two grapevine cultivars under these stress conditions. Plant growth, leaf gas exchange, and photosynthesis were examined to evaluate plant responses to elevated temperature and water deficit. Our findings reveal an interaction between heat and drought stress for gas exchange as well as for proline and ABA biosynthesis. ABA concentration was a suitable marker of heat, drought, and combined stress whereas proline was only good marker for combined stress. Gene expression of P5CS showed the same pattern as proline concentration. Gene expression of NCED1 in leaves was an appropriate marker for drought and combined stress, while NCED2 was not.

**605 - Preharvest salicylic acid application effects on fruit quality and yield in *Aristotelia chilensis* plants subjected to irrigation deficit.**

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Drought is an important abiotic stress that decrease fruit quality and crop yield. Exogenous salicylic acid application (SA) has shown to be a promising tool to cope with drought stress in plants. *Aristotelia chilensis* (Mol.) also known as maqui, is an endemic species of Chile, which has a great interest for farmers due to high phenolic compounds and antioxidant levels in their fruits. However, there are not information in *A. chilensis* in response to SA application. Therefore, we aimed to evaluate the preharvest SA application on fruit quality and yield in *A. chilensis* subjected to irrigation deficit. Three-year-old plants growing under field conditions were subjected to: 1) well-watered plants (WWP: 100% crop evapotranspiration (ET<sub>c</sub>), and Irrigation deficit plants (IDP: 60% ET<sub>c</sub>) based on ET<sub>c</sub> data. A single application of 0.5 mM SA was performed at fruit colour change by spraying fruits and leaves of both irrigation treatments. Fruit quality and yield were determined at fruit maturity stage. IDP showed 20 % higher total soluble solids compared to control plants; however, not differences were observed with SA. Meanwhile, titratable acidity (TA) was significantly reduced in IDP without SA compared to WWP with SA. IDP with SA showed higher equatorial and polar diameter (about 15% and 10%, respectively) compared to IDP without SA. Meanwhile, no changes were observed between WWP and IDP with SA. Same tendency was observed in fruit fresh and dry weight, where IDP with SA exhibited higher (about 15% and 10%, respectively) levels compared to IDP without SA. Water deficit also reduced fruit yield (about 15%) compared to WWP, meanwhile, SA recovers fruit yield in IDP, showing no changes between control and IDP with SA. Thus, SA improve fruit quality and fruit yield in *A. chilensis* plants subjected to drought stress.

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**669 - A polyamine oxidase of *Solanum lycopersicum* controls plant growth, xylem differentiation and drought stress tolerance**

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In plants, the polyamines putrescine, spermidine, spermine (Spm) and thermospermine (T-Spm) are involved in developmental and defense processes. In particular, T-Spm is implicated in the control of plant growth and xylem differentiation interfering with auxin and cytokinin signaling. Polyamine levels are finely regulated through biosynthesis and catabolism. In *Arabidopsis*, five FAD-dependent polyamine oxidases (AtPAO1 to AtPAO5), which exhibit distinct expression patterns, substrate specificity and subcellular localization, are involved in polyamine catabolism. Notably, the cytosolic AtPAO5, which oxidizes Spm and T-Spm, contributes to the control of plant development, xylem differentiation and abiotic stress tolerance. In tomato (*Solanum lycopersicum*), three AtPAO5 homologs were identified (SIPAO2, SIPAO3 and SIPAO4), and CRISPR/Cas9 mediated loss-of-function *slpao3* mutants were obtained. Transgenic tomato plants ectopically expressing AtPAO5 (AtPAO5over) were additionally obtained. Molecular, phenotypical and physiological analyses evidenced that *slpao3* mutants and AtPAO5over tomato transgenic plants present altered Spm and T-Spm levels, growth parameters, number of xylem elements, and expression levels of genes related to auxin (SIPIN1 and SIPIN6) and cytokinin (SIAHP6) signaling pathways, with respect to the wild-type plants. Furthermore, *slpao3* mutants are characterized by an increased tolerance to drought stress compared to wild-type plants, whereas on the contrary the AtPAO5over tomato transgenic plants appear hypersensitive to this stress. Analyses are in progress to unravel the structural and functional determinants of the *slpao3* stress tolerance characteristics. This study suggests that polyamine metabolism can be exploited for transferring stress tolerance traits in crop plants.

**700 - Phyto-courier, a silicon nano particles-based biostimulant – evidence from Cannabis sativa exposed to salinity**

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Global warming and rising sea levels are major crises society is facing in the recent years. Ensuring agricultural productivity under continuously changing environmental conditions is a challenge of the utmost importance. In that matter, salinity deserves special attention in case of land flooding. As an abiotic stress, salinity causes osmotic imbalance, nutrient deficiency and oxidative stress in plants, which strongly impacts growth and development. The resulting plant yield loss will have economic consequences. Utilising plant-derived compounds as payloads of a biodegradable and safe silicon-based nanoparticle (Si-NP) technology referred to as “phyto-courier” represent a sustainable way to fight exogenous stresses in plants. The goal is to develop a leading formulation mitigating stress effects in crops through a novel nanotechnology and to investigate its physiological and molecular effects in plants. The model plant used is industrial hemp (*Cannabis sativa*), a multipurpose crop with importance for several industrial sectors, grown for its fibres and seed oil. Si-NPs were loaded with quercetin and the phyto-courier was applied to *C. sativa* plants grown under salinity stress (250 mM) via foliar application. Quercetin as a secondary plant metabolite has known stress-mitigating properties improving plant performance when subjected to non-favourable conditions. *C. sativa* treated with the phyto-courier displayed a decreased expression of stress-related genes and accumulated higher levels of sucrose, indicating a protective effect of the formulation. The obtained results warrant follow-up studies to evaluate different exposure time windows, frequencies and application routes as well as to extend the analyses to other plant species of economic interest.

## TOPIC:

### The plant microbiome and new strategies for biofertilization

#### **Keynote Lecture**

##### **Plant microbiota controls the function of the root endodermis in *Arabidopsis thaliana***

##### **Gabriel Castrillo**

All living organism have evolved specialized cell layers to control their mineral nutrient and trace element composition. These cells function as a diffusion barrier to water, solutes, and immunoactive ligands while interacting with the local microbiota. The root diffusion barriers in the endodermis, which are critical for the mineral nutrient balance of plants, coordinate with the resident microbiota to exert their function. We demonstrate that genes controlling endodermal function in *Arabidopsis thaliana* contribute to the plant microbiome assembly. We described a new regulatory mechanism of endodermal differentiation driven by the microbiota with profound effects on nutrient balance. This mechanism is linked to the microbiota's capacity to repress responses to the phytohormone abscisic acid in the root. Our findings establish the endodermis as a regulatory hub coordinating microbiota assembly and homeostatic mechanisms.



## TOPIC:

### The plant microbiome and new strategies for biofertilization

#### Oral Communications

**201 - Azospirillum brasilense AF-22 containing multiple plant beneficial traits and colonization potential positively modulates the growth of Helianthus annuus L. under reduced fertilizer inputs**

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Pakistan is the world third largest importer of edible oil, imposing enormous burden on the economy of the country. Sunflower (*Helianthus annuus* L.) has great potential to bridge up the gap between production and consumption of edible oil. The use of plant growth promoting rhizobacteria is a promising strategy for sustainable agriculture production and is a potential alternative to chemical fertilizers and pesticides. Despite its economic importance, a little is known about the response of sunflower towards inoculation with PGPR. A potential *Azospirillum brasilense* AF-22 was isolated from Bandi, Himalayan Mountain region of Dhirkot (subdivision), Azad Jammu and Kashmir. The bacterium produced  $24.67\mu\text{g mL}^{-1}$  indole-3-acetic acid (HPLC),  $137.84\text{nmol mg}^{-1}\text{ protein h}^{-1}$  nitrogenase activity (GC) and solubilized  $40.11\mu\text{g mL}^{-1}$  insoluble phosphorus (Spectrophotometer) showing significant decrease in pH (from 7 to 4.74) due to the production of oxalic acid, malic acid and gluconic acid (HPLC). The *Azospirillum brasilense* AF-22 was metabolically diverse (utilized 68 out of 96 carbon sources), resistant to many antibiotics, and showed antagonistic activity against *Fusarium oxysporum*. Inoculation with this bacterium to sunflower grown in soil-free (hydroponic) medium, sterilized soil and under natural field conditions at two agro-ecologically different locations i.e., Rawalakot, Azad Jammu and Kashmir, and Faisalabad, Pakistan showed a significant increase in sunflower growth, yield, oil contents and achene NP uptake compared with non-inoculated control treatments. AF-22 was able to colonize sunflower roots forming a biofilm like structure; documented through yfp-labelling by confocal laser scanning microscopy as well as through immunogold labeling coupled with transmission electron microscope (TEM). The ultrastructure studies through TEM also confirmed the endophytic nature of AF-22. This study concludes that *Azospirillum brasilense* AF-22 containing multiple plant growth promoting traits can be a potential candidate for production of biofertilizer for sunflower crop to enhance yield with reduced application of chemical (NP) fertilizers.

#### **407 - Plant immune system activation is necessary for efficient interaction with beneficial bacteria**

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Microbes of the phytomicrobiome are associated with every plant tissue. Plants are continuously monitoring the presence of microorganisms to establish an adapted response. Plants use pattern recognition receptors to perceive microbe associated molecular patterns (MAMPs) which are microorganism specific molecules. MAMP detection will lead to a defined immune response called MAMP-triggered immunity (MTI). The plant immune system not only recognize pathogenic bacteria but also beneficial bacteria as well. However, unlike immune recognition of pathogenic bacteria, which was extensively studied, the interaction of beneficial bacteria with the plant immune system is less explored. In my work I studied the colonization of the plant model system *Arabidopsis Thaliana* by *Bacillus vazezensis*, an auxin producing beneficial bacteria. Auxin is a central plant hormone, playing important role during plant root development. Bacterial produced auxin was shown to influence root development process. However, the role played by auxin for the bacterial physiology itself is less clear. In my work I found that

the plant immune system and bacterial produced auxin stimulate each other. Thus, the bacterial colonization triggered an immune reaction in the root, the immune reaction in turn triggered auxin production by bacteria. Auxin promotes bacterial survival and spreading over the root. Finally, efficient spreading over the root and further auxin secretion promotes lateral root formation by the plant. Thus, both the bacteria and the plant are engaged in a positive feedback loop to promote efficient root colonization by the bacteria and root developmental response.

**469 - Proteomic analysis reveals how pairing of a Mycorrhizal Fungus with Plant Growth-Promoting Bacteria modulates growth and defense in wheat**

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Plants rely on associated microbiota for improving their nutritional status and environmental stress tolerance. Previous studies mainly focused on the interaction of a plant with a single microbe; however, changes in plants during simultaneous associations with multiple microbes have not received sufficient attention. We investigated local and systemic changes induced in wheat (*Triticum aestivum* L.) by two plant growth-promoting bacteria (PGPB), *Azospirillum brasilense* and *Paraburkholderia graminis*, either alone or together with an arbuscular mycorrhizal fungus (AMF), *Funneliformis mosseae*. We conducted phenotypic, proteomic, and biochemical analyses to investigate bipartite (wheat–PGPB) and tripartite (wheat–PGPB–AMF) interactions. Results revealed that only AMF and *A. brasilense* (alone and together) promoted plant growth. The bioprotective effect of the PGPB–AMF interactions on wheat plants was also explored against the leaf pathogen, *Xanthomonas translucens*. While PGPB alone did not show any bioprotection, co-inoculation of pathogen-infected plants with PGPB and AMF led to varied responses: *A. brasilense* drastically altered the bioprotective effect of the AMF, whereas *P. graminis* did not affect AMF-induced pathogen resistance. Proteomic changes in wheat plants strongly depended on the inoculum composition (single or multiple microbes) and the organ under study and led to differential growth effects and pathogen resistance. The protein profiles clearly revealed that the AMF was the crucial driver of plant growth and defense priming, under our growth conditions (low P). However, the overall changes induced by the AMF–PGPB consortium can interfere with the final mycorrhizal-induced resistance outcome.

### 531 - Breeding for Plant-Trichoderma compatibility

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Plant growth enhancement through the application of *Trichoderma* species has been shown in many plant species. Inconstant efficiencies have however been seen, and a lack of genetic understanding of the interaction likely limits the present agricultural benefits of the technology. Here we present the characterization of phenotypic variation in inbred breeding lines of sugar beet (*Beta vulgaris*) and candidate gene targeting using genomic resequencing. Our platform for phenotypic characterization of sugar beet inbred lines composed of two assays investigated the direct inoculation of *T. afroharzianum* T-22 in sand and soil as well as volatile stimulation from *T. afroharzianum*, *T. atroviride*, *T. viride*, and *T. reesei*. The evaluation of phenotypic response ranged from -1.4X to enhancements of 2.8X in total dried weight to controls. Interestingly, inbred line contributions to changes in total dried weight varied from tissue specific growth responses in both root and shoot. By resequencing of inbred sugar beet lines we were able to adapt homologous gene candidates from incompatibility assays using *Arabidopsis thaliana*. Unique markers linked to inbred lines with negative growth phenotypes were then used to assay a larger population to isolate a small region on chromosome three with four differential alleles of a peptiabol resistance candidate gene. Confirmed, it would be the first plant gene for the breeding of crop-*Trichoderma* compatibility. This result also provides evidence for conservation of *Trichoderma* compatibility mechanisms between plant species, allowing transfer of knowledge on *Trichoderma* compatibility genes from model species to sequenced crop species.

## 547 - Uncovering factors that modulate the microbiota assembly in Zea mays

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To cope with adverse environmental conditions, such as water scarcity and nutrient-deficient soils, plants have evolved a range of biochemical, molecular and physiological mechanisms. Recent evidence suggests that soil microbiota play a critical role in plant response to environmental stresses. How these two elements, the plant mechanisms and the microbiota, coordinate to respond to an environmental stressor, remain poorly understood. We have designed a holistic approach that combines characterization of a collection of agricultural soils, soil bacterial composition, plant microbiota, and water and nutrition availability to disentangle the main drivers that influence the assembly of plant microbiota in the model plant *Zea mays* (maize). We monitored free-living soil bacteria communities, soil mineral content, and climatic conditions in 24 agricultural soils in the Sahel region (Cabo Verde) for two years. We observed strong structuring of the free-living bacteria according to the soil mineral content, but not with the climatic conditions. Since plants recruit their symbionts from the surrounding free-living microbes, we have further evaluated the factors driving microbiota assembly in root and maize single-leaves in three different soils. We found that individual maize leaves harbour distinct ionome and microbiota compositions, indicating that leaves with different age provide distinct habitats and harbour distinct microbiota populations. Understanding the principles that underpin microbiota adaptation to variable environmental conditions, both at soil level and in association with the plant, will help designing microbial synthetic communities with a predictable effect on the plant host. This will support the development of more efficient and environmentally-friendly agricultural practices.

## TOPIC:

### The plant microbiome and new strategies for biofertilization

#### **Extended Elevator Pitches**

**270 - Effect of seed priming by endophytic *Bacillus subtilis* on growth and drought stress tolerance of *Triticum aestivum* L. cultivars of steppe Volga and forest-steppe West Siberian agroecological groups**

**Oksana Lastochkina<sup>(1)</sup> - Darya Garshina<sup>(2)</sup> - Ludmila Pusenkova<sup>(2)</sup> - Regina Hafizova<sup>(3)</sup> - Sergey Ivanov<sup>(3)</sup>**

**Ufa Federal Research Center of the Russian Academy of Sciences, Institute of Biochemistry and Genetics, Ufa, Russian Federation<sup>(1)</sup> - Ufa Federal Research Center of the Russian Academy of Sciences, Bashkir Research Institute of Agriculture, Ufa, Russian Federation<sup>(2)</sup> - Ufa Federal Research Center of the Russian Academy of Sciences, Ufa Institute of Chemistry, Ufa, Russian Federation<sup>(3)</sup>**

Were investigated the effect of seed priming by endophytic *Bacillus subtilis* (strains 10-4) on growth, drought tolerance and endogenous salicylic acid (SA) of *Triticum aestivum* L. (wheat) cultivars with different drought stress adaptation strategies belonging to steppe Volga (SV) (cv. Saratovskaya-55 (S55), Ekada-70 (E70)) and forest-steppe West Siberian (FSWS) (cv. Omskaya-35 (O35), Salavat Yulaev (SY)) agroecological groups (ecotypes), representing, respectively, Volga and Western Siberia - the largest Russian regions for wheat production. It was discovered that the same strain of *B. subtilis* (10-4) in the same growth conditions exerts multidirectional effects on growth and drought tolerance of wheat cultivars belonging to SV and FSWS ecotypes. Particularly, *B. subtilis* 10-4 promoted better germination of seeds, elongation of seedlings (roots and shoots), fresh and dry biomass accumulation of VS ecotype's cv. S55 and E70 under normal and simulated drought (12%PEG-6000) conditions. In the opposite scenario, strain 10-4 had practically no effect (or in some cases even had an inhibitory effect) on the same growth parameters of the FSWS ecotype's wheat cv. O35 and SY were found. *B. subtilis* 10-4 increased the water holding capacity (WHC) of leaves of all tested plants under normal growth condition, however, the greatest responsiveness under drought stress for cv. S55 and E70 were observed. Also, under normal growth condition *B. subtilis* 10-4 increased (in different level) endogenous SA in all studied plants with the highest accumulation for cv. S55 and E70. The findings indicate the ability of *B. subtilis* 10-4 to influence on growth of different ecotypes' wheat plants has a correlation with *Bacillus*-induced changes in their leaf's WHC and in favor the participation of endogenous SA in the initiation of defense responses, especially for cv. E70 and S55 (SV ecotype).

The reported study was funded by RFBR according to the research project № 19-016-00035.

**458 - Cork oak forests plant growth promoting rhizobacteria (PGPR): key partners to prevent drought stress**  
**Ana João Pereira <sup>(1)</sup> - Cláudia Teixeira <sup>(1)</sup> - Rui M Tavares <sup>(1)</sup> - Teresa Lino-Neto <sup>(1)</sup> - Francisca Reis <sup>(1)</sup>**

**BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Centre, University of Minho, Braga, Portugal <sup>(1)</sup>**

Mediterranean temperate forests have been identified as major biodiversity hotspots. These ecosystems are currently threatened by long term drought imposition, which will be further enhanced by the predicted climate changing. Cork oak (*Quercus suber* L.) is an evergreen species typically distributed within the Mediterranean Basin that has an important economic and social impact for the Iberian Peninsula. Although, well adapted to summer drought season, increasing of temperature and decreasing of precipitation is endangering the sustainability of cork oak forests. Sustainable approaches such as, plant growth promoting rhizobacteria (PGPR) inoculation could play a key role for cork oak adaptation and tolerance to drought. This work intends to evaluate and select cork oak forests PGPR capable to increase drought tolerance.

PGPR isolates were obtained from three different cork oak forests, presenting specific bioclimates (humid, sub-humid, semi-arid). Those PGPR isolates presenting the most promising biochemical traits were identified and selected for in vitro and in vivo studies, including plant inoculation (*Arabidopsis thaliana*, *Phaseolus vulgaris* and *Zea mays*). All selected isolates promoted an increase of *A. thaliana* root hair induction, as well as lateral root formation, and a decrease of primary root growth. A significant positive correlation was registered between the promotion of hair root development and the place of origin of each isolate, namely the local water availability and high temperatures. Important agronomic plant species, such as common bean and maize were also inoculated with most promising PGPR in pot assays and physiologically evaluated. PGPR consortia presented better results than single inoculations. Results obtained will contribute for more sustainable forest and agricultural practices in a near future climatic change scenario.

## TOPIC:

### The plant microbiome and new strategies for biofertilization

#### Posters

**641 - The impact of exogenous application of short-chain chitin oligomers on *Medicago truncatula* root transcriptome.**

**Veronica Volpe** <sup>(1)</sup> - Teresa Mazzarella <sup>(1)</sup> - Andrea Crosino <sup>(1)</sup> - Matteo Chialva <sup>(1)</sup> - Andrea Genre <sup>(1)</sup>

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Arbuscular mycorrhizal (AM) association is an ancient symbiosis providing mineral nutrients and water to most crop plants. Symbiosis establishment is anticipated by a reciprocal exchange of chemical signals between fungi and host plants. The fungal perception of root exuded strigolactones, a class of carotenoid-derived molecules, boosts the release of plant-directed signals, called Myc-factors. Among them, tetra/penta-chitoligosaccharides (CO) activate symbiotic signalling in plant hosts, including  $\text{Ca}^{2+}$  spiking in the nuclei of root epidermal cells. Here we applied exogenous CO, derived from crustacean exoskeleton, to pot-grown *Medicago truncatula* inoculated with the AM fungus *Funneliformis mosseae* during a time-course (10, 14, 21, 28 days) to investigate the early and later root transcriptional responses using an RNAseq approach. Transcriptome analysis was performed on inoculated and non-inoculated plants, with or without CO treatments. A first global analysis revealed a few general trends: a general increase in gene expression correlated with the progressive development of the symbiosis; a time-dependent decrease in the impact of CO treatment; a stronger effect of CO application at 10 days, suggesting an impact of the exogenous molecules on early symbiotic signaling.

In more detail, our analyses indicate an anticipated activation of intracellular accommodation processes in mycorrhizal CO treated plants, in line with the more extensive colonization observed at later time points. Alongside symbiosis promotion, CO treatment caused a general upregulation of the strigolactone biosynthetic pathway and induced a partial repression of plant defense. A strong downregulation was also recorded in several genes belonging to secondary metabolism, which can also be related to defense responses. Overall, our transcriptomic analysis outlined the molecular background for the CO-dependent promotion of AM development.



## **664 - Biotransforming swine slurry with microbial consortiums for Agroforestry purposes**

**Noelia de la Cruz-Gómez <sup>(1)</sup> - Emiliano Enrique Diaz-Portuondo <sup>(2)</sup> - Marta Berrocal-Lobo <sup>(1)</sup>**

**Polytechnic University of Madrid, Polytechnic University of Madrid/Systems and Natural Resources, Madrid, Spain <sup>(1)</sup> - Zoitech Lab S.L., I+D+i, Madrid, Spain <sup>(2)</sup>**

An input from swine manure increased a 140 % from 1961 to 2014 producing until 11 tons of manure inputs in 2014. European nitrate directive limited the use of manure in 2011. The ecotoxicological effects of pig slurry makes necessary its biotransformation for agroforestry purposes. Spain is today the EU's second largest pig producer. With an annual production output of 90.000 metric tons, perennial ryegrass accounts a 50 per cent of total grass production for forage and turfgrasses purposes, been one of the principal crops used for animal feeding. At this work toxic swine slurry directly obtained from farming, was pre-treated with a bacterial consortium, the bio-transformed waste obtained (ZOI), was used as biofertilizer analyzing the effect of ZOI on the development of *Lolium perenne* sp. (Lp) crop. Germination ratio and different growth parameters were measured on plants for two weeks, by hydroponic culture. ZOI did not affect the germination, increasing leaf and root length, fresh and dry weight, and consequently, the total N and C content. The use of ZOI, allowed us recovering pig manures for agroforestry purposes, into a circular global economy strategy.

## 678 - Mycorrhiza-induced plant gene expression in the roots of two terrestrial orchids under field conditions

**Silvia Perotto** <sup>(1)</sup> - **Rafael Valadares** <sup>(2)</sup> - **Fabio Marroni** <sup>(3)</sup> - **Marcio Lambais** <sup>(2)</sup> - **Raffaella Balestrini** <sup>(4)</sup>

University of Torino, Department of Life Sciences and Systems Biology, Torino, Italy <sup>(1)</sup> - Universidade de São Paulo, Departamento de Ciência do Solo, Piracicaba, São Paulo, Brazil <sup>(2)</sup> - University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences, Udine, Italy <sup>(3)</sup> - C.N.R., Institute for Plant Sustainable Protection, Torino, Italy <sup>(4)</sup>

Mycorrhiza is a ubiquitous symbiotic association established between land plants and some soil fungi, in which plants usually provide photosynthesis-derived carbon to the fungal partner in exchange of water and mineral nutrients such as nitrogen and phosphorus. Mycorrhiza in orchids is quite unique because the fungus provides the host plant with carbon at least during early development, i.e. the protocorm stage, or throughout the plant life history stages in achlorophyllous or partially photosynthetic orchid species. Despite their unique trophic relationship, orchids accommodate the fungal partner in their tissues through cellular changes that must imply a signaling process and a modulation of gene expression. Transcriptomic changes in mycorrhizal versus non mycorrhizal tissues have been investigated mainly in laboratory conditions during early plant development, whereas gene expression in adult orchids has been rarely addressed. We have investigated plant gene expression in field collected mycorrhizal and non-mycorrhizal roots of two terrestrial orchid species with different trophic strategies: the photosynthetic *Oeceoclades maculata* and the partially mycoheterotrophic *Limodorum abortivum*. The two plant species associated specifically with two fungal symbionts in the Basidiomycetes, *Psathyrella candolleana* in *O. maculata* and *Russula delica* in *L. abortivum*. In both orchid species, mycorrhizal and non-mycorrhizal roots are often found on the same individual. RNA was extracted from root segments of mycorrhizal and non-mycorrhizal samples for cDNA libraries construction and sequenced by Illumina. The results revealed the upregulation of several molecular markers shared with other endosymbiosis, including arbuscular mycorrhiza and legume nodules. The results also indicate an increased level of plant transcripts encoding sugar and amino acid transporters in mycorrhizal roots, suggesting an important role of the fungus in carbon and nitrogen supply to the host. The results expand our current knowledge on the orchid mycorrhizal symbiosis in adult plants under natural conditions.

## 190 - Symbiotic relationships between *Populus* explants and *Bradyrhizobium* sp. bacterium in vitro

**Greta Striganavičiūtė**<sup>(1)</sup>

**Lithuanian Research Centre of Agriculture and Forestry, Institute of Forestry, Kaunas, Lithuania**<sup>(1)</sup>

Although plant-bacteria symbiosis is usually investigated in natural systems, increasing evidence also points to its possible continuance in vitro. The present study reports a symbiotic bacterium from the in vitro cultures of *Populus* trees. Three *Populus tremula* × *P. tremuloides* genotypes (L191, Wa13, and 174/10) and one *P. tremula* × *P. alba* genotype (IBL 91/78) were cultured in vitro by using apical shoot segments as explants. The presence of a bacterium, whose colonies stretched from the explants onto the nutrient medium, was observed in all three *P. tremula* × *P. tremuloides* genotypes, but not in IBL 91/78. The bacterial DNA was extracted, and 16S rRNA gene analysis classified the bacterium as *Bradyrhizobium* sp. The rooting-related differences between the *Bradyrhizobium*-infected and uninfected *Populus* genotypes were assessed both in the dark and under a 16-hour white-light photoperiod. The uninfected genotype IBL 91/78 showed a much higher adventitious rooting rate in the light than in the dark (62.1 % vs. 25.8 %). This could be explained by light-dependent polar auxin transport from a viable shoot apex, which is a known hormonal mechanism in plants. In contrast, the *Bradyrhizobium*-infected *P. tremula* × *P. tremuloides* genotypes Wa13 and 174/10 had lower rooting rates in the light than in the dark: 21.3 % vs. 68.1 % and 13.3 % vs. 49.5 %, respectively. The genotype L191 had similar rooting rates both in the light and dark (40.0 % vs. 45.6 %) but, interestingly, only 30.4 % of L191 explants had non-browning shoot apices in the dark, in contrast to 95.0 % of viable apices in the light. Thus, the adventitious rooting in the *Bradyrhizobium*-infected genotypes seemed to be independent from the viability of shoot apex and did not require light, indicating a possible role of the symbiotic bacteria rather than plant internal mechanisms only.

### **303 - The multifaceted functions of rhizobacterium, *Bacillus licheniformis*, on plant growth and stress tolerance**

**Huey-wen Chuang**<sup>(1)</sup>

**Department of Bioagricultural Sciences, National Chiayi University, Chiayi, Taiwan**<sup>(1)</sup>

Plant-growth-promoting rhizobacteria play a beneficial role in plant growth through various mechanisms. A rhizobacterial strain, *Bacillus licheniformis* CH102 exhibited antifungal activity and harbored genes required for lantibiotic and 2,3-butanediol production. In the tested plants, CH102 improved root architecture and increased fresh weight. Exposure to CH102 increased the plant tolerance toward high temperatures and water deficits, and reduced disease infection rate of bacterial soft rot. CH102 induced gene expression involved in induced disease resistance, nitrogen assimilation, antioxidants, photosystem II, ion transport and synthesis of secondary metabolites. Arabidopsis transcriptomic analysis revealed the CH102-upregulated genes were associated with several cellular pathways including the PEP-PEPR system, cell wall modification, and biosynthesis of osmoprotectants including choline, proline, and galactinol. The presence of CH102 elicited increased response of Arabidopsis genes associated with the jasmonic acid (JA) and abscisic acid (ABA) signaling pathways. Furthermore, CH102 inoculation increased expression of transcription factors implicated in the regulation of abiotic stress responses, including DDF2, CBF1, DREB2A, HSFA2, WRKY30 and WRKY33. In summary, CH102 exerts effects on multiple cellular pathways involved in defense response, metabolism, antioxidant system and hormone signaling such as JA and ABA to regulate plant growth and tolerance to biotic and abiotic stresses.

### **380 - Endophytic *Bacillus* spp. isolates promote *Nicotiana tabacum* L. shoot growth in vitro**

**Elena Andriunaite<sup>(1)</sup> - Inga Tamosiunė<sup>(1)</sup> - Monika Aleksandraviciūtė<sup>(2)</sup> - Danas Baniulis<sup>(1)</sup>**

**Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Babtai, Lithuania<sup>(1)</sup> - Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania<sup>(2)</sup>**

Despite wide technical application, in vitro plant tissue culture faces a variety of unfavourable conditions such as mechanical damage, osmotic shock, or phytohormone imbalance that could be detrimental to culture viability, growth efficiency, and genetic stability. Recent studies revealed that in vitro plant cultures have a diverse endophytic bacteria community, suggesting that engineering of the endophytic microbiome has the potential to improve their growth and acclimation to stress induced by the in vitro conditions. Therefore, the aim of this study was to identify tobacco endophytic bacteria isolates capable to promote biomass accumulation of in vitro shoots. Forty-nine endophytic bacteria isolates were obtained from greenhouse-grown tobacco plants and were identified as *Bacillus* sp. and *Pseudomonas* sp. Twenty-one of the isolates were used to study endophytic bacteria effect on tobacco shoot growth in vitro. The shoots were inoculated with bacterial suspension and shoot fresh weight was assessed after 3 weeks of cultivation. Isolates of *B. mobilis* (Nt.3.2), *B. mycoides* (Nt.10.1), *B. thuringiensis* (Nt.18, Nt.20.2) promoted shoot growth 11% to 21%. Interestingly, fresh weight of shoots inoculated with isolates of the same species, such as *B. mobilis* (Nt.14.2), *B. mycoides* (Nt.25, Nt.12.1), and *B. thuringiensis* (Nt.37), was reduced from 4% to 7%. Inoculation with the remaining isolates of *B. aryabhattai*, *B. marisflavi*, *B. simplex* and *P. koreensis* had no significant effect on biomass accumulation or was detrimental to tobacco shoot growth. The results suggest that isolates with a contrasting effect on shoot growth represent the capability of multiple bacterial strains to establish different interactions with the host. The isolates of the *Bacillus cereus* group, with shoot growth-promoting properties, have a potential application to improve the growth of plant tissue culture in vitro, and further studies based on their interaction with plant and host specificity would aid practical implementation.

**487 - Pestalotiopsis pini sp. nov., an emerging pathogen on Stone pine (Pinus pinea L.)**

**Ana Cristina Silva** <sup>(1)</sup> - **Eugénio Diogo** <sup>(1)</sup> - **Joana Henriques** <sup>(1)</sup> - **Ana Paula Ramos** <sup>(2)</sup> - **Marcelo Sandoval-Denis** <sup>(3)</sup> - **Pedro W. Crous** <sup>(3)</sup> - **Helena Bragança** <sup>(1)</sup>

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Stone pine (*Pinus pinea* L.) is one of the most important forest tree species in Portugal and in the whole Mediterranean basin. *Pestalotiopsis* species are common endophytes, saprobes or pathogens in a variety of hosts and environments. The objective of the present study was to identify *Pestalotiopsis* species associated with symptomatic stone pine trees. Samples of stone pine trees showing shoot blight and stem necrosis were obtained from stone pine orchards and urban areas in Portugal, and the isolated *Pestalotiopsis* species were identified based on morphology and combined ITS, TEF and TUB DNA sequence data. Artificial inoculations on one-year-old stone pine seedlings were performed with the two species most frequently found in association with shoot blight disease. A total of five *Pestalotiopsis* spp. were isolated. A taxonomic novelty, *Pestalotiopsis pini* is described, representing a new pathogen for stone pine. *Pestalotiopsis* species may represent a threat to the health of pine forests in the Mediterranean basin. New research lines are ongoing in order to address the potential impact extension of pestalotioid species on stone pine plantations, and to understand the effect of other biotic and abiotic factors, so that new management strategies can be developed against these pathogens.

#### **491 - Characterization of olive-associated fungi from olive cultivars with different levels of susceptibility to anthracnose**

**Joana Castro** <sup>(1)</sup> - **Daniela Costa** <sup>(1)</sup> - **Paula Baptista** <sup>(2)</sup> - **Teresa Lino-Neto** <sup>(1)</sup>

**BioSystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center, University of Minho, Braga, Portugal** <sup>(1)</sup> - **CIMO/School of Agriculture, Polytechnic Institute of Bragança, Bragança, Portugal** <sup>(2)</sup>

The olive tree (*Olea europaea* L.) plays an important socio-economic role worldwide, but olives are frequently affected by anthracnose (caused by *Colletotrichum* spp.). This disease affects 30-50% of Portuguese olive groves and leads to premature fall and/or mummification of fruits, with a consequent reduction in production and/or depreciation of olive oil quality. The control of this disease is very difficult and mostly relies on the use of copper-based fungicides. The current awareness of the impact of these products on health and environment has highlighted the importance of creating ecological alternatives, such as biocontrol approaches. The susceptibility of olives to anthracnose is already known to be affected by factors, such as cultivar, production system and olive maturation stage. In addition, the plant-associated microbial community has been increasingly recognized as playing an important role in plant health. Therefore, keeping in mind the potential of native olive tree microbiota to function as biological control of anthracnose, the main objective of this work was to evaluate the endophytic and epiphytic fungal communities in olives. Using a metabarcoding approach, through the sequencing (Illumina MiSeq) of ITS amplicons, fungal diversity was determined in olives from different olive cultivars (Cobrançosa and Madural), from different production systems (organic farming and integrated production) and in two maturation stages (green and semi-ripen). The results allow a better understanding of the effect of the cultivar, the production system of the olives and the maturation stage of the fruit in the structuring of the fungal community of olives. Results are discussed concerning the selection of potential biocontrol agents against anthracnose.

**594 - A fungal endophyte increases plant resilience to nutrient deficiencies: a case of Fe acquisition in legumes**

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An endophytic *Fusarium solani* strain (FsK) was isolated from the roots of tomato plants grown on a suppressive compost. The fungus is capable of protecting the plants against root pathogens and to elicit induced systemic resistance in tomato plants. The protective ability of strain FsK is mediated by the ethylene and ABA signalling pathways. The fungus is capable of promoting plant growth under nutrient deficiency conditions and alleviates water stress. Colonization can be established in other plant species. In the FsK-Lotus japonicus association, FsK is able to colonize the roots without causing any symptoms. Here, we investigated how colonization of Lotus japonicus roots by FsK impacts Fe homeostasis in roots and shoots. Under Fe deficiency conditions, root colonization by FsK is higher compared with control conditions and FsK is still capable of promoting plant growth via shoot fresh weight. The fungus-treated plants showed an earlier flowering time. These results demonstrated that FsK played an important role in enhancing plant growth under iron deficiency conditions, suggesting its potential as an inoculant for legume crops.



**651 - Short-chain chito-oligosaccharide production from different filamentous fungi and their application to stimulate arbuscular mycorrhizal symbiosis**

**Andrea Crosino** <sup>(1)</sup> - **Elisa Moscato** <sup>(1)</sup> - **Marco Blangetti** <sup>(2)</sup> - **Gennaro Carotenuto** <sup>(1)</sup> - **Federica Spina** <sup>(1)</sup> - **Simone Bordignon** <sup>(2)</sup> - **Virginie Puech-Pagès** <sup>(3)</sup> - **Laura Anfossi** <sup>(2)</sup> - **Veronica Volpe** <sup>(1)</sup> - **Cristina Prandi** <sup>(2)</sup> - **Roberto Gobetto** <sup>(2)</sup> - **Giovanna Cristina Varese** <sup>(1)</sup> - **Andrea Genre** <sup>(1)</sup>

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Short-chain chito-oligosaccharides (COs) are chitin-derived molecules composed of 4 to 5 N-acetylglucosamine residues, released by fungi from the phylum Glomeromycota and perceived by their host plant roots during the establishment of arbuscular mycorrhizal (AM) symbiosis. This symbiosis provides plants with water and mineral nutrients, namely phosphate. While longer chain COs (namely CO8) trigger plant defense responses, the exogenous application of short chain COs in the soil has recently been demonstrated to promote AM establishment, with an earlier and more extensive colonization of the host root.

Currently, COs are produced from crustacean shells by fishing waste processing industries, with a process that presents a few limitations in terms of environmental sustainability, seasonality and the relatively high final costs for semi-purified products, free of long-chain COs .

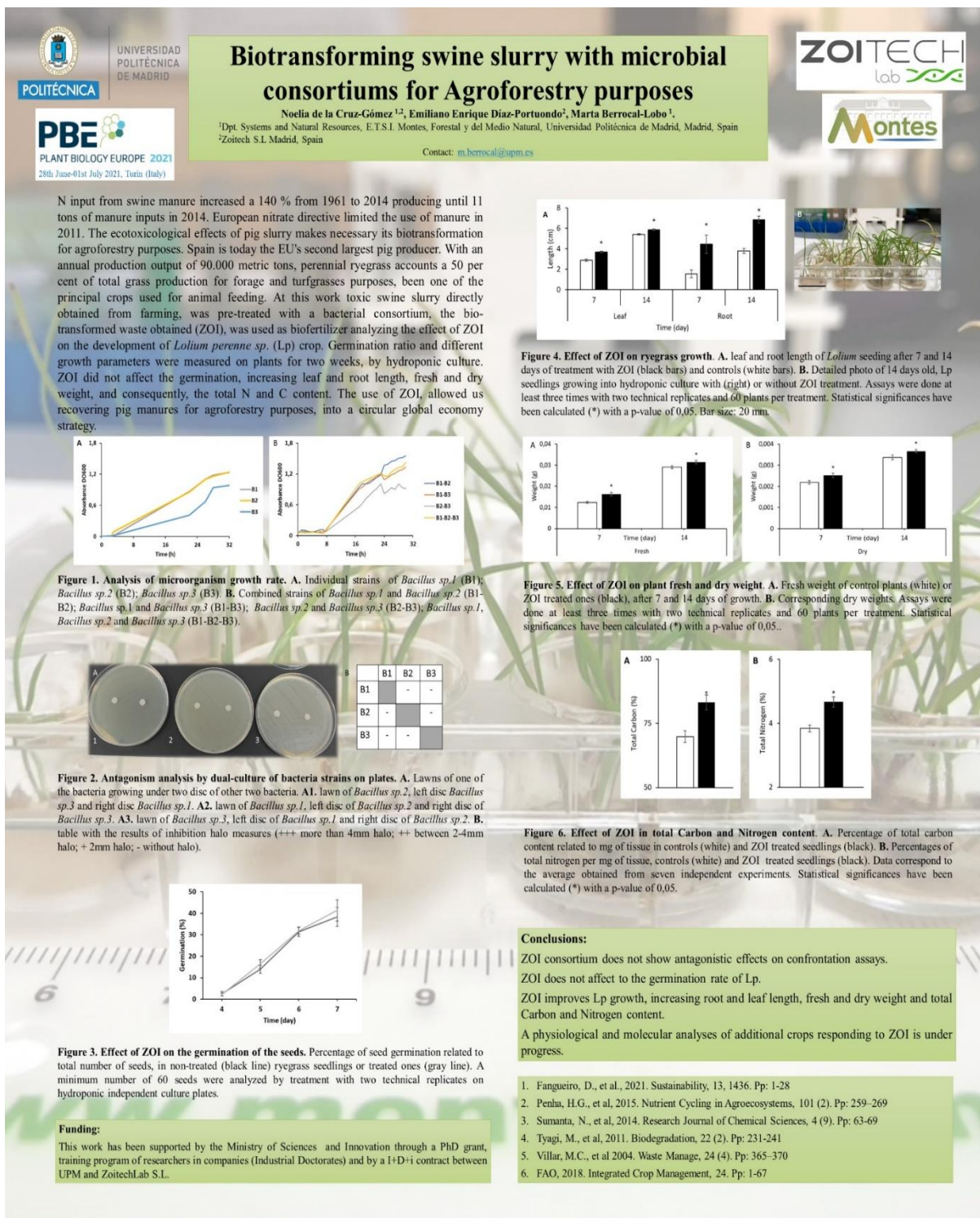
In this work we present a method to purify highly bio-active short-chain COs from three different fungi grown in vitro in liquid media. The bioactivity of extracted COs was tested in *Medicago truncatula* plants by quantifying their ability to activate symbiotic signaling and promote root colonization.

Our results indicate fungal-derived COs as more efficient elicitors of symbiotic responses in the host plant, compared to crustacean COs, and open the way to larger scale production of these molecules in view of their application in sustainable agricultural practices.

## 668 - Biotransforming swine slurry with microbial consortiums for Agroforestry purposes

Noelia de la Cruz-Gómez <sup>(1)</sup> - Emiliano Enrique Diaz-Portuondo <sup>(2)</sup> - Marta Berrocal-Lobo <sup>(1)</sup>

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## TOPIC:

### Trafficking and transport in plant cells

#### **Keynote Lecture**

##### **Exocyst complex functions in plant secretory pathways**

**Viktor Zarsky**<sup>(1)</sup> - Ivan Kulich<sup>(2)</sup> - Martin Potocký<sup>(3)</sup> - Tamara Pečenková<sup>(3)</sup> - Fatima Cvrčková<sup>(2)</sup>

**Dep. of Exp. Plant Biol. and Inst. of Exp. Botany, Charles university and Czech Academy of Sciences, Prague, Czech Republic**<sup>(1)</sup> - **Department of Exp. Plant Biology, Charles university, Prague, Czech Republic**<sup>(2)</sup> - **Inst. of Exp. Botany, Czech Academy of Sciences, Prague, Czech Republic**<sup>(3)</sup>

Targeted delivery of plasma membrane and cell wall components is an essential process in all plant cells – their polarity, morphogenesis and defense. The vesicle tethering complex exocyst assists in localized delivery of exocytosis vesicles to specific PM domains via direct interactions both with membrane lipids and proteins as an effector of small GTPases. Land plant EXO70 exocyst subunits form three distinct subfamilies – EXO70.1, EXO70.2 and EXO70.3. Interestingly, while the basal well conserved EXO70.1 subfamily consists of multi-exon genes, the remaining two subfamilies contain mostly single exon genes. Transcriptomic or proteomic data clearly indicate that most cell types in plants express and also use several different EXO70 isoforms; even closely related EXO70s are often functionally very specific. The exocyst research was transformed substantially by the discovery of exocyst complex version involvement in the autophagy process in both metazoans and plants. We will present a critical overview on the isoforms of subfamily EXO70.2 (esp. EXO70B1, EXO70B2, EXO70C2, EXO70Ds, EXO70E2, EXO70H1 and EXO70H4) we imply in often unconventional secretory processes related to autophagy, secondary metabolites transport, cell growth regulation, signaling, secondary cell wall biogenesis (including callose and silica deposition) and defense. Engagement of EXO70.2 class of EXO70s in biotic interactions and defense correlates well with enormous production and conservation of new protein variants within this subfamily of exocyst EXO70 subunits. We will also discuss possible functions of least studied subfamily EXO70.3 isoforms. Acknowledgement: this work is supported by the GACR/CSF projects 19-02242J, 20-11642S and MSMT proj. OPVVV “Cent. Exp. Biol. Plant” - CZ.oz.1.01/0.0/0.0/16-019/0000738.

## TOPIC:

### Trafficking and transport in plant cells

#### Oral Communications

##### 63 - Functional Interactions of Nuclear RNase P in Arabidopsis

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Ribonuclease P (RNase P) is the essential enzyme that catalyses the maturation of the 5' end of tRNA-precursors. First discovered as a ribonucleoprotein, where the catalytic activity is held by an RNA<sup>1</sup> (by Sidney Altman, who was awarded the Nobel prize for this discovery in 1989), it was long thought to be universally conserved as a ribozyme in the three domains of life. However, in 2008, it was first shown that RNase P also exists in a protein-only form<sup>2</sup> in human mitochondria. In plants, our team at IBMP, Strasbourg, has shown that Protein-Only RNase P (PRORP) enzymes hold RNase P in mitochondria, chloroplasts and the nucleus<sup>3,4</sup>. Since then, recent work from the team has focused on the biophysical characterization of PRORP in complex with its tRNA substrates<sup>5</sup>. Using SAXS and X-ray crystallography we provided a refined model of the PRORP-tRNA complex. Furthermore the protein interaction network of PRORP1 was studied in detail in mitochondria and chloroplasts to understand how the function of PRORP enzymes is integrated with other cellular processes<sup>6</sup>. Similarly, the protein and RNA interaction networks of PRORP2 are investigated in the nucleus. Our results show that PRORP2 interacts with tRNA methyltransferase 1A/B, shedding new lights on nuclear tRNA maturation process. In addition we proved that PRORP2 RNA substrates are not restricted to precursors tRNA but also include mRNAs containing tRNA-like structure (TLS). Among them, PRORP2 cleaves MAF1 precursor mRNA. Since MAF1 is a repressor of RNA polymerase III, PRORP enzymes might indirectly regulate RNA polymerase III activity and thus the transcription of tRNAs (unpublished work). Using transgenic lines we are currently investing the role played by MAF1 TLS on RNA pol. III activity and more largely on plant physiology.

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### 133 - Glutathionylation of plant glyceraldehyde-3-phosphate dehydrogenase triggers late and irreversible collapse into insoluble aggregates

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Protein aggregation is a complex physiological process, primarily determined by stress-related factors revealing the hidden aggregation propensity of proteins that otherwise are fully soluble. Here we report a mechanism by which glycolytic glyceraldehyde-3-phosphate dehydrogenase of *Arabidopsis thaliana* (AtGAPC1) is primed to form insoluble aggregates by the glutathionylation of its catalytic cysteine (Cys149). Intriguingly, no structural alterations have been observed after protein modification by H<sub>2</sub>O<sub>2</sub>-dependent overoxidation and S-nitrosylation. Following a lag phase, glutathionylated AtGAPC1 initiates a self-aggregation process resulting in the formation of branched chains of globular particles made of partially misfolded and totally inactive proteins. GSH molecules within AtGAPC1 active sites are suggested to provide the initial destabilizing signal and Arg231 plays a major role in stabilizing the γ-glutamate moiety of glutathione. The following removal of glutathione by the formation of an intra-molecular disulfide bond between Cys149 and Cys153 reinforces the aggregation process. Physiological reductases, thioredoxins and glutaredoxins, could not dissolve AtGAPC1 aggregates but could efficiently contrast their growth. Besides acting as a protective mechanism against overoxidation, S-glutathionylation of AtGAPC1 triggers an unexpected aggregation pathway with completely different and still unexplored physiological implications.

#### **426 - The post-translational modifications are crucial for Arabidopsis type III ACC synthases regulation**

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ACC synthases are the most rate limiting enzymes in ethylene biosynthesis in plants. ACSs are strictly regulated at transcriptional and post-translational level. Based on the protein structure, ACC synthases are classified into three groups. The mechanism of half-life of type I and II is well recognized but regarding type III, is not. Interplay between phosphorylation and ubiquitination events determines ACS protein fate in ethylene signaling. In Arabidopsis there is a unique member of type III ACC synthases – ACS7. Although it is known that ACS7 is degraded by proteasome 26S in ubiquitin dependent manner, the precise regulation is still not fully understood. We have shown that ABI1 and ABI1-like protein phosphatases affect ACS7 protein stability and launch ACS7 proteasomal degradation. What is more, we demonstrated that besides ubiquitylation, and phosphorylation, ACS7 is also SUMOylated. SUMO proteins (Small Ubiquitin-Like Modifiers) are one of the most important post-translational modifications (PTMs) in plant environmental stress response. SUMO, among others, can influence protein stability, subcellular localisation, ability of protein-protein interaction. BiFC experiments showed that ACS7 interacts with HPY2 E3 SUMO ligase and using in vitro SUMOylation assay we confirmed that ACS7 undergoes HPY2-dependent SUMOylation. To investigate what is the role of ACS7 SUMOylation we tested the recombinant GST-ACS7 protein turnover in hpy2-2 protein extracts, during cell free degradation assay. Our results highlight the complex role of post-translational modification in regulation of ACS7 turnover.

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## TOPIC:

### Trafficking and transport in plant cells

#### Posters

**606 - Root illumination status defines PIN-FORMED2 (PIN2) subcellular distribution and intracellular trafficking**

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The sessile life style of plants forces them to adapt constantly to changing environmental conditions. Establishment of auxin gradients underpin modulation of plant architecture during development and adaptation processes. PIN-FORMED (PIN) auxin efflux carrier facilitate active transport of auxin between cells and their abundance and subcellular distribution is rearranged depending on environmental conditions. Continuous modulation of cellular mechanisms including PIN endocytosis from the PM, followed by subsequent exocytosis to the PM or vacuolar degradation are crucial to orchestrate auxin distribution. Plant-cell-biological experiments are often performed using seedlings grown in full light, a growth condition not representative of real-life lighting levels. Using a new growth system, D-root, to grow shoots in light and roots in dark, we observed altered PIN2 turnover in a pharmacological screen depending on the root illumination status. This prompted us to further investigate the effects of D-root on root growth and PIN2 turnover. We observed significant growth and auxin level differences between full-light and D-root grown roots.

**177 - Potassium deficiency inhibits TOR via SnRK1 in Arabidopsis thaliana: linking K<sup>+</sup> levels to the regulation of autophagy via cell energy metabolism.**

**Elena V. Tyutereva <sup>(1)</sup> - Camilla K. Rabadanova <sup>(1)</sup> - Alexandra V. Murtuzova <sup>(1)</sup> - Ksenia S. Dobryakova <sup>(1)</sup> - Olga V. Voitsekhovskaja <sup>(1)</sup>**

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In plants, autophagy is inhibited by TOR kinase when nutrients are available, while SnRK1 (orthologue of mammalian AMPK) induces autophagy via TOR-dependent and -independent mechanisms. SnRK1 is activated by low energy status. In plants, electrochemical gradients necessary for ATP production and energization of transport processes are based on K<sup>+</sup> and H<sup>+</sup> membrane fluxes. We studied the effects of K<sup>+</sup> deficiency on autophagy and on TOR activity in Arabidopsis. Potassium starvation led to an increase in autophagosome formation; however, due to alkalinization of the vacuoles, the autophagic flux was arrested at the stage of cargo lysis, and no PCD was induced. Strikingly, autophagosomes formed in K<sup>+</sup>-deprived seedlings were present as aggregates. As autophagosomal traffic within plant cells is assisted by microtubules, we studied autophagosome formation in the mor1-1 mutant under conditions leading to disruption of microtubules, and observed similar aggregates. TOR activity was inhibited in K<sup>+</sup>-deprived seedlings in the presence of sucrose. Replacement of sucrose with glucose prevented the inhibition of TOR in K<sup>+</sup>-deprived seedlings, suggesting that K<sup>+</sup> deficiency acts upstream of TOR. Furthermore, K<sup>+</sup> deficiency failed to induce autophagy in plants overexpressing a catalytic subunit of SnRK1, KIN10, as well as in kin10 RNAi lines, suggesting direct involvement of SnRK1. NaCl failed to induce autophagy in K<sup>+</sup>-deprived seedlings, further suggesting action via SnRK1. We propose that low K<sup>+</sup> levels activate SnRK1 via disruption of K<sup>+</sup> and H<sup>+</sup> transmembrane gradients leading to ATP exhaustion; the latter interferes with kinesin function and autophagosomal traffic, leading to aggregation of autophagosomes. Altogether, our data link K<sup>+</sup> levels to the regulation of autophagy via cell energy metabolism.

The study was supported by RSF project No. 18-16-00074.



**183 - Elicitors induce immune priming in *Arabidopsis thaliana* through the plasma membrane-associated Ca<sup>2+</sup>- binding protein PCaP1**

**Moira Giovannoni<sup>(1)</sup> - Simone Ferrari<sup>(2)</sup> - Thomas Ott<sup>(3)</sup> - Giulia De Lorenzo<sup>(2)</sup> - Benedetta Mattei<sup>(1)</sup>**

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Oligogalacturonides (OGs) and flagellin are well-known plant elicitors capable of inducing reversible protein phosphorylation and relocalization of plasma membrane (PM) proteins upon their administration to *A. thaliana* plants. Among these proteins, PCaP1, a PM-anchored protein with actin filament-severing activity, was identified from early phosphoproteome changes upon OG treatment.

Although basal resistance was not affected, two *pcap1* loss-of-function mutants failed in activating the late defence responses typically induced by these elicitors such as protection against *B. cinerea*, up-regulation of late defense genes and induction of callose deposition. Moreover, *pcap1* null mutants showed a lack of responsiveness to additional OG and flagellin treatments indicating that PCaP1 is required for elicitor-induced immune priming against pathogens.

Transgenic plants expressing the PCaP1-GFP fusion under the control of its endogenous promoter also revealed that PCaP1 is localized in PM microdomains being rapidly internalized in endocytic vesicles in response to OGs. Although the functional role of this internalization is still under investigation, the subcellular relocalization of PCaP1 may represent a mechanism for desensitization by removing ligand-bound receptors from the PM.

**218 - The identification of cytoskeletal determinants of PIN-FORMED protein dynamics within the plasma membrane.**

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PIN-FORMED (PIN) auxin efflux carriers are one of the most studied integral plasma membrane (PM) proteins, important for the generation of auxin concentration gradients regulating plant development. PINs undergo trafficking between PM and intracellular compartments, which modulates the process of cell-to-cell auxin transport and its directionality. Although the molecular machinery that regulates plant vesicle trafficking processes and the role of auxin in this process is intensively studied, the mechanisms regulating dynamics of auxin carriers within the PM, where they perform their role, are still poorly understood. Our previously published data using fluorescence recovery after photobleaching and fluorescence correlation techniques indicated that PINs are present in both highly mobile (20%) and largely immobile domains (80%) within PM. Here we address the involvement of cytoskeleton in the PINs nanodomain organization within the PM using total internal reflection fluorescence microscopy in tobacco cultured cells carrying inducible genes for GFP-tagged PINs. Image analysis-based quantification of these distributions showed that 3 tested tobacco NtPINs (NtPIN2, 3 and 11) displayed protein-specific dynamics between individual nanodomains. In agreement, the application of actin and microtubules drugs, i.e. latrunculin B (Lat B) and oryzalin (Ory), resulted in NtPIN-specific changes, ranging from their disappearance from nanodomains observed after LatB in NtPIN2, to almost no changes observed after Ory. Interestingly, auxin transport assays showed that the activities of all tested NtPINs were not influenced after the drug treatments, suggesting that highly mobile and cytoskeleton-dependent fraction does not contribute to the overall auxin transport, which is perhaps the function of immobile fraction. Our work is pioneering in the identification of auxin transporting PM nanodomains, which composition is now identified with co-IP/MS to propose a model for the interaction between cytoskeleton and auxin efflux carriers.

### **345 - Effect of Divalent Ca<sup>2+</sup> ions on subcellular targeting of Sucrose transporter (StSUT4) from *Solanum tuberosum***

**Varsha Garg** <sup>(1)</sup> - Aleksandra Hackel <sup>(1)</sup> - Christina Kuehn <sup>(1)</sup>

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StSUT4 is a low affinity sucrose transporter belonging to the Type III sucrose transporter subfamily. There are several arguments regarding its sub-cellular localization: it is functional in sucrose uptake at the plasma membrane but the StSUT4-GFP fusion protein is localized at the tonoplast. Its sub-cellular localization is highly regulated at post-translational level either by oligomerization, protein-protein interaction and subcellular targeting. Recent findings reported enhanced internalization of StSUT4 when transiently expressed as homodimer. Incubation of StSUT4-YFP expressing tobacco leaf discs with 50mM Calcium chloride (CaCl<sub>2</sub>) leads to an increase in the number and diameter of vesicles as compared to the water control. In our data, we reported the co-localization of StSUT4 with the vacuolar marker in transiently expressing tobacco leaves. Here, we observed a similar effect of calcium ions and the translational inhibitor cycloheximide (CHX) on the sub-cellular redistribution of StSUT4 during transient expression. Mg<sup>2+</sup> as well as Ca<sup>2+</sup> ions were shown to affect sucrose transport activity of plant sucrose transport. It is the question whether regulation of sucrose transport activity occurs via internalization of the protein or via modification of its protein stability. A highly conserved di-acidic motif (DTD) is present in all sucrose transporters and seems to be involved in the Ca<sup>2+</sup>-dependent regulation of sucrose transporter activity.

### 349 - Exocyst complex functions in plant secretory pathways

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Targeted delivery of plasma membrane and cell wall components is an essential process in all plant cells – their polarity, morphogenesis and defense. The vesicle tethering complex exocyst assists in localized delivery of exocytosis vesicles to specific PM domains via direct interactions both with membrane lipids and proteins as an effector of small GTPases. Land plant EXO70 exocyst subunits form three distinct subfamilies – EXO70.1, EXO70.2 and EXO70.3. Interestingly, while the basal well conserved EXO70.1 subfamily consists of multi-exon genes, the remaining two subfamilies contain mostly single exon genes. Transcriptomic or proteomic data clearly indicate that most cell types in plants express and also use several different EXO70 isoforms; even closely related EXO70s are often functionally very specific. The exocyst research was transformed substantially by the discovery of exocyst complex version involvement in the autophagy process in both metazoans and plants. We will present a critical overview on the isoforms of subfamily EXO70.2 (esp. EXO70B1, EXO70B2, EXO70C2, EXO70Ds, EXO70E2, EXO70H1 and EXO70H4) we imply in often unconventional secretory processes related to autophagy, secondary metabolites transport, cell growth regulation, signaling, secondary cell wall biogenesis (including callose and silica deposition) and defense. Engagement of EXO70.2 class of EXO70s in biotic interactions and defense correlates well with enormous production and conservation of new protein variants within this subfamily of exocyst EXO70 subunits. We will also discuss possible functions of least studied subfamily EXO70.3 isoforms. Acknowledgement: this work is supported by the GACR/CSF projects 18-18290J, 19-02242J, 20-11642S and MSMT proj. OPVVV “Cent. Exp. Biol. Plant” - CZ.02.1.01/0.0/0.0/16-019/0000738.

## TOPIC:

### Translating plant research from lab to field

#### **Keynote Lecture**

**Translating plant organ growth from model to crop**

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The focus of our research is to obtain insights in the interplay between cell division and cell expansion in determining final organ size. The data on the molecular networks governing growth will be presented in light of the available knowledge in model organisms, relative to what we learnt in maize. Emphasis will be on a conserved growth regulatory module and how crops and model organisms contribute to our understanding.

## TOPIC:

### Translating plant research from lab to field

#### **Extended Elevator Pitches**

##### **262 - Leveraging international consortia to promote plant sciences**

**Roslyn Gleadow**<sup>(1)</sup> - Isabel Mondoza<sup>(2)</sup> - Bill Davies<sup>(3)</sup> - Barry Pogson<sup>(4)</sup>

**Monash University, Melbourne, School of Biological Sciences, Melbourne, Australia**<sup>(1)</sup> - **Communications Officer, Global Plant Council, Valencia, Spain**<sup>(2)</sup> - **Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom**<sup>(3)</sup> - **School of Biology, Australian National University, Canberra, Australia**<sup>(4)</sup>

The challenges facing plant scientists are enormous. There is an urgent need to develop new crops that are resistant to disease, high temperature, extreme weather event to feed a growing population, yet with a smaller environmental footprint. Against this backdrop, botany and plant sciences courses at universities are being eroded and opportunities for research funding are increasingly limited in many countries. Individual organisations have successfully raised the profile of specific crops (e.g. CYMMYT, IRRI) but more is needed.

The Global Plant Council (GPC) is an umbrella organisation for professional plant science societies and institutions around the world. GPC was first established in 2009 to develop, a coordinated approach internationally to address global challenges where plants are key to the solution. We focus efforts on communication of developments in our understanding of plant biology to aid the exploitation of this understanding to the benefit of society'

In this contribution, we will outline strategies to promote plant sciences and raise awareness of the need for education, outreach and research.

## TOPIC:

### Translating plant research from lab to field

#### Posters

#### 434 - Recombinant protein stability in cyanobacteria

Nico Betterle <sup>(1)</sup> - Xianan Zhang <sup>(2)</sup> - Diego Hidalgo-Martinez <sup>(2)</sup> - Anastasios Melis <sup>(2)</sup>

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The living cell possesses extraordinary molecular and biochemical mechanisms by which to recognize and efficiently remove foreign, damaged, or denatured proteins. This essential function has been a barrier to the overexpression of recombinant proteins in most expression systems. Notable exception is the overexpression in *E. coli* of recombinant proteins, most of which, however, end-up in “inclusion bodies”, i.e., cytoplasmic aggregates of proteins that are inaccessible to the cell’s proteasome. “Fusion constructs as protein overexpression vectors” proved to be unparalleled in their ability to cause substantial accumulation of recombinant proteins from plants, animals and bacteria, as soluble proteins in unicellular cyanobacteria. Recombinant protein levels in the range of 10-20% of the total cellular protein can be achieved. The present work investigated this unique property in the context of recombinant protein stability in *Synechocystis* sp. PCC 6803 by developing and applying an *in vivo* cellular Tobacco Etch Virus cleavage system with the objective of separating the target heterologous proteins from their fusion leader sequences. The work provides new insights about the overexpression, cellular stability, and exploitation of transgenes with commercial interest, highly expressed in a cyanobacterial bio-factory. The results support the notion that eukaryotic plant- and animal-origin recombinant proteins are unstable, when free in the cyanobacterial cytosol, but stable when in a fusion configuration with a highly-expressed cyanobacterial native or heterologous protein. Included in this analysis are recombinant proteins of the plant isoprenoid biosynthetic pathway (isoprene synthase,  $\beta$ -phellandrene synthase, geranyl diphosphate synthase), the human interferon protein, as well as prokaryotic proteins (tetanus toxin fragment C, and the antibiotic resistance genes kanamycin and chloramphenicol). Future success of synthetic biology approaches with cyanobacteria and other systems would require overexpression of pathway enzymes to attain product volume and the work reported in this paper sets the foundation for such recombinant pathway enzyme overexpression.

## 523 - High throughput cloning and protein expression analysis for structural studies of AUX1/LAX protein.

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The phytohormone auxin regulates myriads of developmental processes through its unique property of polar transport facilitated by asymmetric localization of influx and efflux membrane proteins. AUXIN1/LIKE-AUX1 (AUX1/LAX) proteins form a plant-specific sub-class within the amino acid/auxin permease (AAP) super family, encoding auxin uptake carriers. These uptake proteins share extensive sequence similarity regardless of different auxin related developmental responses. Despite being a master player in auxin homeostasis, the molecular mechanism of AUX1/LAX proteins remains poorly understood. Therefore, the goal of this multidisciplinary project is to obtain an atomic structure of AUX1/LAX protein through X-ray crystallography to delineate the novel molecular mechanism of auxin accumulation. To achieve optimum expression, heterologous expression of AUX1/LAX homologues (Dicots: *Arabidopsis thaliana* AtAUX1 & AtLAX3, *Nicotiana tabacum* LAX1, *Medicago truncatula* LAX3, Monocots: *Brachypodium distachyon* AUX1, Ancestral land plants: *Physcomitrella patens* AUX1/LAX and *Marchantia polymorpha* AUX1/LAX) selected over a wide phylogeny of plant genomes, were done on a high-throughput platform. A total of 96 constructs consisting of full length and truncated versions of the selected homologues were taken for high throughput cloning into four vectors with different tag combinations at N & C-terminal of the protein. For expression screening, two expression systems namely Expi293 mammalian system and recombinant baculoviral system in Sf9 insect cell line were explored. Furthermore, a small-scale detergent screening was carried out to select the best purification conditions. In addition, large scale purification using affinity and size exclusion chromatography were optimized to obtain sufficient yields of the protein for crystallography. The predicted outcomes have several implications in food security by providing a tool to explore these influx carriers as potential sites for novel agrochemical inhibitors using rational drug design.



### **38 - Biocontrols for bananas: re-thinking experimental designs according to a meta-analysis**

**Juniper Kiss** <sup>(1)</sup> - **Daniel P. Bebber** <sup>(1)</sup>

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Biological controls are viewed as environmentally friendly alternatives to chemical controls of plant diseases and pests. While publications report 60-90% disease or pest reductions in the greenhouse, biocontrols are considered to be unreliable in the field. Our aim is to identify sources of variation in experimental outcomes due to different setups and using different biocontrol types. Here, we focus on biocontrols used against banana diseases and pests as regulations are becoming stricter on allowed chemical residues.

We carried out an English literature search on Web of Science and Scopus between 2000-2019. In total, we identified 91 publications with 1,093 observations on biocontrol experiments against banana pests and diseases from 26 countries. Most observations were reported for using *Bacillus amyloliquefaciens* (11.3%), *Pseudomonas fluorescens* (8.5%), *B. subtilis* (5.8%), *Glomus mosseae* (4.6%), and non-pathogenic *Fusarium oxysporum* (3.8%) biocontrol stains. Whilst 543 observations were discarded due to insufficient reporting, 516 observations were used to calculate Hedges *d* as effect size. A Bayesian multi-level model was used to investigate the effect of biocontrol group (mycorrhizal, plant growth promoting rhizobacteria, endophytes and mixtures) under different experimental set ups (in vitro, in vivo, greenhouse, field), for different experiment durations for 21 banana diseases and pests. Explained heterogeneity ( $I^2$ ) was calculated for different moderators to investigate the source of variability.

Overall, the mixture of mycorrhiza with PGPR and mycorrhiza under field conditions had the largest effect whilst PGPR and endophytes had the smallest. The biocontrols' effects were larger in controlled environments and in relatively shorter experiments, when the plants were drenched with biocontrol inoculums. We suggest that a fundamental shift is needed in experimental designs from disease suppressions measured by one organism against another in vitro, towards a more field-relevant approach.

#### **74 - Identification of asymptomatic *Zymoseptoria tritici* using remote sensing in winter wheat plots**

**Christopher Adams** <sup>(1)</sup> - **Duncan Robertson** <sup>(2)</sup> - **David Langton** <sup>(3)</sup> - **Oliver Windram** <sup>(1)</sup>

**Imperial College London, Imperial College London, Ascot, United Kingdom** <sup>(1)</sup> - **Agrii, Agrii, Dunmow, United Kingdom** <sup>(2)</sup> - **Origin Enterprises, R&D Innovation, Dublin, Ireland** <sup>(3)</sup>

Wheat (*Triticum aestivum*) is one of the most important crops globally with 776.4 million tonnes produced last year with 155 million tonnes produced in the EU alone. However it is predicted that 10% of all EU wheat yield is lost to *Zymoseptoria tritici* (*Z. tritici*, formerly *Mycosphaerella graminicola*). Current treatment methods for *Z. tritici* include blanket application of broad spectrum fungicides accounting for 70% of all fungicide applied to wheat, at key growth stages that is both expensive, costing \$1.2 billion annually in Europe, and damaging to the environment. *Z. tritici* is a hemibiotrophic pathogen that has two distinct phases in its infection cycle, an 11-14 day asymptomatic latent phase followed by a necrotrophic phase. During the later necrotrophic phase fungal biomass increases this leads to the formation of necrotic lesions which are then recognised as *Septoria tritici* blotch (STB). If *Z. tritici* could be detected early before necrotrophic damage has occurred a precise reactive targeted fungicide regime could be used. We have developed a remote sensing method to detect the asymptomatic phase using wheat plots in field. First hyperspectral sensing is used to detect presence and stage of lifecycle of *Z. tritici*, as well as to infer multispectral image data to capture. The multispectral images are processed and analysed with a semi-supervised machine learning approach which can detect *Z. tritici* infection with high accuracy. Use of this method in the field could result in reactive targeted fungicide application which could i.) increase yield through reduced crop loss to infection, ii.) reduce the cost of fungicide application iii.) reduce damage to the environment through reduced fungicide application and iv.) increase profit for a farmer. There is also potential in the future to apply the method to other diseases and crops.

## 165 - Volatile organic compounds as novel non-invasive markers for sensing ripening stages of apple

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Apple (*Malus x domestica*) is one of the most widely consumed fruits across the world. One of the most important needs for apple growers is to determine optimal harvesting time in order to assure the correct fruit ripening stage, high nutritional value and extended post-harvesting self life as expected by consumers for maximum salability. My research focuses on identifying especial signature volatile organic compounds (VOCs) which shows differential emission pattern during apple ripening. In the present investigation, apple fruits (cv. 'Shireen') were classified into four ripening stages (HS-60: commercial harvesting time minus 60 days; HS-30: commercial harvesting time minus 30 days; HS-15: commercial harvesting time minus 15 days, and HS: commercial harvesting time). In case of cv. 'Shireen, commercial harvesting time was  $120 \pm 10$  days after flowering. The VOCs were profiled using solid phase micro-extraction coupled Gas Chromatography Mass spectrometry (SPME-GC-MS) based volatomics. Volatomics conditions were first optimized and then VOCs were identified by matching to NIST library (2017) with more than 70% similarity, kovat index and their mass spectrum pattern. A total of 102 VOCs were identified that includes terpenes, esters, alcohols, aldehydes, higher alkanes, and others. The output for PCA (Principal Component Analysis) data consisted of score plots for visualizing the contrast between all the four ripening stages and discrimination analyses using loading plots. Finally six signature VOCs were identified showing substantial variation among ripening stages which could serve as potential non-invasive markers for tracking ripening. These signature VOCs will be used as sensing target by non-invasive sensor to be used by the apple growers in field. The proposed solution has economic significance.

## 292 - Towards the development of herbicides with a new mode of action

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This study investigates the potential of inhibiting lysine biosynthesis in plants as a novel herbicide discovery approach. Specifically, we focus on the enzyme dihydrodipicolinate synthase (DHDPS), which catalyses the first committed and rate limiting step of the lysine biosynthesis pathway. By employing a combination of X-ray crystallography and rational inhibitor design, we have generated a novel class of inhibitors that target a previously unknown binding site within plant DHDPS. Furthermore, we have shown that these inhibitors exhibit low-mid micromolar potency both in vitro and in planta using enzyme kinetics and pre- and post-emergence assays, respectively. Thus, this study provides proof-of-concept that inhibiting lysine biosynthesis represents an attractive target for the development of herbicides with a novel mode of action to combat the rise of resistance.

#### **490 - Global Regulatory Harmonization for Agricultural Biotechnology**

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Government policies and data requirements for the safety assessments of genetically modified (GM) crops vary across regulatory authorities globally. In addition, technology developers use different study designs to generate the required data, resulting in varying data submitted. Differing timelines for regulatory approvals can cause delays in getting new and innovative products into farmer hands, while asynchrony in approvals can lead to trade disruption. After more than 25 years of safe use and numerous benefits to farmers, consumers and the environment, the time has come to modernize the safety assessment process for GM crops through the global alignment of these regulatory policies.

The CropLife International regulatory harmonization project has aligned our members on the studies and approaches which constitute a science-based data package given the history of safe use of these crops and our familiarity with the technology. In our approach, data from a set of core studies is used to identify the need for supplementary hypothesis-driven studies. Employing this approach for the evaluation of GM plants will remove regulatory data requirements that do not provide value to the safety assessment and provide a consistent framework for global regulation. Regulatory harmonization encompasses a range of alignment between national regulatory frameworks. Alignment could be anything from governments having consistent requirements for the regulatory review process, to sharing data and assessments, to mutual recognition or approving a product if it has already received approval from another regulatory agency.

### 630 - Development of waxy wheat cultivars for bio-based industry applications

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Starch is a main component of wheat grain and consists of two glucan polymers amylose and amylopectin with the ratio ranging between 20-30 and 70-80%, respectively. The amylose / amylopectin ratio has a major effect over the physiochemical properties of starch and determines its optimal application in the industry. The isoforms of granule-bound starch synthase (GBSS) are responsible for the biosynthesis of amylose, whereas amylopectin synthesis is a complex pathway that involves at least three starch synthases (SSI, SSII, SSIII) and several branching (SBEI, SBEIIa and SBEIIb) and de-branching enzymes (DBE). Recently, amylose-free (Waxy) and high-amylose wheats, consisting of up to 100% amylopectin and 70% amylose, respectively, were produced through the development of new biotechnology techniques. Here we present the development of new waxy winter wheat cultivars 'Eldija' and 'Sarta' through conventional introgression of waxy alleles and marker-assisted selection to follow the introgression. Both cultivars were characterized by lower than standard grain yield however, as expected in fully waxy wheats, their amylose content was close to zero, 0.68% and 0.36% for 'Eldija' and 'Sarta', respectively. Starch pasting characteristics measured by Rapid Visco Analyzer also differed considerably from the standard and were typical for waxy wheat starches with higher viscosity (at peak) and lower retrogradation rate (setback) with shorter peak time and lower gelatinization temperature. Protein and gluten content of 'Eldija' and 'Sarta' was similar to the high-quality standard cultivar. The release of waxy wheat cultivars 'Eldija' and 'Sarta' opens up availability of novel raw material for bio-based industry applications.



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