

International Conference of the German Society for Plant Sciences

# BOTANIK-TAGUNG

15-19 September 2024 | Halle/Saale



## PROGRAMME

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MARTIN-LUTHER  
UNIVERSITÄT  
HALLE-WITTENBERG



Deutsche  
Botanische  
Gesellschaft

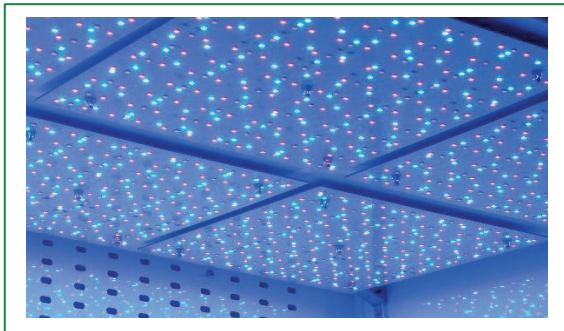
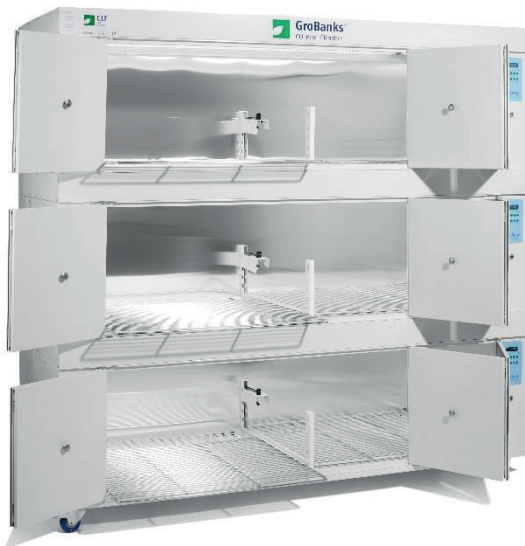
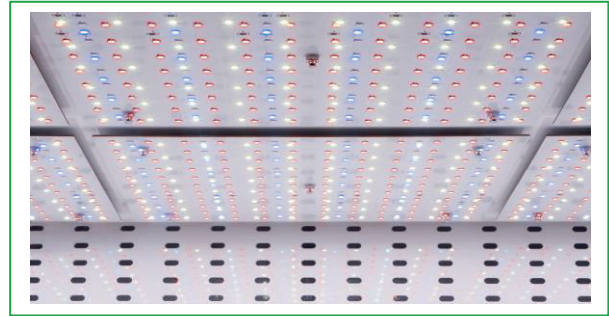
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## WELCOME ADDRESS FROM THE CONFERENCE CHAIR OF THE BOTANIK-TAGUNG 2024



Welcome to Halle!

We cordially invite you to the Botanik-Tagung 2024 – International Conference of the German Society for Plant Sciences (DBG), which will be held at the Martin Luther University Halle-Wittenberg on 15–19 September 2024.

This year's conference is jointly organized by groups of the Biology, Biochemistry, and Agriculture Departments of Halle University, the Leibniz Institutes of Plant Biochemistry (IPB) and of Plant Genetics and Crop Plant Research (IPK), as well as the German Centre for Integrative Biodiversity Research (iDiv). In addition, all DBG sections, DFG-funded consortia, and the German Society of Plant Nutrition (DGP) have been involved in shaping an exciting programme that spans the entire field of plant science - from proteins to ecosystems.

Nearly 30 internationally leading plenary and keynote speakers will highlight seminal current developments across different research areas. The conference motto - Growing Solutions for Growing Challenges - reflects that plant science needs to mobilize its full potential to counter existential problems of our planet. Photosynthetic organisms are the only means to sequester CO<sub>2</sub> in large amounts, but environmental conditions in their habitats, from oceans to rainforests, are turning increasingly unfavourable.

Accelerating climate change also demands further adaptation of crops to growth constraints, which benefits from a profound mechanistic understanding of gene and protein functions, as provided by molecular and cell biological approaches in model plants. This year's Botanik-Tagung will bridge basic and application-oriented plant research to foster the scientific exchange required to improve our fundamental understanding of plants and to translate it into resilient crops and ecosystems. Beyond the scientific programme, workshops for early career researchers will support the next generation of leading plant scientists. Childcare during the entire conference will enable parents to fully participate. During and after the conference, there will be the opportunity of excursions to experimental facilities, botanical gardens, and stunning local ecosystems.

The conference will be located in the centre of the beautiful and historic university town of Halle (Saale), also home of the German Academy of Sciences, Leopoldina. With around 20,000 students, Halle has a great variety of pubs, bars, and restaurants to socialize and to continue scientific discussions until late.

We look forward to welcoming you at an exciting Botanik-Tagung in September 2024,

Edgar Peiter  
Conference Chair



## Venue

Martin-Luther-University Halle-Wittenberg  
Universitätsplatz 1/9  
06108 Halle a. d. Saale  
Germany

## Date

15–19 September 2024

## Scientific organiser

Prof. Dr. Edgar Peiter – Plant Nutrition, MLU Halle-Wittenberg

## Scientific organizing committee

Debora Gasperini – Jasmonate Signaling, IPB Halle a. d. Saale  
Helge Bruehlheide – iDiv & Geobotany, MLU Halle-Wittenberg  
Ingo Heilmann – Plant Biochemistry, MLU Halle-Wittenberg  
Kristina Kühn – Cell Physiology, MLU Halle-Wittenberg  
Marcel Quint – Crop Physiology, MLU Halle-Wittenberg  
Nicolaus von Wirén – Physiology and Cell Biology, IPK Gatersleben  
Sascha Laubinger – Plant Genetics, MLU Halle-Wittenberg  
Steffen Abel – Molecular Signaling Processing, IPB Halle a. d. Saale

## Administration

Julia Grimmer – CRC 1664 (SNP2Prot) & RTG 2498, MLU Halle-Wittenberg

## Conference organisation

Conventus Congressmanagement & Marketing GmbH  
Anja Kreuzmann/Julian Unger  
Carl-Pulfrich-Straße 1, 07745 Jena, Germany  
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anja.keutzmann@conventus.de/julian.unger@conventus.de  
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**conventus**  
CONGRESSMANAGEMENT

## Design/Layout

Satz/Layout                      Conventus Congressmanagement & Marketing GmbH  
Editorial deadline              8 September 2024



## GENERAL INFORMATION



### Registration fees

DBG member	455 EUR
Non-member	565 EUR
Student/PhD student (DGB member)	245 EUR
Student/PhD student (Non-member)	300 EUR
BSc/MSc student (University Halle a. d. Saale)	20 EUR
Industry/Non-academic	670 EUR
Day ticket (15–19 September/per day)	195 EUR

### Workshops

#### 16 September, 12:45–13:45

WS 1 – Wie Forschungs- und Selbstpräsentation gelingen (language: German)	15 EUR
WS 2 – Assay Design Guidelines for qPCR and dPCR	free of charge
WS 3 – Plant Lighting and Energy Efficiency	free of charge

#### 17 September, 14:45–16:45

WS 4 – Reproducibility in Plant Biology	15 EUR
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#### 18 September, 12:45–14:15/14:30–16:30

WS 5 – Writing and Publishing	free of charge
WS 6 – Phenotyping Workshop	free of charge

### Excursions

#### 19 September, 14:30–18:00

Ex 1 – Leibnitz Institute of Plant Genetics and Crop Plant Research (IPK)	10 EUR
Ex 2 – Leipzig Canopy	10 EUR
Ex 3 – Botanical Garden of Halle a. d. Saale	free of charge

#### 20 September, 09:00–14:00

Ex 4 – Ecotrons, GCEF & MyDiv	10 EUR
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### Botany Party

Regular	50 EUR
BSc/MSc/PhD student	30 EUR



### General terms and conditions

Please find our General Terms and Conditions at [www.botanik-tagung.de](http://www.botanik-tagung.de).



### Registration

Please follow the on-site signs to find our desk/check-in. You will be provided with your name badge, the pocket planner and all other useful information. Please visit the check-in before attending the lectures.



### Opening hours check-in

Sunday, 15 September	15:00–19:00
Monday, 16 September	07:30–19:00
Tuesday, 17 September	08:00–18:00
Wednesday, 18 September	08:00–18:00
Thursday, 19 September	08:00–13:00



### WIFI access

SSID: event-net	
User name:	bota2024@uni-halle.de
Password:	jO@5kM{5



#### Certificate of attendance

Certificates of attendance will be available on request on your last conference day at the check-in desk.



#### DBG Poster prize

The German Botanical Society will offer 10 prizes for the best posters. The awarded authors will get 142 EUR each, this is the actual age of the society in Euro.



#### Poster session

The session for all posters with odd poster numbers will take place on Monday 16 September, 17:15–18:45.

The session for posters with even numbers will take place on Tuesday, 17 September, 17:30–19:00.

Authors are requested to be present at their posters during the poster session. Please remove your poster until Thursday, 19 September at 10:00.

The pinboards will be numbered and should be used only with the designated pins. You will find your poster number in the programme book.



#### Catering

During coffee breaks drinks and small snacks will be provided in the catering tent and in the Aula, 1<sup>st</sup> floor of the Löwengebäude, where the industrial exhibition and the posters are situated. A small, packed vegetarian and vegan lunch will be offered at our lunch station in the catering tent on the campus courtyard.



#### Smoking

Smoking is prohibited inside the entire conference area.



#### Taxi

Taxi Zentrale Halle a. d. Saale +49 (0)345 525252



#### Public transport

The Audimax of the University of Halle is only a few minutes' walk from the bus and tram station "Neues Theater". If you would like to plan your journey within the city by public transport, you are welcome to visit the HAVAG website.



#### Parking

Parkhaus Händelhaus-Karree  
Dachritzstraße 10, 06108 Halle a. d. Saale  
Distance to venue: 250 m / 4 minute walk

B+B Parkhaus Hanserin  
Hansering 21, 06108 Halle a. d. Saale  
Distance to venue: 600 m / 10 minute walk

More information as well as an interactive routing planner can be found on the conference website.



## SOCIAL AND CULTURAL PROGRAMME

### Welcome reception | Sunday, 15 September

Come together for some snacks and drinks at the university campus in Halle. Start your conference experience in a relaxed atmosphere.

Start/admission	16:00 (end 18:00)
Venue	Audimax der Martin-Luther-Universität Halle-Wittenberg Universitätsplatz 11, 06108, Halle a. d. Saale, Germany
Fee	included in the conference fee accompanying person: 15 EUR



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### Conference dinner\* "Botany Party" | Wednesday, 18 September

Meet your colleagues and friends in a pleasant atmosphere at the "Botany Party" on Wednesday evening. Enjoy music, snacks and socializing at the historic Volkspark building.

For the evening, a jam session featuring jazz standards is planned, and all conference participants are warmly invited to join in and play. Any other musical contributions for this evening are also very welcome.

Start	19:30
Venue	Volkspark, Schleifweg 8a 06114 Halle a. d. Saale, Germany
Fee*	Regular: Students (PhD, MSc, BSc): Accompanying person:



© Volkspark Halle

50 EUR
30 EUR
50 EUR

\*Due to limited number of places, registration is required.



## Submitting your presentation/technical information

Please prepare your presentation either in MS PowerPoint, Open Office or as a PDF file with the 16:9 widescreen format. A presentation notebook with a PDF reader and MS Office Power-Point 2010/2007 will be provided. Notebook, presenter and laser pointer will be available at the speaker's podium in the lecture hall. The use of Macintosh formats as well as the use of a personal laptop for presenting your lecture is possible upon agreement. However, it may interrupt the flow of the programme in the lecture hall. A technical supervisor will assist you. For video and audio files, please provide AVI, WMV and MPG files only as a separate file.

To guarantee a smooth running programme please upload your presentation on time – at least two hours before the start of the session.

For submission, please use a USB flash drive that is not protected by any software. Professional staff and equipment will be available for you to arrange and preview your presentation.

Please note: certain encodings for video and audio files could lead to technical problems.

## Time allotment

Please prepare your presentation for the allotted amount of time. Chairs and moderators may interrupt should you overrun your time limit.

Allotted time is assigned as follows:

Plenary lectures	40 minutes + 5 minutes discussion
Invited lectures	5 minutes + 5 minutes discussion
Abstract lectures	14 minutes + 4 minutes discussion



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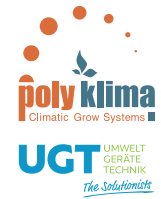
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### Contributing Institutions

Martin Luther University Halle-Wittenberg (MLU)



Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)



Leibniz Institute of Plant Biochemistry (IPB)



German Centre for Integrative Biodiversity Research (iDiv)



### Scientific sponsors

We thank the German Research Foundation for their friendly support of our international speakers.



### Further sponsors

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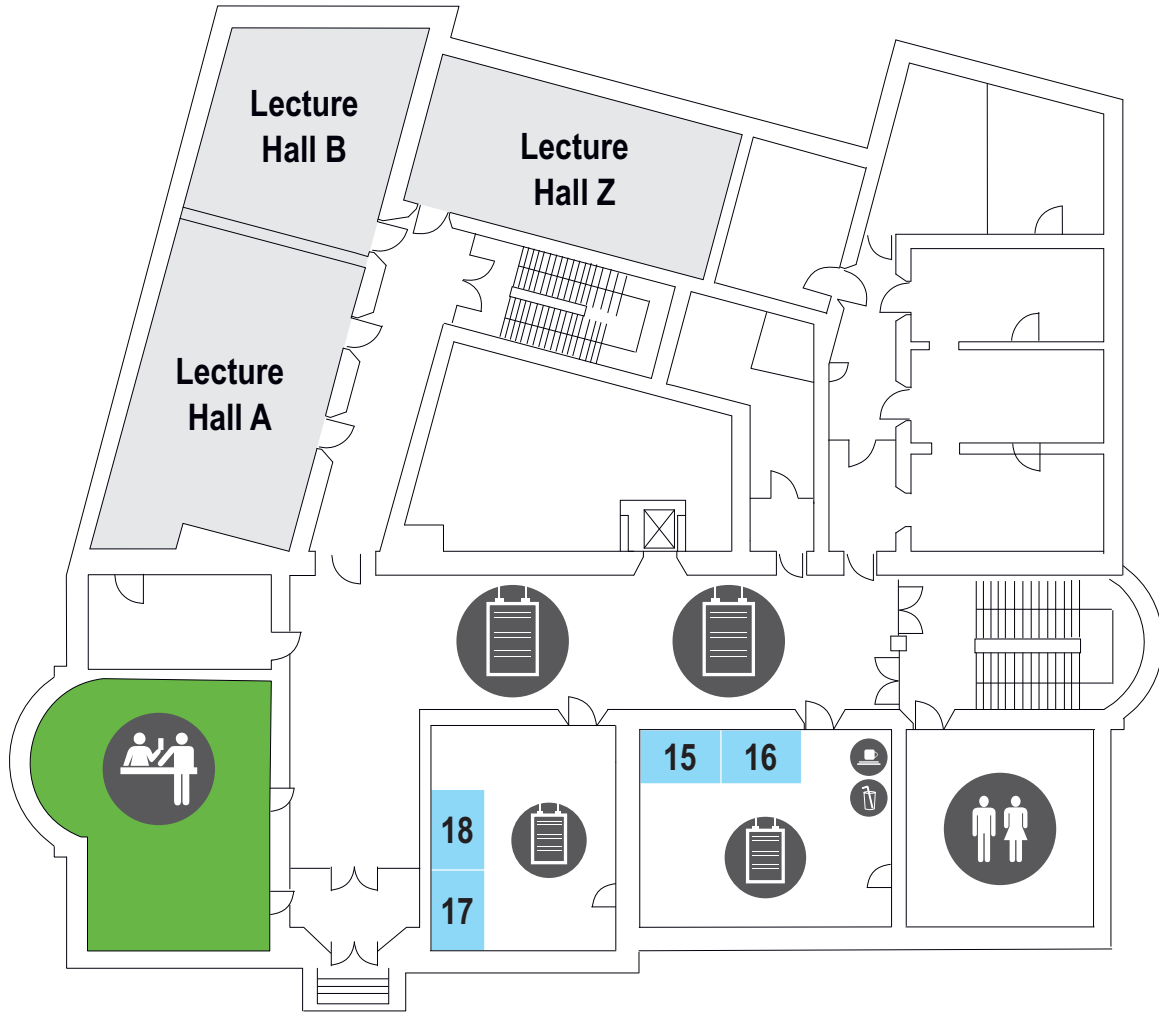


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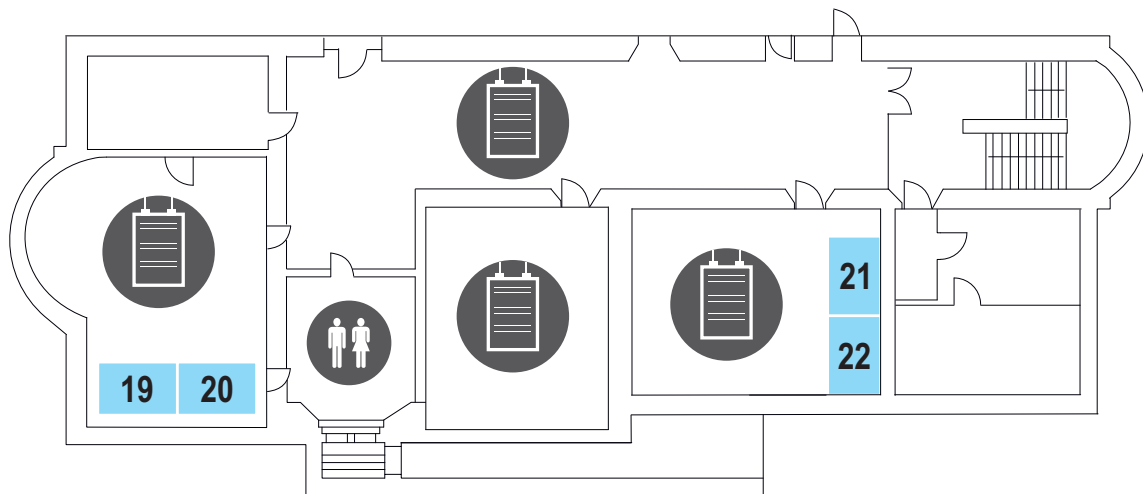


Exhibitors	Booth number
Azenta life Sciences	18
Agrisera Antibodies	13
Berthold Technologies GmbH & Co. KG	17
Bilberry sp. z o.o.	22
CLF PlantClimatics GmbH	5
DH Licht GmbH   Elektro- und Automatisierungsanlagen Pierre Ambrozy	1
Hansatech Instruments Ltd.	21
Heinz Walz GmbH	2
INTEGRA Biosciences Deutschland GmbH	8
Labdeers S.R.O.	19
LemnaTec GmbH	15
MACHEREY-NAGEL GmbH & CO. KG	10
New England BioLabs GmbH	9
Novogene Europe	6
poly klima GmbH   Umwelt-Geräte-Technik GmbH	7
Proteintech Germany	16
PSI (Photon Systems Instruments), spol. S.R.O.	11
Schweizerbart / Borntraeger Science Publishers	4
Springer-Verlag GmbH	14
Stilla Technologies	20
Verband Botanischer Gärten / Deutsche Botanische Gesellschaft	23
Wittich & Visser	3
Xceltis GmbH	12

### Ground floor Melanchthonium



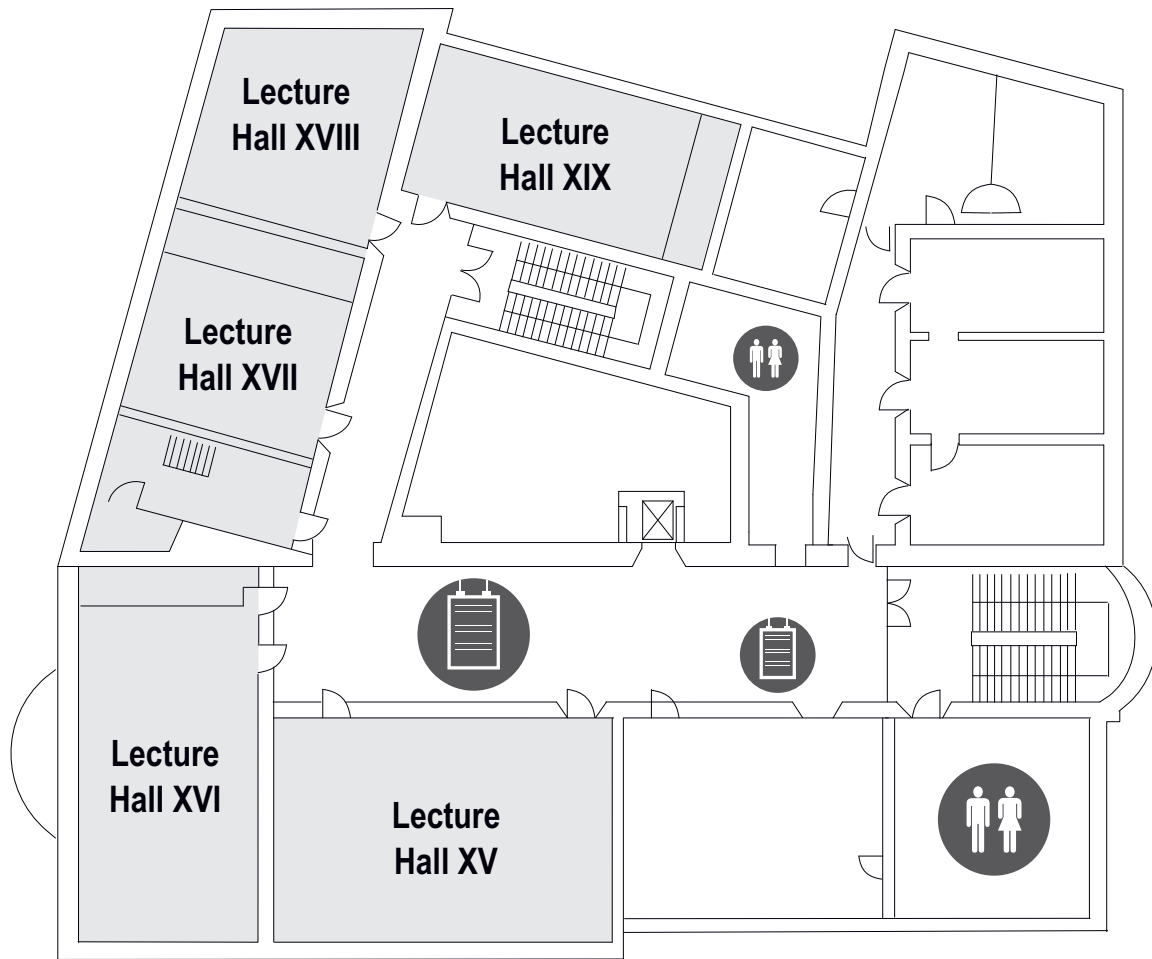
### Basement Melanchthonium



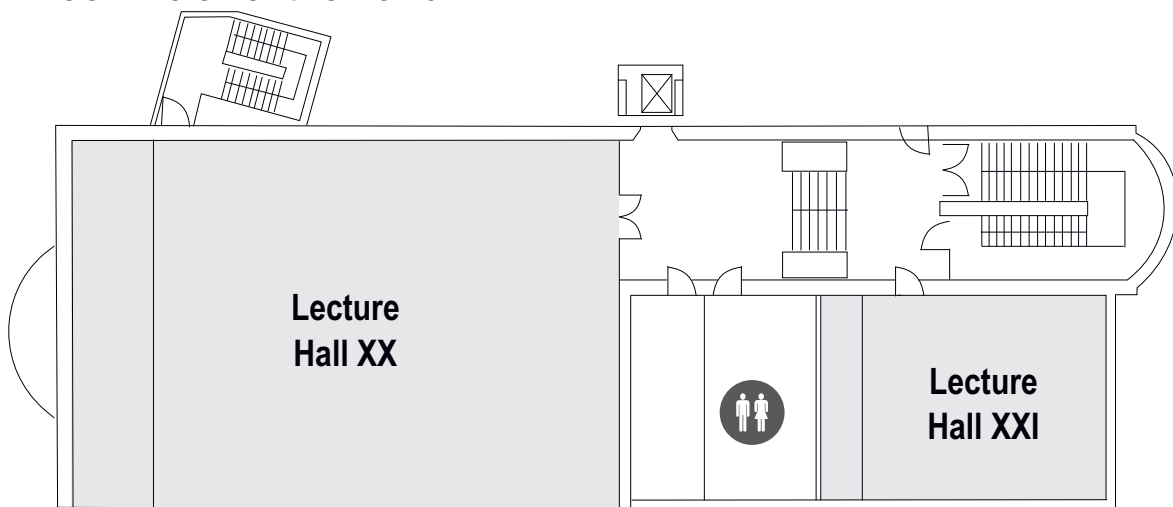
#### Legend

- Exhibitor
- Check-in
- Poster exhibition
- Beverage station
- Toilet

### 1<sup>st</sup> floor Melanchthonianum



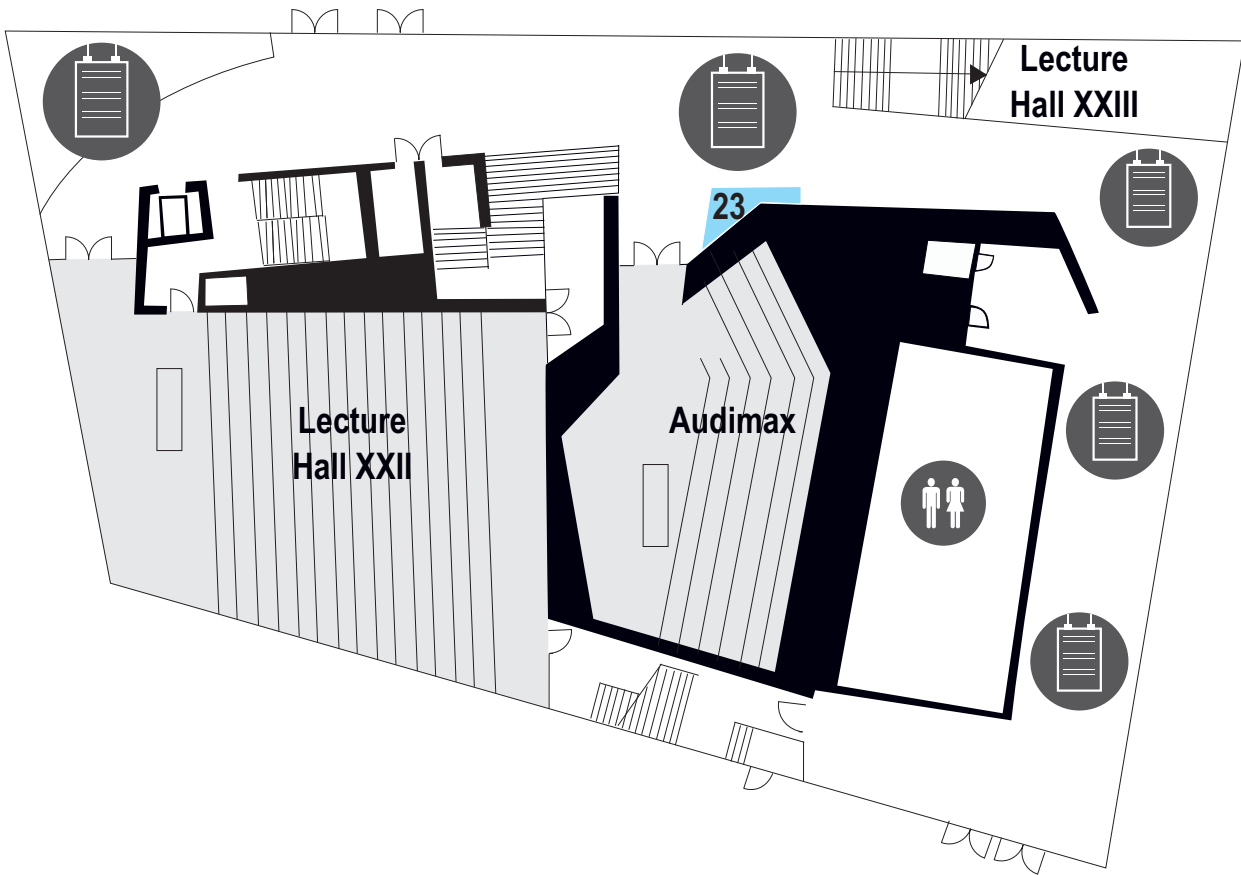
### 2<sup>nd</sup> Floor Melanchthonianum



#### Legend

- Exhibitor
-  Check-in
-  Poster exhibition
-  Beverage station
-  Toilet

Ground floor Audimax



Legend



Exhibitor



Check-in



Poster exhibition

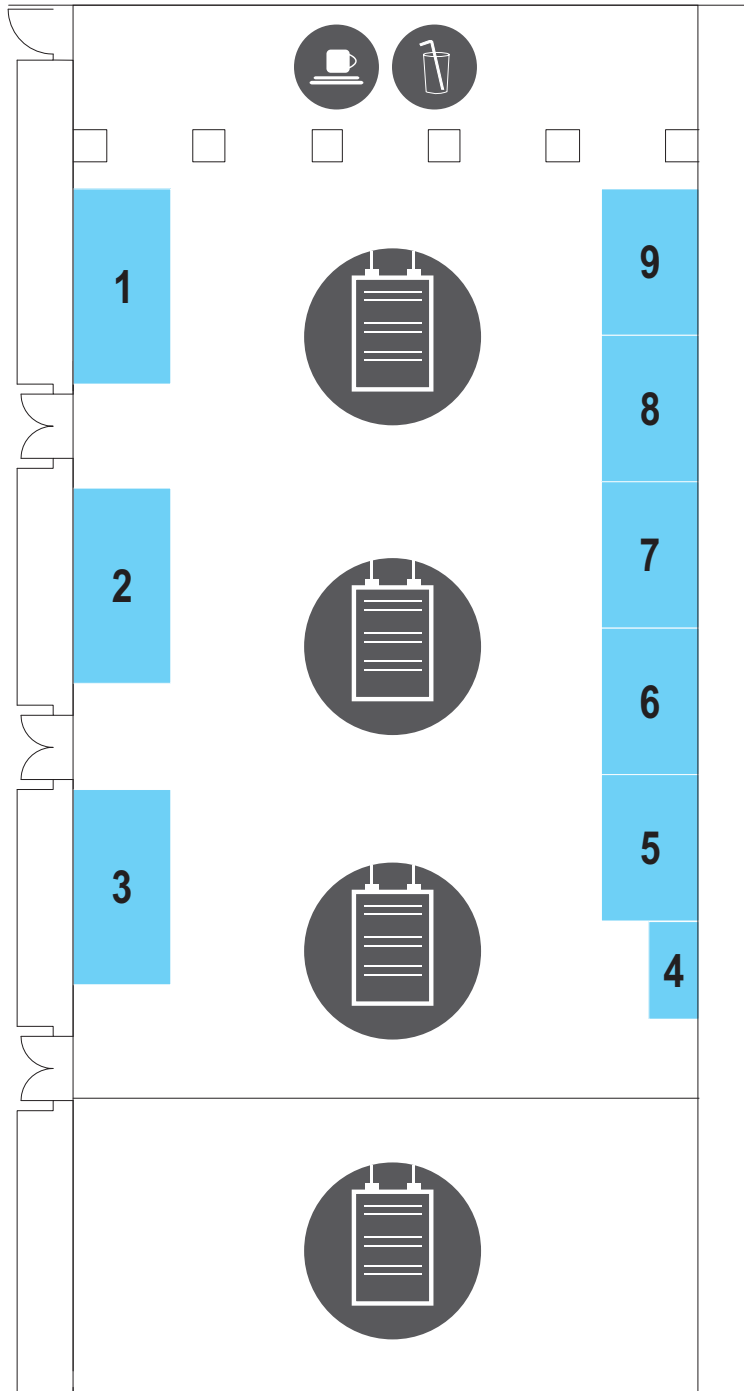


Beverage station



Toilet

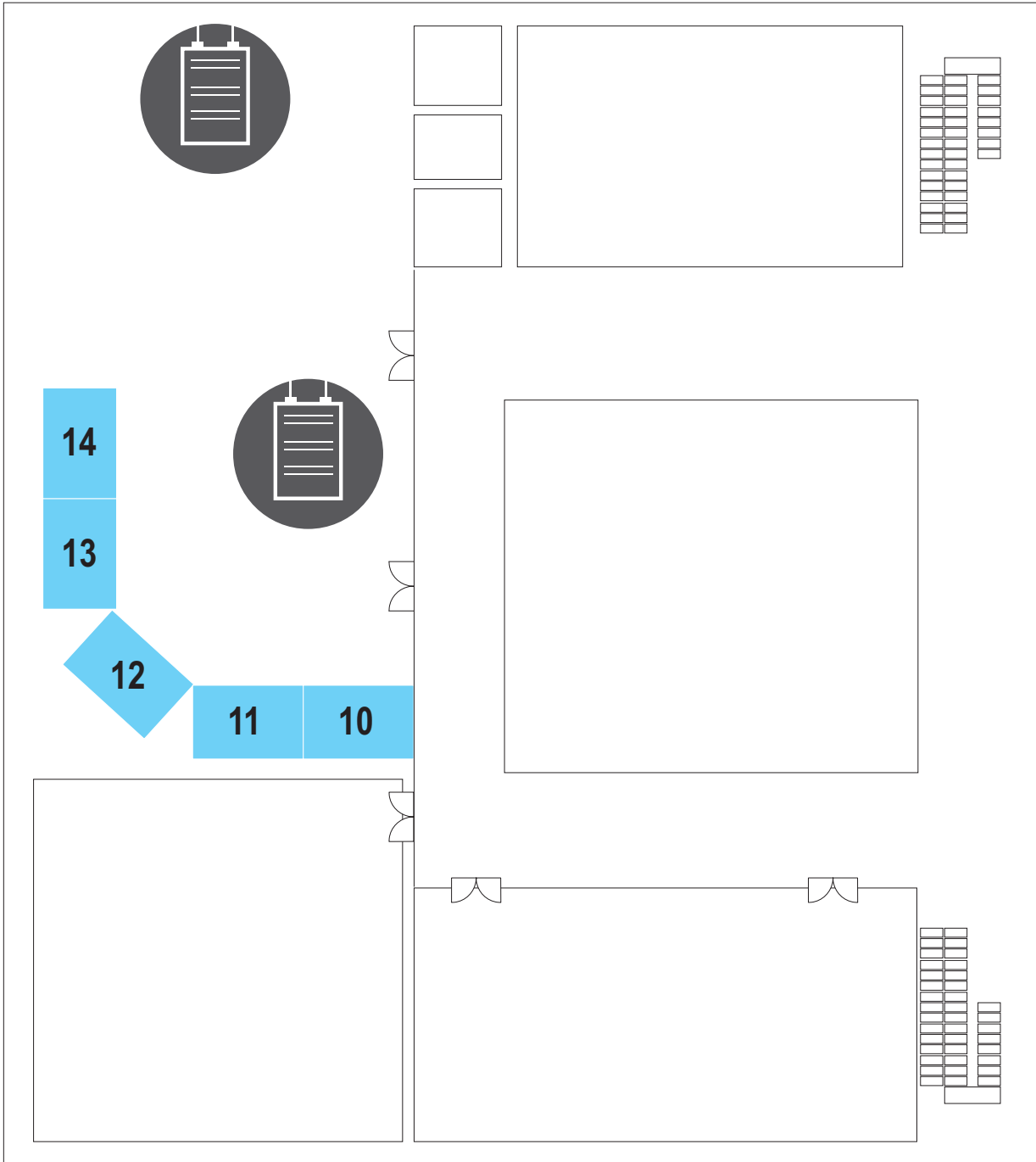
# 1st floor Löwengebäude



## Legend

-  Exhibitor
-  Check-in
-  Poster exhibition
-  Beverage station
-  Toilet

3<sup>rd</sup> floor Löwengebäude



Legend

- Exhibitor
- Check-in
- Poster exhibition
- Beverage station
- Toilet



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# PROGRAMME OVERVIEW | SUNDAY, 15/MONDAY, 16

Audimax	Audimax	Lecture hall XXII	Lecture hall XXIII	Lecture hall XVII	Lecture hall XVIII	Lecture hall XIX	Lecture hall XX
	08:30–10:00 Plenary Lecture 2 with Meredith Schuman p. 23						
	<i>Coffee break</i>						
	10:30–12:30 Navigating abiotic challenges 1 p. 23	10:30–12:30 Photosynthesis and general metabolism p. 23	10:30–12:30 Molecular evolution of plant form and function p. 24				
	<i>Lunch break</i>				12:45–13:45 Wie Forschungs- und Selbstpräsentation gelingen p. 25	12:45–13:45 Assay Design Guidelines for qPCR and dPCR p. 25	12:45–13:45 Plant Lighting and Energy Efficiency p. 25
	14:00–16:00 Beneficial plant-microbe interactions p. 25	14:00–16:00 Plant development 1 p. 26	14:00–16:00 Plant functional diversity in a changing world p. 26				
16:00–18:00 Welcome p. 8	<i>Coffee break</i>						
	16:30–17:15 Plenary Lecture 3 with J. Peñuelas p. 27						
	17:15–18:45						
18:00–20:00 Opening lecture with Giles Oldroyd p. 22	Posters odd						p. 39
						19:00–20:00 Public Lecture p. 27	



Audimax	Lecture hall XXII	Lecture hall XXIII	Lecture hall XVII
08:30–09:45			
Plenary Lecture 4 with Bipin Pandey			
p. 28			
<i>Coffee break</i>			
10:15–12:15	10:15–12:15	10:15–12:15	
Plant development 2	Molecular mechanisms of plant nutrition	Crop biology and genetics	
p. 28	p. 28	p. 29	
<i>Lunch break</i>			
12:30–14:30			
DBG General Assembly			
p. 29			
14:45–16:45	14:45–16:45	14:45–16:45	14:45–16:45
Biology of algae and cyanobacteria	Communication and dynamics of plant organelles	Applied botany for food security	Reproducibility in Plant Biology
p. 29	p. 30	p. 30	p. 31
<i>Coffee break</i>			
17:30–19:00			
Posters even			
p. 52			
19:00–21:00			
DBG Section Meetings			
p. 31			



# PROGRAMME OVERVIEW | WEDNESDAY, 18 SEPTEMBER

Audimax	Lecture hall XXII	Lecture hall XXIII	Lecture hall XX
08:30–09:45			
Plenary Lecture 5 with Sabeeha Merchant			
p. 32			
<i>Coffee break</i>			
10:30–12:30			
Navigating abiotic challenges 2	Biotechnology and genome editing	Plant Hormones and Chemical Mediators	
p. 32	p. 33	p. 33	
12:45–14:15			
Writing and Publishing			
p. 34			
<i>Lunch break</i>			
14:30–16:30			
Specialized metabolism	Gene regulation	Cell biology	Phenotyping workshop
p. 34	p. 34	p. 35	p. 35
<i>Coffee break</i>			
17:00–18:15			
Plenary Lecture 6 with Cyril Zipfel			
p. 35			
19:30–23:00			



Audimax	Lecture hall XXII	Lecture hall XXIII
08:30–09:15		
Plenary Lecture 7 with Staffan Persson p. 36		
<i>Coffee break</i>		
09:45–11:45		
Resisting pathogens and pests p. 36	Plant systematics, evolution and biodiversity p. 36	Plant proteins - structure to function p. 38
<i>Lunch break</i>		
12:45–13:45		
Closing Lecture with Keiko Torii p. 38		

14:30–18:00  
ECR satellite meeting



# PROGRAMME OVERVIEW | THURSDAY, 19 SEPTEMBER

Social Programme	Lecture hall XXII	Social Programme
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09:00–17:00	09:00–14:00
ECR satellite meeting	Excursion

14:30–18:30
Excursions

Plenary Lecture	
Scientific Lecture	
Workshop	
Special	
Poster Session	
Social Programme	

18:00–20:00	Opening lecture with musical interludes by members of the MLU Academic Orchestra
Room	Audimax
Chair	Edgar Peiter (Halle a. d. Saale/DE), Giles Oldroyd (Cambridge/GB)
18:00	Welcome Address – Conference Chair Edgar Peiter (Halle a. d. Saale/DE)
18:20	Welcome Address – Member of the Bundestag Karamba Diaby (Halle a. d. Saale/DE)
18:30	Welcome Address – Mayor of Halle a. d. Saale Egbert Geier (Halle a. d. Saale/DE)
18:40	Welcome Address – Rector of the Martin Luther University Halle-Wittenberg Claudia Becker (Halle a. d. Saale/DE)
18:50	Welcome Address – President of the German Society for Plant Sciences Andreas P. M. Weber (Düsseldorf/DE)
19:00	Using beneficial microbial associations to enhance agricultural sustainability Giles Oldroyd (Cambridge/GB)



## SCIENTIFIC PROGRAMME | MONDAY, 16 SEPTEMBER

- 08:30–10:00      **Plenary Lecture 2, Wilhelm Pfeffer Award and Best Paper Award**  
Room              **Audimax**  
Chair              Meredith Schuman (Zürich/CH), Sascha Laubinger (Halle a.d. Saale/DE)
- 08:30              Mapping and managing causes and consequences of plant biodiversity  
Meredith Schuman (Zürich/CH)
- 09:15              Wilhelm Pfeffer Award
- 09:45              Best Paper Award
- 10:30–12:30      **Navigating abiotic challenges 1**  
Room              **Audimax**  
Chair              Carolin Delker (Halle a. d. Saale/DE), Ana Cano-Delgado (Barcelona/ES)
- 10:30              Spatiotemporal analysis of brassinosteroid receptors unveils novel mechanisms to unlock drought adaptation in crops  
Ana Cano-Delgado (Barcelona/ES)
- 11:00              Feeling the warmth in spring: Elucidating molecular mechanisms underlying the morphogenetic response of spring barley to warmer temperatures  
Jiaying Zhu, Marie Richter, Jason Lourantos, Roice George, Anja Hartmann, Yusheng Zhao  
Yongyu Huang (Seeland/OT Gatersleben/DE), Thomas Hartwig, Natalka Bajaczky (Köln/DE)
- 11:18              Identification of core, conditional, and crosstalk components of tomato heat stress response using integrative transcriptomics and orthology  
Dennis Psaroudakis (Gatersleben/DE), Abul Khayer, Leke V. Aiyesa (Gatersleben, Göttingen/DE), Nick Bergau  
Alain Tissier (Halle a. d. Saale/DE), Yunlong Lu, Philip A. Wigge (Großbeeren/DE), Alon Israeli  
Naama Teboul (Jerusalem/IL), Andrea Bräutigam (Bielefeld/DE), Jędrzej Szymanski (Gatersleben, Jülich, Düsseldorf/DE)
- 11:36              Metabolic acclimation to low temperature: a central role for C1 transfer  
Thomas Naegele, Anastasia Kitashova, Martin Lehmann, Serena Schwenkert (Planegg-Martinsried/DE)
- 11:54              Cytosolic class I glutaredoxins integrate glutathione homeostasis and stress-related redox signalling  
Michelle Schlößer, José Manuel Ugalde, Andreas Meyer (Bonn/DE)
- 12:12              Understanding the antagonistic regulation of microRNA858a in response to biotic and abiotic stress factors  
Zheng Zhou, Daguang Cai (Kiel/DE)



- 10:30–12:30    **Photosynthesis and general metabolism**  
 Room            Lecture hall XXII  
 Chair            Etienne Meyer (Halle a. d. Saale), Ute Armbruster (Potsdam/DE)
- 10:30            PSII Assemble! – About some of the many hands that make LIGHT work  
 Ute Armbruster (Potsdam/DE)
- 11:00            Metabolic maintenance in photosynthesis  
Dario Leister (Planegg-Martinsried/DE), Anurag Sharma (Kopenhagen/DK, New York, NY/US), Natalia Kerber  
 Thomas Nägele, Bennet Reiter, Viviana Pasch (Planegg-Martinsried/DE), Simon Beeh (Planegg-Martinsried, Tübingen/DE)  
 Peter Jahns (Düsseldorf/DE), Roberto Barbato (Vercelli/IT), Mathias Pribil (Kopenhagen/DK)  
 Thilo Rühle (Planegg-Martinsried/DE)
- 11:18            Spliceosomal complex components are critical for adjusting the C:N balance during high-light acclimation  
Gali Estopare Araguirang (Rostock/DE), Benedikt Venn (Kaiserslautern/DE), Nadja-Magdalena Kelber (Rostock/DE)  
 Regina Feil, John Lunn (Potsdam-Golm/DE), Tatjana Kleine, Dario Leister (München /DE)  
 Timo Mühlhaus (Kaiserslautern/DE), Andreas S. Richter (Rostock/DE)
- 11:36            Integrating spatial constraints with metabolic models predicts C3/C4 and intermediate photosynthesis  
Tiago Moreira Machado, Nadine Töpfer (Köln/DE)
- 11:54            Synthetic redesign of plant photorespiration: photorespiratory bypasses towards more efficient carbon fixation  
Laia Segura Broncano, Philipp Westhoff (Düsseldorf/DE), Tobias Erb (Marburg/DE), Andreas P.M. Weber (Düsseldorf/DE)
- 12:12            Discovery and characterization of a chloroplast ribose transporter in *Phaseolus vulgaris* and *Arabidopsis thaliana*  
Luisa Voß (Hannover/DE), Isabel Keller (Kaiserslautern/DE), Rebekka Schröder, Denise Mehner-Breitfeld, Marco Herde  
 Nieves Medina-Escobar, Thomas Brüser (Hannover/DE), Ekkehard Neuhaus (Kaiserslautern/DE)  
 Claus-Peter Witte (Hannover/DE)
- 10:30–12:30    **Molecular evolution of plant form and function**  
 Room            Lecture hall XXIII  
 Chair            Jan de Vries (Göttingen/DE), James H. Leebens-Mack (Athens, GA/US)
- 10:30            Macroevolutionary and genomic investigations of sex chromosome evolution in seed plants  
 James H. Leebens-Mack (Athens, GA/US)
- 11:00            Transcription factor binding motifs have been conserved for 500 million years  
Sanja Zenker, Anja Meierhenrich, Andrea Bräutigam (Bielefeld/DE)
- 11:22            Hornworts reveal a spatial model for pyrenoid-based CO<sub>2</sub>-concentrating mechanisms in land plants  
 Fay-Wei Li (Ithaca, NY/US)
- 11:45            Evolution of arabinogalactan-proteins: Independent evolutionary history of protein backbone and glycosylation  
 Lukas Pfeifer, Birgit Classen (Kiel/DE)
- 12:07            Gene space travels in the Arabideae – approaches for detecting genomic footprints of life history evolution  
Christiane Kiefer, Nora Walden, Marcus Koch (Heidelberg/DE)



## SCIENTIFIC PROGRAMME | MONDAY, 16 SEPTEMBER

12:45–13:45 **Wie Forschungs- und Selbstpräsentation gelingen**  
Room Lecture hall XVII  
Chair Esther Schwarz-Weig (Mistelgau/DE)

Immer mehr Menschen informieren sich direkt in der Wissenschaft, auf Websites und in Sozialen Medien. Denn die Öffentlichkeit verlangt Aufklärung, gerade weil Wissenschaftsfeinde Falschmeldungen lancieren. Was Sie tun können, damit sich die Scientific Community, Medien, Drittmittelgeber und Außenstehende für Ihre Arbeit interessieren und sie verstehen, ist Thema dieses Impuls-Workshops. Erfahren Sie, wie Sie andere auf Ihre Expertise aufmerksam machen und für Ihr Forschungsthema gewinnen.

12:45–13:45 **Assay Design Guidelines for qPCR and dPCR (Logo Stilla einfügen)**  
Room Lecture hall XVIII  
Chair Martin Becker (Villejuif/FR)

PCR is a cornerstone of scientific research, with applications that reach far beyond agarose gel analysis. In this comprehensive workshop, participants will gain expertise in designing PCR assays and exploring chemical modifications of oligos. Additionally, the fundamental principles of quantitative PCR (qPCR) and digital PCR (dPCR) will be thoroughly explained.

12:45–13:45 **Plant Lighting and Energy Efficiency (Logo Billberry einfügen)**  
Room Lecture hall XIX  
Chair Krzysztof Dobrynin (Lodz/PL)

To what extent can a light spectrum influence growth and development of plants? The presentation will introduce an LED lighting system with multiple spectra and a control software application that enables research on photobiology of plants. Aspects of energy efficiency in LED lighting systems will also be addressed.

14:00–16:00 **Beneficial plant-microbe interactions**  
Room Audimax  
Chair Martina Ried (Halle a. d. Saale), Caroline Gutjahr (Potsdam/DE)

14:00 Form and function of a plant-fungal symbiosis  
Caroline Gutjahr (Potsdam/DE)

14:48 Plant-derived ROS licenses co-habitation with a potentially pathogenic leaf microbiota member  
Frederickson Entila (Köln/DE), Xiaowei Han (Wuhan/CN), Akira Mine (Kyoto/JP), Paul Schulze-Lefert (Köln/DE)  
Kenichi Tsuda (Wuhan/CN; Köln/DE)

15:06 Ectomycorrhiza-induced systemic defenses involve CERK-dependent and -independent pathways  
Andrea Polle, Steven Dreischhof, Mascha Muhr, Franklin Rezende, Dennis Janz, Jacob Schmidt  
Mareike Jakobi (Göttingen/DE), Birgit Kersten (Großhansdorf/DE), Volker Lipka (Göttingen/DE)  
Jörg-Peter Schnitzler (Neuherberg/DE), Matthias Fladung (Großhansdorf/DE), Thomas Teichmann (Göttingen/DE)

15:24 The *Medicago truncatula* GRAS transcription factor SCL1 – a new regulator of cortical cell size and arbuscule development during mycorrhiza symbiosis  
Diana Schwarz, Stephanie Voß, Christine Seemann, Carolin Heck, Natalia Requena (Karlsruhe/DE)

15:42 The fungal plant pathogen *Verticillium dahlia* engages in stable interactions with terrestrial microalgae  
Hanna Rovenich, Eva Schnell, Anton Kraege, Heidrun Haeweker, Ole Nielsen, Edgar Chavarro-Carrero  
Bart Thomma (Köln/DE)

- 14:00–16:00 **Plant development 1**  
 Room **Lecture hall XXII**  
 Chair Katharina Bürstenbinder (Marburg/DE), Viola Willemsen (Wagedingen/NL)
- 14:00 Unraveling the Secrets of Root Architecture: From Cell Division to Organ Formation  
 Viola Willemsen (Wagedingen/NL)
- 14:30 High-resolution single-cell RNA sequencing dataset of primary root xylem reveals new developmental regulators  
 Claudia von der Mark, Akshay Gokulendran Nair, Thomas Depuydt, Thomas Eekhout, Carolin Grones, Max Minne  
 Maite Saura-Sanchez, Jos Wendrich, Jonah Nolf, Jasper Staut, Klaas Vandepoele, Bert De Rybel (Ghent/BE)
- 14:48 Reproducibly oriented cell divisions pattern the first flat body structures to set up dorsoventrality and de novo meristem  
 formation in *Marchantia polymorpha*  
 Eva-Sophie Wallner, Liam Dolan (Vienna/AT)
- 15:06 Why are some sugars toxic to plants? – Changes in root development  
 Raimund Tenhaken, Eva Ivanov Kakova, Martina Althammer, Margit Höftberger, Christoph Regl (Salzburg/AT)  
 Klaus Herburger (Rostock/DE)
- 15:24 Splitting Hairs- Investigating the Role of Root Hairs in Perceiving Nutrient Availability and Local Plastic Physiological  
 Responses  
 Dylan Jones (Gatersleben/DE), Caroline Marcon (Bonn/DE), Hannah Schneider (Gatersleben/DE)
- 14:00–16:00 **Plant functional diversity in a changing world**  
 Room **Lecture hall XXIII**  
 Chair Christine Römermann (Jena/DE), Helge Bruelheide (Halle a. d. Saale)
- 14:00 Some answers are hidden underfoot – belowground traits and their power to predict plant functioning  
 Joana Bergmann (Müncheberg/DE)
- 14:48 Linking plant diversity-productivity relationships to plant functional traits and changes in soil properties  
 Peter Dietrich (Halle a. d. Saale/DE), Nico Eisenhauer, Christiane Roscher (Leipzig/DE)
- 15:06 Relationship between endophytic fungi and leaf traits in a temperate biodiversity experiment  
 Michael Köhler (Halle a. d. Saale/DE)
- 15:24 Role of plant functional traits in the naturalization and invasion success of Asteraceae  
 Amarpreet Kaur, Aditi Sharma, Daizy Rani Batish (Chandigarh/IN)
- 15:42 Effects of Tree Species Richness on Within-Individual Leaf Trait Variation in the Tropical Rainforest  
 Tobias Proß, Helge Bruelheide (Halle a. d. Saale/DE), Catherine Potvin (Montreal/CA), Maria Sporbert  
 Stefan Trogisch (Halle a. d. Saale/DE), Sylvia Haider (Lüneburg/DE)



## SCIENTIFIC PROGRAMME | MONDAY, 16 SEPTEMBER

16:30–17:15	Plenary Lecture 3
Room	Audimax
Chair	Josep Peñuelas (Barcelona/ES), Helge Bruelheide (Halle a. d. Saale/DE)
16:30	How long can plants prevent more severe climate change? Josep Peñuelas (Barcelona/ES)
17:15–18:45	Poster walk - odd
19:00–20:00	Public Lecture – in German
Room	Lecture hall XX
Chair	
19:00	Biodiversität im Umbruch - Status, Trends, Folgen, Handlungsbedarf Helge Bruelheide (Halle a. d. Saale/DE)

Der Abendvortrag der Botanik-Tagung nimmt Sie mit auf eine faszinierende Reise durch die Welt der Biodiversität. In den letzten Jahren haben wir eine alarmierende Abnahme der Biodiversität beobachtet. Dieser Rückgang betrifft nicht nur einzelne Arten, sondern ganze Ökosysteme, die essenziell für unser Wohlergehen und unsere Lebensgrundlagen sind. Der Vortrag geht auf die neuesten Erkenntnisse über den aktuellen Stand der Biodiversität ein und zeigt auf, wie sich diese Vielfalt im Laufe der Zeit verändert hat, welche Rolle dabei Landnutzungsänderungen und Klimawandel spielen und welche Folgen der Verlust an Biodiversität für die Umwelt und die Gesellschaft hat. Es wird deutlich werden, dass ein Umdenken und Handeln auf verschiedenen Ebenen erforderlich sind, um den rapiden Rückgang der Biodiversität zu stoppen.

- 08:30–09:45 **Plenary Lecture 4 and Eduard Strasburger Award**  
 Room **Audimax**  
 Chair Bipin Pandey (Leicestershire/GB), Nicolaus von Wirén (Gatersleben/DE)
- 08:30 Understanding how plant roots sense and respond to soil compaction  
 Bipin Pandey (Leicestershire/GB)
- 09:15 Eduard Strasburger Award
- 10:15–12:15 **Plant development 2**  
 Room **Audimax**  
 Chair Christopher Grefen (Bochum/DE), Michael Lenhard (Potsdam/DE)
- 10:15 How do plants tell left from right?  
 Michael Lenhard (Potsdam/DE)
- 10:45 Two weeks to survive: molecular mechanisms harmonizing seed conditioning, germination, and haustoriogenesis in parasitic weeds of the Orobanchaceae family  
 Guillaume Brun (Berlin/DE), Florian Schindler (Vienna/AT), Amal Bouyrakhen, Olivier Dayou (Berlin/DE)  
 Wolfram Weckwerth (Vienna/AT), Susann Wicke (Berlin/DE)
- 11:03 Zauderer1 and Zauderer2 Encode Two F-box Proteins Involved in Embryo/seed Development and Timing of Flowering  
 Denys Tysiachnyi (Leipzig/DE, Kyiv/UA), Jan Erik Leuendorf, Thomas Schmülling (Berlin/DE)  
 Wolfram Brenner (Leipzig/DE)
- 11:21 Exploring the role of peptide hormones in plant growth and fruit development by altering their maturation processes  
 Carlotta Francese, Anna Pavanello, Marco Boschin, Marco Armellin (Padova/IT)  
 Umberto Salvagnin (Padova, San Michele all'Adige/IT), Livio Trainotti (Padova/IT)
- 11:39 Deciphering the 3D cellular basis of morphogenesis: how the ovule bends into its final shape  
 Tejasvinee Atul Mody, Ratula Ray (Freising/DE), Gabriella Mosca (Tübingen/DE), Kay Schneitz (Freising/DE)
- 11:57 Uncovering Gene-Phenotype Associations in Leaf Development within a Vast Wild Arabidopsis thaliana Population by Landscape Transcriptomics  
 Eneza Yoeli Mjema, Sascha Laubinger (Halle a. d. Saale/DE)
- 10:15–12:15 **Molecular mechanisms of plant nutrition**  
 Room **Lecture hall XXII**  
 Chair Patrick Bienert (Freising/DE), Wolfgang Busch (La Jolla, CA/US)
- 10:15 Kill the messenger - spatial regulation of iron acquisition in plant microbe interactions  
 Wolfgang Busch (La Jolla, CA/US)
- 10:45 Natural variation in a node-expressed MTP-type Zn transporter gene modulates shoot development and Zn allocation to grains in barley  
 Jingyi Guo (Gatersleben/DE), Zhongtao Jia (Gatersleben/DE, Beijing/CN), Jochen Kumlehn  
 Martin Mascher (Gatersleben/DE), Edgar Peiter (Halle a. d. Saale/DE), Nicolaus von Wirén (Gatersleben/DE)
- 11:03 Involvement of auxin in nutritropism of rice  
 Toru Fujiwara, Kiyoshi Yamazaki (Tokyo/JP)
- 11:21 Navigating Iron Deficiency: The Dynamic Role of MTP10 in Arabidopsis  
 Bastian Meier (Halle a. d. Saale/DE), Stefanie Höller (Düsseldorf/DE), Moritz Friesch (Halle a. d. Saale/DE)  
 Dennis Brueckner, Gerald Falkenberg (Hamburg/DE), Edgar Peiter (Halle a. d. Saale/DE)
- 11:39 Deciphering novel mechanisms of sulfur homeostasis  
 Daniela Ristova, Suvajit Basu, Stanislav Kopriva (Köln/DE)



## SCIENTIFIC PROGRAMME | TUESDAY, 17 SEPTEMBER

- 11:57      Role of novel plant inositol pyrophosphate synthases and phosphohydrolases in phosphate signaling  
Gabriel Schaaf, Esther Lange, Robin Schneider, Klea Lami, Verena Gaugler (Bonn/DE)
- 10:15–12:15      **Crop biology and genetics**  
Room      Lecture hall XXIII  
Chair      Nils Stein (Seeland OT Gatersleben/DE), Laura Dixon (Leeds/GB)
- 10:15      Fine-tuning vernalization in hexaploid bread wheat  
Laura Dixon (Leeds/GB)
- 10:45      Phytochrome C and Photoperiod Response 1 Interact to Control Floral Development in Barley Under High Temperatures  
Kumsal Ecem Colpan Karisan, Maria Von Korff Schmising (Düsseldorf/DE)
- 11:03      Effects of light fluctuations on kernel number and compensation of yield potential via thousand kernel weight are cultivar- and phase-specific in winter wheat  
Khadija Sabir, Hartmut Stützel (Hannover/DE), Tsu-Wei Chen (Berlin/DE)
- 11:21      Homeologue-aware binding analysis of Brassica napus seed development transcription factors  
Anja Meierhenrich, Bart Verwaaijen (Bielefeld/DE), Dominic Knoch, Thomas Altmann (Seeland / OT Gatersleben/DE)  
Andrea Bräutigam (Bielefeld/DE)
- 10:39      The pan-epigenome of barley reveals epigenetic consequences of structural variations  
Zihao Zhu, Sudharsan Padmarasu (Seeland/DE), Thomas Lux, Manuel Spannagl (Neuherberg/DE), Axel Himmelbach  
Martin Mascher, Nils Stein (Seeland/DE)
- 11:57      Genome-wide analysis of 24-nt siRNA-mediated de novo DNA methylation and its role in regulating plant growth-defence trade-offs in wheat (*Triticum aestivum*)  
Lingyue Han, Zheng Zhou, Markus Schemmel, Henning Kage, Daguang Cai (Kiel/DE)
- 12:30–13:45      **DBG General Assembly**  
Room      Audimax
- 14:45–16:45      **Biology of algae and cyanobacteria**  
Room      Audimax  
Chair      Severin Sasso (Leipzig/DE), Thomas Mock (Norwich/GB)
- 14:45      Diatom genome evolution in real time  
Thomas Mock (Norwich/GB)
- 15:15      Developmental pathways underlying sexual differentiation in a U/V sex chromosome system  
Daniel Liesner, Guillaume Cossard, Min Zheng (Tübingen/DE), Olivier Godfroy (Roscoff/FR), Josué Barrera-Redondo  
Fabian B. Haas, Susana M. Coelho (Tübingen/DE)
- 15:33      The effect of flavonoids on cyanobacterial motility and their role in symbiotic relationship with land plants  
Deren Büyüktas, Willie D. Macedo Mesquita, Armin Dadras, Clara G. Köhne, Anja Pöhlein, Corinna Herfurth, Ivo Feussner  
Rolf Daniel, Tatyana Darienko, Maike Lorenz, Sophie de Vries (Göttingen/DE)
- 15:51      Starch reorganizes and becomes more accessible in maturing cells of the green alga *Zygnema* sp.  
Qian Wang (Rostock/DE), Clarisse Uwizeye, Pierre-Henri Jouneau, Denis Falconet, Eric Marechal (Grenoble/FR)  
Andreas Holzinger (Innsbruck/AT), Klaus Herburger (Rostock/DE)
- 16:09      Unraveling adaptive responses of the green alga *Chlamydomonas reinhardtii* and its bacterial interactions in a nature-like environment  
Trang Vuong, Ece Kurtoglu, Constanze Schultz, Laura Schrader, Patrick Then, Jan Petersen, Martin Westermann  
Anxhela Rredhi, Somak Chowdhury, Ruchira Mukherji, Michael Schmitt, Jürgen Popp, Pierre Stallforth  
Maria Mittag (Jena/DE)

- 16:27 Surviving in an extreme environment: Cyanobacteria and microalgae of biological soil crusts in Spitsbergen (High Arctic)  
Burkhard Becker, Ekaterina Pushkareva (Köln/DE), Eva Hejdkukova, Anastasiia Kologmiiets, Oleksandr Bren, Pavel Pribyl  
Josef Elster (Ceske Budejovice/CZ)
- 14:45–16:45 **Communication and dynamics of plant organelles**  
Room Lecture hall XXII  
Chair Oriana Mariani, Helene Röhricht, Sandra Schüler, Vera Wagner, Ivana Mladenovic (Halle a. d. Saale)
- 14:45 Probing organelle interactions using optical tweezers  
Imogen Sparkes (Bristol/GB)
- 15:15 Phosphoinositides modulate auxin-dependent transcription by controlling the histone acetyltransferase GCN5 in Arabidopsis  
Franziska Daamen, Mareike Heilmann (Halle a. d. Saale/DE)
- 15:33 Role of the ER-bound transcription factor ANAC013 and its cleavage by rhomboid-like protease RBL2 in mitochondrial retrograde signalling under hypoxia stress  
Tilo Renziehausen (Bielefeld/DE), Emese Eysholdt-Derzsó (Kiel/DE), Stephanie Frings (Bielefeld/DE)  
Stephanie Frohn (Seeland/DE), Margret Sauter (Kiel/DE), Inge De Clercq (Ghent/BE), Joost van Dongen (Aachen/DE)  
Jos Schippers (Seeland/DE), Romy Schmidt-Schippers (Bielefeld/DE)
- 15:51 *In vivo* detection of dynamic light-induced H<sub>2</sub>O<sub>2</sub> release from chloroplasts for putative retrograde communication  
José Manuel Ugalde, Andreas Meyer (Bonn/DE)
- 16:09 Defining a novel peroxisome-mediated strategy of plant viruses to combat RNA interference  
Stefan Wirling, Lara-Marie Halscheid, Cy Jeffries (Hamburg/DE), Ute Krämer (Bochum/DE), Sigrun Reumann (Hamburg/DE)
- 16:27 Plastidial amino acid transporter proteins in *A. thaliana*  
Franziska Kuhnert, Karolina Vogel, Philipp Westhoff, Peter Lundquist, Christian Rosar, Tatjana Goss, Vanessa Valencia  
Andreas Weber (Düsseldorf/DE)
- 14:45–16:45 **Applied botany for food security**  
Room Lecture hall XXIII  
Chair Jutta Papenbrock (Hannover/DE), Ian Dodd (Lancaster/GB)
- 14:45 Applications of phytohormone signalling: from deficit irrigation to microbial inoculants  
Ian Dodd (Lancaster/GB)
- 15:15 Reducing the sinapine levels of *Camelina sativa* seeds through targeted genome editing of REF1  
Kirstin Feussner, Amélie Kelly, Martin Fulda, Merle Aden, Ilka Abreu, Ivo Feussner (Göttingen/DE)
- 15:33 Exploring the Micromechanical and Self-Healing Properties of Apple Fruit Cuticles as Influential Factors Preserving Fruit Quality  
Dag Heinemann, Timm Landes, Bishnu P. Khanal, Hans Bethge, Miroslav Zabic (Hannover/DE)
- 15:51 Molecular analysis of *Salicornia* spp. to better exploit its potential as new crop plant  
Andre Fussy, Jutta Papenbrock (Hannover/DE)
- 16:09 Impact of changing air temperatures on the growth and phytochemical contents in spearmint (*Mentha spicata*)  
Dipanjali Chatterjee, Adinpunya Mitra (Kharagpur/IN)
- 16:27 Agroforest systems with grapevines and trees, advances and disadvantages  
Christian Zörb, Patrick Pascal Lehr (Stuttgart/DE)



## SCIENTIFIC PROGRAMME | TUESDAY, 17 SEPTEMBER

14:45–16:45    **Reproducibility in Plant Biology**  
Room            Lecture hall XVII  
Chair            Susann Auer (Dresden/DE)

Rigor and reproducibility are the core of modern science and set apart scientific inquiry from pseudoscience. Many researchers have never received formal training on how to work in a way that enables reproducibility for themselves and other researchers. This workshop will introduce reproducible workflows and a range of tools for plant biologists and everybody interested in working more reproducibly along the themes of organization, documentation, analysis and dissemination.

17:30–19:00    **Poster walk - even**

19:00–21:00    **DBG Section Meetings**

Room            **Pflanzenphysiologie und Molekularbiologie**  
Chair            Lecture hall XX  
                     Stefan Rensing

Room            **Biodiversität und Evolutionsbiologie**  
Chair            Lecture hall XVIII  
                     Elvira Hörandl (Göttingen/DE)

Room            **Phykologie**  
Chair            Lecture hall XIX  
                     Claudia Büchel (Frankfurt/DE)

Room            **Angewandte Botanik**  
Chair            Lecture hall Z  
                     Jutta Papenbrock (Hannover/DE)

Room            **Pflanzliche Naturstoffe**  
Chair            Lecture hall B  
                     Ute Wittstock (Braunschweig/DE)

Room            **Interaktionen (ehemals Sektion Mykologie und Lichenologie)**  
Chair            Lecture hall A  
                     Sophie de Vries (Göttingen/DE)



08:30–09:45	<b>Plenary Lecture 5 and Horst Wiehe Award</b>
Room	Audimax
Chair	Sabeeha Merchant (Berkeley, CA/US), Kristina Kühn (Halle a. d. Saale/DE)
08:30	Tales of algae - from new biochemistry to synthetic biology Sabeeha Merchant (Berkeley, CA/US)
09:15	Horst Wiehe Award
10:30–12:30	<b>Navigating abiotic challenges 2</b>
Room	Audimax
Chair	Hannah Schneider (Seeland OT Gatersleben/DE), Peng Yu (Bonn/DE)
10:30	Maize domestication resolves root formation and abiotic stress resilience Peng Yu (Bonn/DE)
11:00	An interdisciplinary study to investigate the plant-soil-microbiome continuum under drought Roman Hartwig (Stuttgart/DE), Michael Santangeli (Vienna/AT), Henrike Würsig, María Martín Roldán (Halle a. d. Saale/DE) Bunlong Yim (Braunschweig/DE), Eva Lippold (Halle a. d. Saale/DE), Ariel Tasca (München /DE), Eva Oburger (Vienna/AT) Mika T. Tarkka, Doris Vetterlein (Halle a. d. Saale/DE), Patrick Bienert (München/DE) Evgenia Blagodatskaya (Halle a. d. Saale/DE), Kornelia Smalla (Braunschweig/DE), Stefanie Wienkoop (Vienna/AT) Monika Wimmer (Stuttgart/DE)
11:18	Mechanisms of root branching under heterogenous water availability Poonam Mehra (Nottingham/GB)
11:36	Old but gold: exploiting the underutilized oilseed <i>Camelina sativa</i> to uncover and promote tolerance to abiotic stress for improving climate resilience in crops Dominik Großkinsky (Tulln an der Donau/AT), Susana Silvestre, Aline Forgatti-Hell, Richard Haslam (Harpending/GB) Anais Da Costa, Jean-Denis Faure (Versailles/FR), Federica Zanetti, Andrea Monti (Bologna/IT), Javier Prieto Ruiz Paloma León (Madrid/ES), Björn Usadel (Jülich/DE), Malo Le Boulch, Cédric Cassan, Pierre Pétriacq, Sylvain Prigent Yves Gibon (Bordeaux/FR), Claudia Jonak (Tulln an der Donau/AT)
11:54	A constitutive drought-stressed status is provoked by the complete loss of plasma membrane aquaporins in <i>Arabidopsis thaliana</i> Ting Zhu (Neuherberg/DE), Po-Kai Hsu (La Jolla, CA/US), Zhenyu Yang (Freising/DE), Komal Jhala Birgit Geist (Neuherberg/DE), Erwin Grill (Freising/DE), Julian I. Schroeder (La Jolla, CA/US) Anton Schäffner (Neuherberg/DE)
12:12	Reinforcement Learning-Supported Metabolic Modeling Enables Dynamic Simulation of Plant Seed-to-Seed Growth Cycle in a Changing Environment Daniel Koch (Seeland/DE), Stefano Camborda La Cruz, Jan-Niklas Weder (Köln/DE), Dennis Psaroudakis (Seeland/DE) Nadine Töpfer (Köln, Düsseldorf/DE), <u>Jedrzej Szymanski</u> (Seeland, Jülich, Düsseldorf/DE)



## SCIENTIFIC PROGRAMME | WEDNESDAY, 18 SEPTEMBER

- 10:30–12:30 **Biotechnology and genome editing**  
Room Lecture hall XXII  
Chair Tom Schreiber (Halle a. d. Saale), Holger Puchta (Karlsruhe/DE)
- 10:30 Applying CRISPR/Cas to plants: From gene editing to chromosome engineering  
Holger Puchta (Karlsruhe/DE)
- 11:00 Agrobacterium-based protein transfer system for transgene-free genome engineering  
Julia Macholl, Tobias Jores (Düsseldorf/DE)
- 11:18 Engineered CRISPR endonucleases favoring homology-directed repair in plants  
Tom Schreiber, Anja Prange, Petra Schäfer (Halle a. d. Saale/DE), Thomas Iwen (Marburg/DE)  
Ramona Grützner, Sylvestre Marillonnet (Halle a. d. Saale/DE), Aurélie Lepage, Marie Javelle, Wyatt Paul (Chappes/FR)  
Alain Tissier (Halle a. d. Saale/DE)
- 11:36 Taking the Next Step in Plant Genome Editing: Precision and Efficiency with Advanced Base Editors  
Lucas Lang, Vincent Albinus, Malte Rumpf, Dirk Becker, Hermann Schmidt (Hamburg/DE)
- 11:54 SpaceEx – Developing a new platform for spatial transcriptomics in plants and beyond  
Paride Rizzo (Seeland/DE), Erika Schaudy (Vienna/AT), Maya Giridhar (Freising/DE), Ivo Grosse  
Matthias Müller-Hannemann, Antonia Schmidt (Halle a. d. Saale/DE), Benjamin Chavez, Isabel Mora-Ramirez  
Nandhakumar Shanmugaraj, Thorsten Schnurbusch, Axel Himmelbach, Lothar Altschmied (Seeland/DE)  
Mark Somoza (Freising/DE)
- 12:12 Creating an artificial CMS system by knocking out a functional gene in tobacco mitochondria  
Joachim Forner, Dennis Kleinschmidt (Potsdam/DE), Etienne H. Meyer (Halle a. d. Saale/DE)  
Jürgen Gremmels (Potsdam/DE), Robert Morbitzer, Thomas Lahaye (Tübingen/DE), Mark A. Schöttler  
Ralph Bock (Potsdam/DE)
- 10:30–12:30 **Plant Hormones and Chemical Mediators**  
Room Lecture hall XXIII  
Chair Debora Gasperini (Halle a. d. Saale), Eva Benkova (Vienna/AT)
- 10:30 Hormonal regulation of plant development - auxin and cytokinin cross-talk and beyond  
Eva Benkova (Vienna/AT)
- 11:00 The influence of the circadian clock and environmental conditions on calcium-dependent responses to pathogens  
Marc Knight, Bryony Jacobs (Durham/GB)
- 11:18 Inositol pyrophosphates are master regulators of arbuscular mycorrhiza  
Kiran Raj (Halle a. d. Saale/DE), Verena Gaugler, Gabriel Schaaf (Bonn/DE), Martina K. Ried-Lasi (Halle a. d. Saale/DE)
- 11:36 Ethylene-mediated reduction of root colonization by arbuscular mycorrhiza fungi requires the repressor of the karrikin signalling pathway, SMAX1  
Kartikye Varshney (Potsdam-Golm, Freising-Weihenstephan/DE), Debatosh Das (Planegg-Martinsried/DE, Burley, ID/US)  
Satoshi Ogawa (Riverside, CA/US, Yokohama/JA), Salar Torabi (Potsdam-Golm, Freising-Weihenstephan/DE)  
Regina Hüttl (Freising-Weihenstephan/DE), David Nelson (Riverside, CA/US)  
Caroline Gutjahr (Potsdam-Golm, Freising-Weihenstephan, Planegg-Martinsried/DE)
- 11:54 The interplay between aba, ja and sa during drought stress response in arabidopsis and potato  
Ute Vothknecht, Sabarna Bhattacharyya (Bonn/DE), Sakil Mahmud (Columbia, SC/US), Elena Rodriguez (Bonn/DE)  
Bernhard Wurzing, Markus Teige (Vienna/AT), Carissa Bleker, Kristina Gruden (Ljubljana/SI), Fatima Chigri (Bonn/DE)
- 12:12 A new connection with the matrix: Structural role of peptides to sustain pollen tube expansion  
S. Moussu, Hyung Kyung Lee (Lausanne/CH), K. Haas (Versailles/FR), C. Broyart, - Ursina, D. de Bellis (Lausanne/CH)  
Thomas Levasseur, E. Bonnin (Nantes/FR), N. Geldner (Lausanne/CH), B. Cathala (Nantes/FR), H. Höfte (Versailles/FR)  
Julia Santiago (Lausanne/CH)

12:45–14:15    **Writing and Publishing**  
 Room            **Audimax**  
 Chair            Mary Williams (Glasgow/UK)

You may have heard the expression, “Science isn’t finished until it’s communicated,” and to a certain extent this is true. When we look back at the history of (Western) science, publications provide our evidence and our records. More importantly, publications remain the primary tool through which scientists are evaluated for career advancement and obtaining grant funding. This workshop provides tips and practice for improving your writing skills from a professional writer and editor. The workshop will also look at what happens during the peer-review process, including how to respond to reviewer comments.

14:30–16:30    **Specialized metabolism**  
 Room            **Audimax**  
 Chair            Ute Wittstock (Braunschweig/DE), Dietrich Ober (Kiel/DE), Deyang Xu (Copenhagen/DK)

- 14:30            Harnessing plant transporters for sustainable agriculture  
 Deyang Xu (Copenhagen/DK)
- 15:00            Dissecting gene-metabolite relationships in the *Medicago truncatula* terpenome after *Aphanomyces euteiches* infection  
 Esther Harding, Heena Yadav, Sylvestre Marillonnet, Sabine Rosahl, Alain Tissier, Bettina Hause  
 Paola Ochoa (Halle a. d. Saale/DE)
- 15:18            The biosynthesis of thymol, carvacrol, and thymohydroquinone in Lamiaceae proceeds via cytochrome P450s and a short-chain dehydrogenase  
 Sandra T Gohr (Halle a. d. Saale/DE), Pan Liao (West Lafayette, IN/US), Christoph Crocoll (Kopenhagen/DK)  
 Benoit Boachon (Saint-Etienne/FR), Jonathan Gershenzon (Jena/DE), Natalia Dudareva (West Lafayette, IN/US)  
 Jörg Degenhardt (Halle a. d. Saale/DE)
- 15:36            Neighbourhood matters: Consequences of individual versus neighbourhood plant chemodiversity on visiting insects  
 Caroline Müller, Dominik Ziaja, Rohit Sasidharan, Elisabeth Eilers, Ruth Jakobs (Bielefeld/DE)
- 15:54            Selecting for Quantity and Quality of Acylsugars in Tomato Whitefly Resistance Breeding  
 Jan-Willem de Kraker, Jurre Bleeker (Enkhuizen/NL), David Cano (Almería/ES), Ilona Degeling (Enkhuizen/NL)  
 Teresa Montoro (Almería/ES), Janita Romers, Ilya Roobeek, Nejra Solo (Enkhuizen/NL)  
 Francisco Villanueva (Almería/ES), Marieke Ykema (Enkhuizen/NL)
- 16:12            Synthetica Botanica: Engineering the Future of Plant Metabolism  
 John D’Auria (Seeland/DE)
- 14:30–16:30    **Gene regulation**  
 Room            **Lecture hall XXII**  
 Chair            Selma Gago-Zachert (Halle a. d. Saale), Martin Crespi (Paris/FR)
- 14:30            Long non-coding RNAs in the regulation of gene expression  
 Martin Crespi (Paris/FR)
- 15:00            Plant enhancers: how they work and where to find them  
 Tobias Jores (Düsseldorf/DE, Seattle, WA/US, Düsseldorf/DE), Jackson Tonnies, Nicholas A Mueth, Sayeh Gorjifard  
 Josh T Cuperus, Stanley Fields (Seattle, WA/US), Christine Queitsch (Seattle, WA/US, Seattle/DE)
- 15:18            Positioning of pyrimidine motifs around cassette exons defines their PTB-dependent splicing in *Arabidopsis*  
 Rica Burgardt (Mainz/DE), Dorothee Lambert, Christina Heuwieser (Tübingen/DE), Maximilian Sack (Leipzig/DE)  
 Gabriele Wagner (Tübingen/DE), Zasha Weinberg (Leipzig/DE), Andreas Wachter (Mainz, Tübingen/DE)
- 15:36            Control of *Arabidopsis* immune and developmental gene expression through phosphorylation of VQ-motif containing transcriptional co-regulators  
 Jolina Marx, Pascal Pecher, Martin Weyhe, Justin Lee (Halle a. d. Saale/DE)



## SCIENTIFIC PROGRAMME | WEDNESDAY, 18 SEPTEMBER

- 15:54      Single-cell sequencing of C3-C4 intermediate Brassicaceae species  
Sebastian Triesch, Urte Schlüter, Andreas Weber (Düsseldorf/DE)
- 16:12      panomiX: a panomics data integration and interpretation tool  
Ankur Sahu, Dennis Psaroudakis (Gatersleben/DE), Jędrzej Szymanski (Gatersleben, Jülich/DE)
- 14:30–16:30      **Cell biology**  
Room      **Lecture hall XXIII**  
Chair      Mareike Heilmann (Halle a. d. Saale), Jürgen Kleine-Vehn (Freiburg/DE)
- 14:30      Growth coordination from subcellular to organ scale  
Jürgen Kleine-Vehn (Freiburg/DE)
- 15:00      How do viscoelastic properties differ in Arabidopsis root tissues and zones?  
Luis Alonso Baez, Astrid Bjørkøy, Bjørn Stokke, Thorsten Hamann (Trondheim/NO)
- 15:18      Phytochrome B photobody live imaging - how do phytochrome B photobodies form?  
Franziska Stamm, Subiya Haque, Manisha Sahu, Omar Heliel, Janina Schmidt, Ivan Zubcic  
Kasper van Gelderen (Heidelberg/DE)
- 15:36      Characterizing the function of the VIPP1/2 auxiliary VPL proteins in the chloroplast unfolded protein response in Chlamydomonas reinhardtii  
Katharina König, Anna Probst, Saskia Zeilfelder, Lara Weber, Miriam Kubik, Raffaella Hüttmann, Katrin Hieronimus  
Frederik Sommer, Michael Schroda (Kaiserslautern/DE)
- 15:54      Being in the right place – Peripheral membrane protein(s) during polar growth of tobacco pollen tubes  
Carolin Fritz, Benedikt Kost (Erlangen/DE)
- 16:12      Calmodulin, IQDs, and Microtubules: Integrating Calcium Signaling with Cytoskeletal Regulation during Cell Division  
Jonas Buhl, Pradeep Dahiya, Gina Stamm, Katharina Bürstenbinder (Marburg/DE)
- 14:30–16:30      **Phenotyping workshop**  
Room      **Lecture hall XX**  
Chair      Simone Gatzke (Jülich/DE)
- Plant phenotyping is an emerging science that links genomics with plant ecophysiology and agronomy. The relationship between the genes, the environment and the phenotype of a plant determines the structure, function and efficient utilization of resources of that plant and ultimately its performance. While molecular and genetic methods experienced significant advances in recent years, quantitative phenotype analysis became the limiting factor.
- 17:00–18:15      **Plenary Lecture 6 and Poster Awards**  
Room      **Audimax**  
Chair      Cyril Zipfel (Zurich/CH), Debora Gasperini (Halle a. d. Saale/DE)
- 17:00      Deciphering the molecular basis of plant receptor kinase-mediated immune signaling  
Cyril Zipfel (Zurich/CH)
- 17:45      Poster Awards
- 19:30–01:00      **Botany Party**  
**Volkspark**

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## SCIENTIFIC PROGRAMME | THURSDAY, 19 SEPTEMBER

- 08:30–09:15 **Plenary Lecture 7**  
Room **Audimax**  
Chair Staffan Persson (Copenhagen/DK), Steffen Abel (Halle a. d. Saale/DE)
- 08:30 Regulation of carbon allocation and cell wall synthesis  
Staffan Persson (Copenhagen/DK)
- 09:45–11:45 **Resisting pathogens and pests**  
Room **Audimax**  
Chair Sophie de Vries (Göttingen/DE), Alison Bennett (Columbus, OH/US)
- 09:45 The presence of arbuscular mycorrhizal fungi in plant rhizospheres drives changes in plant and herbivore microbiome composition  
Alison Bennett (Columbus, OH/US)
- 10:15 Epidermal bladder cells of *Chenopodium quinoa* as a herbivore defense mechanism  
Max William Moog (Frederiksberg C/DK)
- 10:33 A metabolic arms race between barley (*Hordeum vulgare* L.) and two fungal pathogens  
Dario Esposito, Yiming Liu (Halle a. d. Saale/DE), Lisa Mahdi (Köln/DE), Andrea Porzel, Pauline Stark, Hussein Hidayat Anja Scherr-Henning, Ulschan Bathe (Halle a. d. Saale/DE), Ivan Acosta (Golm/DE), Alga Zuccaro (Köln/DE) Gerd Balcke, Alain Tissier (Halle a. d. Saale/DE)
- 10:51 Exploring the Functional Diversity of NLR Immune Receptors in Divergent Land Plant Lineages  
Khong-Sam Chia, Philip Carella (Norwich/GB)
- 11:09 Carbohydrate availability and homeostasis modulate pathogen susceptibility of *Arabidopsis thaliana*  
Kristina Sandra Munzert, Lena Zanoni (Marburg/DE), Henriette Leicher (Freising/DE), Carsten Rautengarten Berit Ebert (Bochum/DE), Martin Stegmann (Freising, Ulm/DE), Lars Voll, Timo Engelsdorf (Marburg/DE)
- 11:27 Characterisation of polygalacturonases in the interaction of parasitic plants and phytopathogens with their host plant  
Wiebke Häger (Trondheim/NO), Thomas Bawin, Kirsten Krause (Tromsø/NO), Thorsten Hamann (Trondheim/NO)
- 09:45–11:45 **Plant systematics, evolution and biodiversity**  
Room **Lecture hall XXII**  
Chair Natalia Tkach (Leipzig/DE), Alexandra Müllner-Riehl (Halle a. d. Saale), Susann Wicke (Berlin/DE)
- 09:45 Evolution and development of parasitism in the Broomrape family (Orobanchaceae)  
Susann Wicke (Berlin/DE)
- 10:15 How do different ploidies adapt? A case of *Arabidopsis* in a non-extreme edaphic environment  
Sonia Celestini (Prague/CZ), Veronika Konečná (Zurich/CH), Filip Kolář (Prague/CZ)
- 10:33 Fragrant Phylogenies: Decoding Daffodil scents with GC-IMS  
Tim Böhnert, Florian Losch, Maximilian Weigend (Bonn/DE)
- 10:51 Testing different strategies to efficiently assemble organellar and nuclear plant genomes – a case study on *Ranunculus auricomus* (Ranunculaceae)  
Kevin Karbstein (Jena/DE), Salvatore Tomasello, Natascha Wagner, Ting Xie, Birthe H. Barke (Göttingen/DE) Claudia Pätzold (Göttingen, Dresden/DE), John P. Bradican (Göttingen/DE), Michaela Preick (Potsdam/DE) Axel Himmelbach, Nils Stein (Seeland/DE), Iker Irisarri (Göttingen, Hamburg/DE), Boas Pucker (Braunschweig/DE) Jan de Vries, Argyris Papantonis, Elvira Hörandl (Göttingen/DE)
- 11:09 Landscape effects on the spatial distribution of genetic lineages in a widespread, generalist herb  
Dirk Albach, Mareike Daubert (Oldenburg/DE)
- 11:27 Hidden promiscuity explains duckweed diversity and evolution  
Anton Stepanenko (Gatersleben/DE, Kyiv/UA), Phuong TN Hoang (Gatersleben/DE, Dalat/VN), Jörg Fuchs, Veit Schubert Ingo Schubert (Gatersleben/DE)



09:45–11:45	Plant proteins - structure to function
Room	Lecture hall XXIII
Chair	Mariana Schuster (Halle a. d. Saale), Michael Hothorn (Geneva/CH)
09:45	Connecting hormone biosynthesis and signal transduction – the molecular basis for brassinosteroid bioactivity Michael Hothorn (Geneva/CH)
10:15	Engineering an Exo70 integrated domain of a barley NLR for improved blast resistance Indira Saado, Helen J. Brabham, Josh W. Bennett, Anson Ho Ching Lam (Norwich/GB) Matthew J. Moscou (Minnesota, MN/US), Juan Carlos De la Concepcion (Vienna/AT), Mark Banfield (Norwich/GB)
10:33	Imaging of plant calcium-sensor kinase conformation monitors real time calcium decoding <i>in vivo</i> Anja Liese (Halle a. d. Saale/DE), Bernadette Eichstädt (Berlin/DE), Sarah Lederer, Susanne Matschi (Halle a. d. Saale/DE), José A Feijó (College Park, MD/US), Waltraud Schulze (Hohenheim/DE), Kai Konrad (Würzburg/DE) Tina Romeis (Halle a. d. Saale/DE)
10:51	Structural differences in functional relatives over the panproteome of barley Amanda Souza Câmara, Victor Henrique Rabesquine Nogueira (Gatersleben/DE)
11:09	Effect of changes in different AGO2 proteoforms on antiviral protection in <i>Arabidopsis thaliana</i> Selma Gago-Zachert, Maoyu Xiang, Florian Müller, Anne Winkler, Simon Fay, Torsten Gursinsky, Ralph Golbik, Sven-Erik Behrens (Halle a. d. Saale/DE)
11:27	Structural studies of the transmembrane domain of ethylene receptor ETR1 Buket Ruffer (Düsseldorf/DE)
12:45–14:00	Closing Lecture and Farewell
Raum	Audimax
Chair	Keiko Torii (Austin, TX/US), Marcel Quint (Halle a. d. Saale/DE)
12:45	Environmental control of stomatal development - From intricate modulation to innovation Keiko Torii (Austin, TX/US)
13:45	Farewell



### 17:15–18:45 Photosynthesis and general metabolism

- P 001 Chlamydomonas reinhardtii alpha-amylase 2 is activated by glutamine  
Lisa Scholtysek, Ansgar Poetsch, Eckhard Hofmann, Anja Hemschemeier (Bochum/DE)
- P 003 A kinetic model for temperature effects on the non-photochemical quenching process in tomato  
Quang Huy Nguyen (Düsseldorf/DE), Thekla von Bismarck (Wageningen/NL), Ute Armbruster (Düsseldorf/DE)  
Anna Matuszyńska (Aachen, Düsseldorf/DE)
- P 005 A versatile outdoor-protocol to acquire photosynthesis of microalgae using stable isotope ratios  
Sven Gindorf, Daniel Remias (Salzburg/AT), Katharina Schott, Andrea Watzinger (Vienna/AT)
- P 007 Adaptive potential of the Leaf Economics Spectrum in the Brassicaceae  
Luis Chausson Garate, José Miguel Valderrama-Martín, Urte Schlüter, Andreas Weber (Düsseldorf/DE)
- P 009 Biochemical and physiological characterization of dual specific N-acetyltransferases in plastids of *Ara bidopsis*  
Jens Mühlenbeck, Jürgen Eirich (Münster/DE), Julia Sindlinger, Dirk Schwarzer (Tübingen/DE), Iris Finkemeier (Münster/DE)
- P 011 Characterization of C3-C4 intermediate *Diplotaxis* species  
Jana Peter, Urte Schlüter, Andreas Weber (Düsseldorf/DE)
- P 013 Chloroplast positioning and its role in metabolic acclimation to a changing environment  
Sophia Bagshaw, Anastasia Kitashova, Thomas Naegele (Planegg-Martinsried/DE)
- P 015 Compartment-specific metabolic acclimation in *Arabidopsis thaliana*  
Vladimir Brodsky, Thomas Nägele (München /DE)
- P 017 Elevating embryo energy status: A novel approach to improve storage starch biosynthesis in dicotyle donous plants  
Reza Chamansara, Manish Raorane, Björn H. Junker (Halle a. d. Saale/DE)
- P 019 Eukaryote-specific assembly factor AT2G48070 is required for efficient assembly of Photosystem II  
Juan Carlos Dávila Frantzen (Düsseldorf/DE), Sandrine Kappel (Potsdam/DE), Ute Armbruster (Düsseldorf/DE)
- P 021 Facultative Crassulacean Acid Metabolism in *Talinum fruticosum*  
Hanna Göhlmann, Andreas Weber (Düsseldorf/DE)
- P 023 Functional interaction of STN7/8 and pCK2 in photosynthetic acclimation  
Tim Demmig, Anja Rödiger (Bochum/DE), Thomas Pfannschmidt (Hannover/DE), Sacha Baginsky (Bochum/DE)

### 17:15–18:45 Specialized metabolism

- P 025 Understanding the Protein Quality Control of Antennae Complex Proteins Under Heat and High-Light-Cold stress  
Dipanshu Ghosh, Twinkle, Vivek Dogra (Palampur, Ghaziabad/IN)
- P 027 An insight of anti – psoriatic potential of *Wrightia tinctoria* leaf phytochemicals  
Dhanya Thankappan, Archana Muttanolla, Vaagdevi Narayangari, Santosh R. Kanade (Hyderabad/IN)
- P 029 Assessing the effects of glucosinolates and their different breakdown product types on the rhizosphere bacteria community  
Annika Hielscher, Eleanor C. M. Chroston (Braunschweig/DE), Nina Bziuk (Graz/AT; Braunschweig/DE)  
Einar J. Stauber (Braunschweig/DE), Beena M. Ravindran (Braunschweig, Leopoldshöhe/DE), Kornelia Smalla  
Ute Wittstock (Braunschweig/DE)
- P 031 Biosynthetic Pathway Discovery in Plants Based on Omics Data Integration  
Felicia Wolters (Wageningen/NL)
- P 033 Comparative analysis of *Taraxacum* hybrids with focus on the rubber biosynthesis  
Jakob Wiemann, Nicole van Dam, Christian Schulze Gronover, Dirk Prüfer (Münster/DE)



- P 035 Comparative genomics reveals a conserved biosynthetic gene cluster in withanolide-producing Solanaceae species  
Nancy Choudhary, Ronja Friedhoff (Braunschweig/DE), Samuel Edward Hakim, Karan Malhotra, Jian Peng  
 Arne Bueltemeier, Ahmed Arafa, Jakob Franke (Hannover/DE), Boas Pucker (Braunschweig/DE)
- P 037 Different flavonoid biosynthesis R2R3-MYB transcription factors recognize distinctive elements in the chalcone synthase promoter  
Lennart Sielmann, Ralf Stracke, Bernd Weisshaar (Bielefeld/DE)
- P 039 Elucidation of early withanolide biosynthesis using an engineered *Nicotiana benthamiana* platform  
Arne Bültemeier, Karan Malhotra, Samuel Edward Hakim (Hannover/DE), Nancy Choudhary (Braunschweig/DE), Jian Peng  
 Ahmed Arafa (Hannover/DE), Boas Pucker (Braunschweig/DE), Jakob Franke (Hannover/DE)
- P 041 Elucidation of herbivore-induced volatile terpenoid formation in leaves of Japanese orange cherry (*Idesia polycarpa*)  
 Melina Panagoulas, Maya Bach, Miriam Fernández-Giro Muñoz, Markus Krischke, Nathalie D. Lackus (Würzburg/DE)
- P 043 Gene clustering in barley reveals cryptic oxidative rearrangement in gramine biosynthesis  
Sara Leite Dias (Seeland/OT Gatersleben/DE), Ling Chuang, Shenyu Liu, Benedikt Seligmann (Hannover/DE)  
 Fabian L. Brendel, Benjamin Chavez, Robert E. Hoffie, Jochen Kumlehn (Seeland/OT Gatersleben/DE), Johanna Wolf  
 Arne Bültemeier, Marco Herde, Claus-Peter Witte (Hannover/DE), John D'Auria (Seeland/OT Gatersleben/DE)  
 Jakob Franke (Hannover/DE)
- P 045 Identification and characterisation of desulfoglucosinolate sulfotransferases from *Tropaeolum majus*  
Katharina Hartelt, Miriam von Barga, Ronja Friedhoff, Boas Pucker, Islam El-Awaad, Ute Wittstock (Braunschweig/DE)
- P 047 Identification of the intermediates and the mechanisms involved in the crosstalk of isoprenoid biosynthesis pathways  
Jara M. Al-Mousawi, Manish Raorane (Halle a. d. Saale/DE), Toranj Rahpeyma, Peter Nick (Karlsruhe/DE)  
 Björn H. Junker (Halle a. d. Saale/DE)
- P 049 Investigation on BAHD Acyltransferases in *Neoblechnum brasiliense*  
Maximilian Ufland, Maike Petersen (Marburg/DE)
- P 051 Learning more about the complex machinery involved in glucosinolate breakdown in *Arabidopsis thaliana*  
Louisa Behnsen, Cindy Seeling, Anita Backenköhler, Kathrin Meier, Einar J. Stauber, Ute Wittstock (Braunschweig/DE)
- P 053 Molecular and biochemical investigations of acyltransferases and esterases in the formation of phenolic compounds in  
*Anthoceros agrestis* and *Mesotaenium endlicherianum*  
Janik Marks, Maike Petersen (Marburg/DE)
- P 055 Production of bioactive compounds from plant cell suspension cultures in bioreactors  
Luca Meink, Björn H. Junker, Manish Raorane (Halle a. d. Saale/DE)
- P 057 Subcellular localization of homospermidine oxidases involved in pyrrolizidine alkaloids biosynthesis  
Adila Shahid, Dietrich Ober (Kiel/DE)
- P 059 Hiding in plain sight: Bacterial gibberellin biosynthesis  
Raimund Nagel (Leipzig/DE, Ames, TX/US), Liza E. Alexander, Charles E. Stewart Jr., Reuben J. Peters (Ames, TX/US)
- PL 002 A chemical effector influencing stomatal development  
Arvid Herrmann, Krishna Sepuru, Pengfei Bai, Keiko Torii (Austin, TX/US), Hitoshi Endo, Ayami Nakagawa, Asraa Ziadi,  
 Hiroe Kato, Ayato Sato, Kenchichiro Itami, Naoyuki Ushida, Shinya Hagihara (Nagoya/JP), Shuhei Kusano (Wako/JP),  
 Jun Liu, Libo Shan (College Station, TX/US), Seisuke Kimura (Kamigamo-Motoyama/JP)
- PL 003 The Program Center MetaCom @ IPB  
Jörg Ziegler, Gerd Balcke, Pauline Stark, Khabat Vahabi, Steffen Neumann, Henriette Uthe (Halle a. d. Saale/DE)

17:15–18:45 Gene regulation

- P 061 Genome Sequence of the ornamental plant *Digitalis purpurea* reveals the molecular basis of flower traits  
Jakob Maximilian Horz, Boas Pucker, Katharina Wolff, Ronja Friedhoff (Braunschweig/DE)
- P 063 Genome wide identification of heat shock proteins from *Prosopis cineraria* and their interaction studies  
Hansa Sehgal, Mukul Joshi (Pilani/IN)
- P 065 Investigating a bHLH transcription factor as marker and regulator of saponin biosynthesis in *Chenopodium quinoa*  
Marius Kollmar, Sophie Otterbach, Lukas John, Sandra Schmöckel (Stuttgart/DE)
- P 067 Investigating plasticity trade-offs and the role of spliceosome factors in flower size plasticity to light and temperature  
Gregory Andreou, Jan Hoffmann, Roosa Laitinen (Helsinki/FI), Zoran Nikoloski (Potsdam/DE)
- P 069 Post-transcriptional RNA modification (m6A) influences the hormone-mediated drought stress response in *Arabidopsis thaliana*  
Yasira Shoaib, Fatima Chigri, Ute Vothknecht (Bonn/DE)
- P 071 Peculiar evolution of an ancient microRNA presumably regulating class B floral homeotic genes  
Lydia Gramzow, Christian Gafert, Günter Theißen (Jena/DE)
- P 073 Redox signaling to chromatin during stress responses in plants  
Mansi Sharma, Michael Wrzaczek (České Budějovice/CZ), Pavel Kerchev (Brno/CZ)
- P 075 Telescripting in Plants: A Novel Mechanism Ensuring Complete mRNA Transcription  
Luise Nagel (Halle a. d. Saale/DE)
- P 077 The *Arabidopsis* Rna-processing Factor Serrate is Involved in Polyadenylation and U1-mediated Telescripting  
Manon Adler (Halle a. d. Saale/DE)
- P 079 Transcriptomic dissection of the H<sub>2</sub>O<sub>2</sub>-Ca<sup>2+</sup> crosstalk in the leaves and roots of barley  
Sabarna Bhattacharyya (Bonn/DE), Bastian Meier (Halle a. d. Saale/DE), Carissa Bleker, Kristina Gruden (Ljubljana/SI)  
Edgar Peiter (Halle a. d. Saale/DE), Ute Vothknecht, Fatima Chigri (Bonn/DE)
- P 081 Sugar-dependent regulation of the flavonoid biosynthesis during high light acclimation: Interplay between SnRK1 and PAP1  
Josephine Dieckmann (Rostock/DE), Ralf Stracke (Bielefeld/DE), Andreas S. Richter (Rostock/DE)

17:15–18:45 Plant proteins: structure to function

- P 083 Characterizing the role of the transamidosome complex to understand the biological relevance of the indirect Glu-tRNA<sup>Gln</sup> synthesis pathway in plants  
Sebastian Schwartz, Benjamin Brandt, Hans-Henning Kunz (Planegg-Martinsried/DE), Franz Hagn, Robert Janowski, (Munich/DE)
- P 085 Computational analysis of protein interactions during the photosystem II assembly  
Milena Lange, Ute Armbruster (Düsseldorf/DE), Till Rudack (Bochum/DE)
- P 087 Cryo-EM structure of photosystem II supercomplex from *Chlorella ohadii*, a green microalga with extreme phototolerance  
Rameez Arshad, Ioannis Skalidis, Panagiotis Kastiris (Halle a. d. Saale/DE), David Kopecny, Monika Opatiková, Petr Ilík, Pavel Pospíšil, Sanja Čavar Zeljković, Dušan Lazár, Roman Kouřil (Olomouc/CZ), Sylvia Brabencová, Pavel Roudnický (Brno/CZ), Eduard Elias, Roberta Croce (Amsterdam/NL)
- P 089 Establishment of BioID-based proximity labelling for in-depth proteome analyses of *Arabidopsis* peroxisomes  
Ebenezer Ntiriakwa, Athina Parasyri, Ipek Dinler, Thomas Mair, Hartmut Schlüter, Sigrun Reumann (Hamburg/DE)
- P 091 Evolutionary conservation of a lipid-droplet anchoring protein-protein interaction in angiosperms  
Pauline Prüsener, Till Ischebeck, Janis Dabisch (Münster/DE)

- P 093 Functional analysis of TatA and TatB subunits of the Tat machinery in chloroplasts  
Ming Zhou, Mario Jakob, Ralf Bernd Klösger (Halle a. d. Saale/DE)
- P 095 Functional characterization of the coiled-coil domain of the viral silencing suppressor (P15) of the peanut clump virus in dimerization and siRNA binding  
Lara-Marie Halscheid, Stefan Wirling, Marvin Bolz, Sigrun Reumann (Hamburg/DE)
- P 097 How to decode cellular signals: Investigating the biochemical mechanism-of-action of calcium-dependent protein kinases  
Kamélia Maguemoun, Jacqueline Monaghan (Kongston/CA), Melissa Bredow (Ames, IA/US)
- P 099 Impact of Natural LPR1 Proteoforms on Fe-dependent Phosphate Sensing  
Natalie Leutert, Christin Naumann, Steffen Abel, Milton T. Stubbs (Halle a. d. Saale/DE)
- P 101 Investigating the function of Arabidopsis HISTONE DEACETYLASE 14 in chloroplasts  
Claudia Markiton, Florian Kotnik, Jürgen Eirich, Iris Finkemeier (Münster/DE)
- P 103 Molecular basis of the structural changes acquired by the C4 photosynthetic NADP-malic enzyme from its housekeeping ancestor  
Jonas Matteo Böhm, Simone Willms, Martin Buitrago Arango, Oja Ferrao, Meike Hüdig, Veronica Graciela Maurino-Larcher (Bonn/DE), Clarisa Alvarez (Rosario/AR)
- P 105 Structural Phloem Proteins: Exploring the Reaction Mechanism of the Plant Defense System  
Lisa Wrobel, Dirk Prüfer, Gundula Noll (Münster/DE)
- PL 004 Evolutionary conservation of the interplay between COP1 and GLKs during terrestrialization  
Miriam Neidert, Maike Hansen, Melanie Kreiss, Ute Hoecker (Cologne/DE)
- 17:15–18:45 Cell biology
- P 107 Elucidating the role of actin-depolymerizing factors (ADFs) in regulating *Nicotiana tabacum* pollen tube polarity  
Marta Fratini, Gottfried Hamm, Mareike Heilmann, Ingo Heilmann (Halle a. d. Saale/DE)
- P 109 Exploring manganese function and biology with genetically encoded Mn<sup>2+</sup>-Biosensor  
Nan Zhou, Lukas Wallrad (Münster/DE), Lutz Schmitt (Düsseldorf/DE), Jörg Kudla (Münster/DE)
- P 111 The Ca<sup>2+</sup>-signaling associated protein kinases CIPK23 and CPK21 have an opposite impact on stomatal movements  
 Shouguang Huang (Würzburg/DE), Triinu Arjus, Hannes Kollist (Tartu/EE), Dietmar Geiger, Rob Roelfsema (Würzburg/DE)
- P 113 Altered Ca<sup>2+</sup> signature promotes side branch formation via actin cytoskeleton modification in the moss *Physcomitrium patens*  
Johanna Knab, Benedikt Kost (Erlangen/DE)
- P 115 Development of a high-throughput split-nanoLUC screen for novel protein interaction partners in *Ara bidopsis* protoplasts  
 Matthias Reimers, Carolin Delker (Halle a. d. Saale/DE), Johannes Stuttmann (Aix en Provence/FR), Marcel Quint  
Lennart Eschen-Lippold (Halle a. d. Saale/DE)
- P 117 Plant cells can form and retain protrusions – Are plant and animal cells more alike than we think?  
Johanna E. M. Dickmann, Marjolaine Martin, Claire Lionnet, Olivier Hamant (Lyon/FR)
- P 119 Investigating contributions of GIPCs to fundamental cell functions using mutants of the moss *Physcomitrium patens*  
Tegan Haslam (Göttingen/DE), Linus Wegner, Katrin Ehlers (Gießen/DE), Cornelia Herrfurth, Ivo Feussner (Göttingen/DE)
- P 121 Using plant pathogen effectors to identify genes regulating stress driven plastid morphology adaptation  
Theresa Staps, Martin Schattat, Christina Lampe, Jolina Marx, Simon Ortmann, Jessica Erickson (Halle a. d. Saale/DE)
- P 123 Biosynthesis and cell biology of piperine accumulation in black pepper, *Piper nigrum*  
Wafa Kouas (Halle a. d. Saale/DE)
- PL 006 Elucidation of Exocyst-Driven Tethering in Plants  
Alaa Allahham, Moritz Werres, Marco Trujillo (Aachen/DE)

17:15–18:45 Communication and dynamics of plant organelles

- P 125 Monitoring the Activity of a Dually-targeted Rna Polymerase *in Vivo*  
Tim Kiesel, Sarlita Dwiani, Kristina Kühn (Halle a. d. Saale/DE)
- P 127 *Plastid morphology impacts jasmonate biosynthesis*  
Ranjit Baral, Hagen Stellmach, Bettina Hause (Halle a. d. Saale/DE)
- P 129 *Quantitative 3D Modelling of Organelle Ultrastructure in Native Guard Cells Using Cryo-FIB-SEM*  
Xudong Zhang (Stuttgart/DE), Bastian Franzisky (Geisenheim/DE), Claus Jakob Burkhardt (Tübingen/DE)  
Endre Majorovits, Eric Hummel, Andreas Schertel (Oberkochen/DE), Christoph-Martin Geilfus (Geisenheim/DE)  
Christian Zörb (Stuttgart/DE)
- P 131 *A quantitative study of the mitochondrial transcriptome in the model plant Arabidopsis thaliana*  
Michelle Marofke, Kristina Kühn (Halle a. d. Saale/DE)
- P 133 *Investigation of novel membrane contact sites in plants*  
Vanessa Valencia, Andreas Weber (Düsseldorf/DE)
- P 135 *mTERF21 – an essential protein for RNA processing in Arabidopsis mitochondria*  
Theresa Schoeller, Carolin Goldhardt, Lea Brings, Minsoo Kim, Martin Schattat, Etienne H. Meyer, Elizabeth Vierling  
Kristina Kühn (Halle a. d. Saale/DE)

17:15–18:45 Molecular mechanisms of plant nutrition

organized by the German Society of Plant Nutrition (DGP)



- P 139 Characterization of a copper deficiency-sensitive natural accession in barley  
Lijuan Yang, Nicolaus von Wirén (Seeland/DE)
- P 141 Characterization of evolutionary conserved PFA-DSP-type phosphohydrolases in Arabidopsis thaliana  
Klea Lami, Philipp Gaugler, Robin Schneider, Gabriel Schaaf, Verena Gaugler (Bonn/DE), Guizhen Liu, Danye Qiu  
Henning J. Jessen (Freiburg i. Br./DE), Debabrata Laha (Bengaluru/IN)
- P 143 CIPK25 as a direct or indirect regulator of the NRT2.1  
Rebecca John, Tatsiana Straub, Waltraud Schulze (Stuttgart/DE)
- P 145 Exploring Uncharted Territories: New Genes for Sulfur Starvation Responses in Plants  
Suvajit Basu, Stanislav Kopriva, Daniela Ristova (Köln/DE)
- P 147 Genetic approach to acquire new insights into members of the Tracheophyta-specific HIPP protein family  
Olaf Barth (Halle a. d. Saale/DE), Athina Parasyri (Halle a. d. Saale, Hamburg/DE)  
Wiebke Zschiesche (Halle a. d. Saale/DE), Laura Rehneke (Halle a. d. Saale, Gießen/DE)  
Klaus Humbeck (Halle a. d. Saale/DE)
- P 149 Identification and Functional Analysis of Ion Transporters Providing Salinity Tolerance in Halophytic Barley Relatives  
Stanislav Isayenkov, Bastian Meier, Edgar Peiter (Halle a. d. Saale/DE)
- P 151 Metabolic responses to phosphate deficiency in barley (*Hordeum vulgare*)  
Magdalena Kuczkowska, Gabriel Schaaf, Peter Dörmann (Bonn/DE)
- P 153 Nitrogen stress-induced alterations in the leaf proteome of rice varieties  
Sana Basri, Altaf Ahmad (Aligarh/IN)
- P 155 Anion Uptake by Guard Cells  
Namrah Ahmad, Rob Roelfsema (Würzburg/DE)
- P 157 Calcium and CDPK in the longevity of plants  
Oliver Nagel, Sarah Lederer, Anja Liese, Tina Romeis, Fabian-Philipp Sylvester, Philipp Schulz (Halle a. d. Saale/DE)

## 17:15–18:45 Plant development

- P 159 Deciphering tomato photoperiodic flowering and its interactions with age and gibberellin pathways  
Mateus Henrique Vicente (Piracicaba/BR), Gloria Serrano-Bueno (Seville/ES), Lilian Ellen Pino (Piracicaba/BR)  
 Fernando Baile (Seville/ES), Lázaro Eustaquio Pereira Peres (Piracicaba/BR), Myriam Calonje  
 Federico Valverde (Seville/ES), Fabio Nogueira (Piracicaba/BR)
- P 161 Genome editing reveals that the transcription factor BRANCHED1 is not a primary determinant of dimorphic fruit ratio in *Aethionema arabicum*  
Jessica Patzer, Frania Kleeberg (Jena/DE), Subha Suvetha Kathalingam (Jena/DE, Singapore/SG), Lena Gauthier, Günter Theißen (Jena/DE)
- P 163 Genome-wide association study reveals the molecular determinants of pre-anthesis tip degeneration in barley  
Kenan Tan, Yongyu Huang, Roop Kamal (Gatersleben/DE), Thorsten Schnurbusch (Gatersleben, Halle a. d. Saale/DE)
- P 165 Glutathione affects plant growth indirectly by controlling the amount of nitric oxid  
Mohammad Taheb Safi, Andreas Meyer, José Manuel Ugalde (Bonn/DE)
- P 167 Modulation of the developmental seminal root lifespan in barley  
Cevza Esin Tunc, Nicolaus von Wirén (Gatersleben/DE)
- P 169 Molecular characterisation of cryptochrome 1-mediated suppression of the Arabidopsis COP1/SPA E3 ubiquitin ligase  
Laura Trimborn, Pengxin Yu, Jathish Ponnu, Ute Hoecker (Köln/DE)
- P 171 Patterning the Shoot Meristem Stem Cell Niche by mir394 Signalling  
Frauke Garbsch, Fei Liu, Chenchen Wang, Thomas Laux (Freiburg i. Br./DE)
- P 173 Photoperiod-dependent modulation of plant development and physiology by mitochondrial metabolic signals  
Maria del Pilar Martinez, Ioana Nica (Bonn/DE), Ke Zheng, Markus Schwarzländer (Münster/DE)  
 Veronica Graciela Maurino-Larcher (Bonn/DE)
- P 175 PRC2 rewires developmental and metabolic programs during seedling emergence before and after the initiation of photosynthesis  
Naseem Samo, María Guadalupe Trejo-Arellano, Fatemeh Aflaki (České Budějovice/CZ), Alina Ebert  
 Alexander Erban (Potsdam/DE), Lenka Gahurová, Jiří Kubásek, Helena Hömig Mondeková (České Budějovice/CZ)  
 Armin Schlereth (Potsdam/DE), Quentin Riviere, Mingxi Zhou (České Budějovice/CZ), Daniel Bouyer (Lyon/FR)  
 Jiří Šantrůček (České Budějovice/CZ), Ondřej Novák (Olomouc/CZ), Joachim Kopka (Potsdam/DE)  
 Iva Mozgová (České Budějovice/CZ)
- P 177 Control of stomatal plasticity in fluctuating environmental conditions  
Sara Forlani, Kaotar Elhazzime, Anne Vaten (Helsinki/FI)
- P 179 Chilling of germinating seeds accelerates flowering in *Aethionema arabicum* involving repression of FLC  
Renu Sharma, Lydia Gramzow, Florian Rümpler, Günter Theißen (Jena/DE)
- PL 005 The Role of the Florigen Activation Complex (FAC) in Floral Development in Arabidopsis  
Maida Romera Branchat, Chloé Pocard, Coral Vincent, Martina Cerise, He Gao, Rainer Franzen,  
 George Coupland (Cologne / DE)
- PL 007 Epigenetics protects male germline development in plants  
Hua Jiang, Sida Zhou, Jinping Cheng (Potsdam-Golm/DE)

## 17:15–18:45 Plant hormones

- P 181 A metathesis-derived library of 12-OPDA variants for scrutinizing and tuning OPDA functions  
Madita Sophie Knieper, Maike Bittmann, Tim Lukas Guntelmann, Andrea Viehhauser, Harald Gröger  
 Karl-Josef Dietz (Bielefeld/DE)
- P 183 A role of jasmonate in root elongation of wheat  
Li Wang, Markus Meier, Martin Mascher, Nicolaus von Wirén (Gatersleben/DE)
- P 185 A zinc finger protein is involved in the response to RES and ROS and the tolerance of abiotic stress  
 Alexandra Fössel (Würzburg/DE), Maximilian Fuchs (Hannover/DE), Leonie Titze, Susanne Berger (Würzburg/DE)
- P 187 Deciphering the Regulation of Abscisic Acid Transport and Homeostasis under Water Deficit in *Ara bidopsis*  
Rainer Waadt, Robin Rasiah (Münster/DE)
- P 189 Dissecting jasmonic acid and auxin cross-talk in tomato flower development and fruit set  
Moritz Friesch, Henrikje Smits (Halle a. d. Saale/DE), Naomi Ori (Rehovot/IL), Bettina Hause (Halle a. d. Saale/DE)
- P 191 Regulation of *Arabidopsis thaliana* 13-LOX enzymes involved in root jasmonate biosynthesis  
Frieda Rößler, Debora Gasperini, Yunjing Ma (Halle a. d. Saale/DE)

## 17:15–18:45 Navigating abiotic challenges

- P 193 *Arabidopsis thaliana* adapts its sphingolipid profile to different temperatures  
Frank Waller, Kristina Erwardt, Pamela Korte, Alina Voss, Markus Krischke, Agnes Fekete, Martin J. Mueller (Würzburg/DE)
- P 195 Changes of leaf water relations during severe drought stress through silicon application to spring wheat (*Triticum aestivum* L.)  
Tebea Selzer (Gießen/DE), Manuela Pérez Rodriguez (Gießen/DE, Valencia/ES), Jakob Santner (Gießen/DE)
- P 197 Differential expression of Methanol Inducible Protein, Pectin Methylesterases, and Pectin Methylesterase Inhibitor/s induced by Hormetic level of Cadmium in *Solanum lycopersicum*  
Manish Yadav, Risha Ravi, Santosh R. Kanade (Hyderabad/IN)
- P 199 Elucidation of Non-Histone Protein Acetylation in *Arabidopsis* Under High Light Stress  
Jie Shen, Jürgen Eirich, Iris Finkemeier (Münster/DE)
- P 201 Grain yield of wheat as affected by individual and combined heat and drought stress during different growth stages with emphasis on source-sink relations  
Dorit Ehrhardt, Jakob Santner, Birgit Hütsch (Gießen/DE)
- P 203 Molecular signatures in the induction of UV protection by chlorogenic acid accumulation in sunflower leaves  
 Jana Stelzner (Kiel/DE), Eva M. Molin (Tulln an der Donau/AT), Hans-Peter Mock (Seeland/DE), Wolfgang Bilger (Kiel/DE)
- P 205 Myrosinase TGG1 regulates guard cell glucosinolate levels with significance for drought-related stomatal closure  
Bastian Franzisky (Geisenheim/DE), Gyöngyi Bárdos (Hohenheim/DE), Katja Witzel, Franziska Hanschen (Großbeeren/DE)  
 Bärbel Kroschewski (Berlin/DE), Christian Zörb (Hohenheim/DE), Christoph-Martin Geilfus (Geisenheim/DE)
- P 207 Na-preferential ion transporter HKT1;1 mediates salt tolerance in blueberry  
Huifang Song (Würzburg/DE)
- P 209 PGPR-induced OsNAM2 gene provides salt tolerance in *Arabidopsis* by AFP2 and SUS protein interaction  
Harshita Joshi (Lucknow/IN), Klaus Harter (Tübingen/DE), Puneet Singh Chauhan (Lucknow/IN)
- P 211 Physiological and genetic mechanisms underlying silicon-mediated drought tolerance in barley  
Gabriel de Oliveira Ragazzo, Sara Beier, Anja Hartmann, Nicolaus von Wirén (Gatersleben/DE)

- P 213 Proteome alterations in bread wheat cultivars differing in their drought tolerance  
Olha Lakhneko (Nitra/SK, Kyiv/UA), Oleg Stasik (Kyiv/UA), L'udovít Škultéty (Bratislava/SK), Dmytro Kirizi  
Oksana Sokolovska-Sergiienko, Maria Kovalenko (Kyiv/UA), Maksym Danchenko (Nitra/SK)
- P 215 Sugar and salt: How seagrass cell walls adapted to the marine habitat  
Lukas Pfeifer, Birgit Classen (Kiel/DE)
- P 217 Synergistic effects of root-associated arbuscular mycorrhizal fungi and green compost on the growth and salt stress tolerance of tomato plants  
Soumaya Zaidi, Mohammad-Reza Hajirezaei, Nicolaus von Wirén (Gatersleben/DE)
- P 219 Systemic effects of combined arsenic and hypoxia exposure in *Arabidopsis thaliana* L. roots  
Lara Vogelsang, Vijay Kumar, Marcus Persicke (Bielefeld/DE), Iris Finkemeier (Münster/DE), Karl-Josef Dietz (Bielefeld/DE)
- P 221 Taurine – the key plant regulator in cadmium hormesis  
Risha Ravi, Manish Yadav, Santosh R. Kanade (Gopanpalle/IN)
- P 223 The dynamic function of the cytosolic redox regulatory network and the sensory role of the type II peroxiredoxins B/C/D  
Kishan Gurjar (Münster/DE)
- P 225 The impact of drought stress on growth and cucurbitacins accumulation in different lines of *C. pepo* subsp. *pepo*  
Anika Wiese-Klinkenberg, Franziska Genzel (Jülich/DE)
- P 227 Transcriptional reprogramming during developmental and stress-induced leaf senescence in barley  
Wiebke Zschiesche (Halle a. d. Saale, Wittenberg/DE), Christina Mohr, Nazeer Fataftha (Halle a. d. Saale/DE)  
Klaus Humbeck (Halle a. d. Saale, Wittenberg/DE)
- PL 008 Wild cabbage (*Brassica incana*) as potential gene pool for climate change adaptation in Brassica crops  
Branka Salopek Sondji, K. Baotić, I. Orehovec, K. Majsec, N. Bauer, J. Drmić, M. Tkalec (Zagreb/HR),  
N. Jasprica (Dubrovnik/HR), N. Major, T. K. Kovačević, S. Goreta Ban (Poreč/HR)
- 17:15–18:45 **Resisting pathogens and pests**
- P 229 Clarifying the molecular defense mechanisms of tomato against dodder  
Arnaud Blaquiere, Peter Slaby, Ronja Burggraf, Isabell Albert, Markus Albert (Erlangen/DE)
- P 231 Degradation of plant cell wall components by type II-secreted enzymes – how phytopathogenic bacteria turn the plant apoplast into a habitat  
Samuel Goll, Jessica Erickson, Daniela Büttner (Halle a. d. Saale/DE)
- P 233 Differential proteomics and post-translational modifications in the defense response of *Zea mays* to multitrophic biotic stresses  
Augusto Penteriche (Münster/DE, Piracicaba/BR), Diego Gallan, Giovanna Veronez (Piracicaba/BR), Jürgen Eirich  
Iris Finkemeier (Münster/DE), Marcio de Castro Silva Filho (Piracicaba/BR)
- P 235 Elucidation of the molecular networks of *Arabidopsis thaliana* SHRKs  
Anna Bannmüller, Athanasios Makris, Martina K. Ried-Lasi (Halle a. d. Saale/DE)
- P 237 Exploring the Molecular Mechanism and Functional Diversity of NEP1-like proteins in Plant Pathogenesis  
Giacomo Giuliani (Erlangen-Nürnberg/DE), Dietmar Geiger, Rob Roelfsema (Würzburg/DE)  
Petra Dietrich (Erlangen-Nürnberg/DE), Thorsten Nürnberger (Tübingen/DE), Isabell Albert (Erlangen-Nürnberg/DE)
- P 239 Ferricrocin: The concealed virulence factor of the corn anthracnose pathogen *C. graminicola*  
Lala Aliyeva-Schnorr, Holger B. Deising, Bennet Rohan Fernando, Christoph Goldbach, René Csuk (Halle a. d. Saale/DE)
- P 241 Identification and characterization of miR398GGT of *Gaeumannomyces graminis* var. *tritici* and its possible role in plant-fungus interactions  
Johanna Stehle, Markus Schemmel, Lingyue Han, Daguang Cai (Kiel/DE)



## POSTERS ODD | MONDAY, 16 SEPTEMBER

- P 243 Loss of pgm, sweet11 and sweet12 alters local and systemic carbohydrate allocation and pathogen susceptibility  
Julia Seufer (Marburg/DE), Samuel Goll (Halle a. d. Saale/DE), Timo Engelsdorf, Alexander Frey, Lars Voll (Marburg/DE)
- P 245 Role of calcium signalling in chitosan-induced immune response in Arabidopsis mesophyll cells  
Pallegama Tennakoon, Rob Roelfsema (Würzburg/DE)
- P 247 The phytosulfokine pathway as a target for host immune suppression by the wheat pathogenic fungus Zymoseptoria tritici  
Maxim Faroux, Elisha Thynne, Eva Holtgrewe-Stukenbrock (Kiel, Plön/DE)
- P 249 The role of Nitrogen in the context of Vitis-Esca crosstalk  
Elnaz Zareei, Peter Nick (Karlsruhe/DE)
- P 251 Understanding the proteolytic processing of plant receptor like kinases  
Adithya Acharya, Mariana Schuster (Halle a. d. Saale/DE)
- P 253 Allelopathic interactions of Parthenium hysterophorus: Implications for the use of Parthenium hysterophorus as soil amendment to combat Macrophomina phaseolina causing charcoal rot in maize  
Muhammad Akbar, Nazir Aslam, Tayyaba Khalil (Gujrat/PK)
- P 255 Enhancing viticulture resilience against trunk diseases by harnessing Interkingdom crosstalks among grapevine, pathogenic endophytic fungi, and soil prokaryotes  
Islam Khattab (Eggenstein-Leopoldshafen/DE)
- P 257 Characterization of CPK Ti in signal propagation after wounding  
Sarah Lederer (Halle a. d. Saale/DE), Jennifer Bortlik (Berlin/DE), Vinzenz Handrick, Philipp Weckwerth, Susanne Matschi (Halle a. d. Saale/DE), Tina Romeis (Halle a. d. Saale, Berlin, Halle a. d. Saale/DE)
- PL 010 Investigating BaYMV isolates in Germany: analyzing the diversity of the VPg-regions  
Claudia J. Strauch, Petra Bauer, Annette Niehl (Braunschweig/DE)
- 17:15–18:45 **Beneficial plant-microbe interactions**
- P 259 Characterization of Plant Growth Promoting Abilities of Pseudomonas argentinensis SA190 Mutants Under Drought Stress  
Büdra Elkatmis (Köln/DE), Maged Saad (Thuwal/SA), Stanislav Kopriva (Köln/DE), Heribert Hirt (Thuwal/SA)
- P 261 Did barley-associated fungi evolve core effectors with antimicrobial activity to target barley keystone microbes?  
Martha Bauer, Fantin Mesny, Bart Thomma (Köln/DE)
- P 263 Establishing a molecular toolkit for the ectomycorrhizal symbiosis between Paxillus involutus and poplar  
Victoria Kreszies, Hendrik Reichelt, Samia Tasnim, Hanna Wege, Ines Teichert (Göttingen/DE)
- P 267 Inositol pyrophosphates - Master regulators of plant root endosymbioses  
Kiran Raj (Halle a. d. Saale/DE), Verena Gaugler (Bonn/DE), Maren Schädel (Halle a. d. Saale/DE), Henning J. Jessen (Freiburg i. Br./DE), Gabriel Schaaf (Bonn/DE), Martina K. Ried-Lasi (Halle a. d. Saale/DE)
- P 269 Interacting proteins of master regulators of arbuscular mycorrhiza development  
Yanina Rizzi (Potsdam-Golm, Freising/DE), Miriam Abele, Christina Ludwig (Freising/DE), Caroline Gutjahr (Potsdam-Golm, Freising/DE)
- P 271 Interactive Effects of Maize Roots and Rhizosphere Microorganisms Under Drought  
Henrike Würsig (Halle a. d. Saale/DE), Roman Hartwig (Stuttgart/DE), Michael Santangeli (Vienna/AT), Doris Vetterlein (Halle a. d. Saale/DE), Eva Oburger (Vienna/AT), Monika Wimmer (Stuttgart/DE), Mika T. Tarkka (Halle a. d. Saale, Leipzig/DE)
- P 273 LysM receptor function in successful ectomycorrhiza formation between poplar and Laccaria bicolor  
Stephanie Werner, Keziah Omenge (Quedlinburg/DE), Khira Deecke (Großhansdorf/DE), Thomas Irving (Madison, WI/US), Tomas Rush (Oak Ridge, TN/US), Carriel Cristobal (Madison, WI/US), Fort Sébastien (Grenoble/FR), Christine Hallmann (Halle a. d. Saale/DE), Matthias Fladung (Großhansdorf/DE), Jean-Michel Ané (Madison, WI/US)



- P 275 Molecular mechanism of PHO2 regulating arbuscular mycorrhiza development  
Ziming Ma, Caroline Gutjahr, Ivan F. Acosta (Potsdam/DE), Debatosh Das (München /DE), Jessica Alpers (Potsdam/DE)  
 Regina Hüttl (München /DE)
- P 277 Effectiveness of different doses of bacterial insecticide against *Trogoderma granarium* (Everts)  
 Fatima Houda Hallak (Aleppo/SY)
- P 279 Identifying the genetic components controlling root nodule symbiosis in *Phaseolus vulgaris* under phosphate deficient conditions  
Oswaldo Valdés-López (Tlalnepantla/MX), Mariel Carolina Isidra-Arellano (London/GB), Martina K. Ried-Lasi (Leipzig/DE)
- P 281 A survey of beneficial rhizobacteria for grain legumes from sub-saharan africa  
 Douglas Bruno Kagambo (Bremen/DE)
- P 283 Beneficial interaction between plant secondary indole metabolites and soil-microbiome  
Seungwoo Jeong (Bonn/DE), Vadim Schütz (Seoul/KR), Margot Schulz, Peter Dörmann (Bonn/DE)
- 17:15–18:45 **Plant Systematics, Evolution and Biodiversity**
- P 285 Diversity atlas of root system dynamics Inter- and intraspecific diversity of root growth and development dynamics  
 Markus Kuhlmann (Stadt Seeland/DE), Christiane Seiler (Stadt Seeland, Quedlinburg/DE), Salar Shaaf, Narendra Narisetti  
 Rongli Shi, Evgeny Gladilin, Andreas Börner (Stadt Seeland/DE), Gerd Bienert (Stadt Seeland, München /DE), Beate Fraust  
 Ricardo Giehl (Stadt Seeland/DE), Astrid Junker (Stadt Seeland, Halle a. d. Saale/DE), Dominic Knoch, Rhonda Meyer  
 Lars-Gernot Otto, Dennis Psaroudakis, Manuela Nagel, Kerstin Neumann, Evelin Willner, Nicolaus von Wirén, Jochen Reif  
 Thomas Altmann (Stadt Seeland/DE)
- P 287 Diversity of Hessian orchids, their artificial cultivation, culture and replanting - first results  
Julia Metzsch, Kardelen Cilgin, Volker Wissemann, Christina M. Müller (Gießen/DE)
- P 289 DNA markers targeting three cellular genomes for the discrimination between *Taxus baccata* *T. cuspidata* and *T. × media*  
Daniel Bross, Hilke Schröder (Großhansdorf/DE), Emilia Pers-Kamczyc (Kórnik/PL), Birgit Kersten (Großhansdorf/DE)
- P 291 Exploring the genetic adaptation of SnRK1 and sugar-signalling for the high light-induced stress response in streptophyte algae and early land plants.  
Christine Kühn, Andreas S. Richter (Rostock/DE)
- P 293 Genetic information for the conservation of segetal plants  
 Philipp Tran, Veit Herklotz (Görlitz/DE), Christiane M. Ritz (Görlitz, Leipzig/DE), Björn Usadel (Jülich/DE), Heiko Schmied  
 Laura Fortmann (Bonn/DE), Stefan Meyer (Görlitz, Göttingen/DE), Dörte Harpke (Gatersleben/DE)  
 Karsten Wesche (Görlitz, Leipzig/DE)
- P 295 Global genomic diversity of a South American oil-palm species: *Acrocomia totai* (Arecaceae)  
Francisco Agustin Vergara, Fei He (Bonn/DE), Jonathan Morales M. (Campinas/BR), Monica Moraes (La Paz/BO)  
 Diego Wassner (Buenos Aires/AR), Maria Zucchi (Piracicaba/BR), Annaliese Mason (Bonn/DE)
- P 297 Investigating Phenolics in Response to UV Exposure in the Zygnematophyceean Alga *Mesotaenium endlicherianum*  
Cäcilia Kunz, Janine Fürst-Jansen, Tatyana Darienko, Ilka N Abreu, Maike Lorenz, Jan de Vries (Göttingen/DE)
- P 299 Phylo- and cytogenetics unravel the complex evolutionary history of autumn-flowering Iberian crocuses  
Ruifang An, Dörte Harpke (Gatersleben/DE)
- P 303 Population genetics of *Crassula helmsii* (T. KIRK) COCKAYNE (Crassulaceae) within two regions of Germany  
Benjamin Feller, Christina M. Müller, Volker Wissemann (Gießen/DE)
- P 305 Revolver flowers – from morphology to pollinator behaviour  
 Julius Jeiter (Dresden/DE)

- P 307 Genetic diversity and structure of natural *Shorea robusta* populations in India as revealed by microsatellites markers  
Garima Mishra, Shailesh Pandey, Rama Kant, Rajendra K. Meena, Maneesh S. Bhandari (Dehradun/IN)
- P 309 The role of LEUNIG and SEUSS transcriptional regulators during land plant evolution  
Julian Vincent Garrecht (Gießen/DE), Julian Ingelfinger (Kaiserslautern/DE), Annette Becker (Gießen/DE)
- P 311 Cryptic diversity in the Prasiolaceae (Prasiolales, Trebouxiophyceae, Chlorophyta) revealed by classic isolation and cultivation  
Svenja Heesch (Rostock/DE)
- P 313 Plant species composition and spatial distribution patterns of taxonomic and phylogenetic heterogeneity along an altitudinal gradient in Sham Valley, Ladakh  
Deachen Angmo, Shalinder Kaur (Chandigarh/IR), Harminder Pal Singh (Chandigarh/IN)
- PL 012 Transgenerational plasticity in clonal plants is fitness relevant for *S. polyrhiza* and its herbivore the waterlily aphid.  
Alexandra Mireya Chávez Argandoña, Anne Schreyer, Meret Huber (Mainz/DE), Pauline Prüsener (Münster/DE)

## 17:15–18:45 Biotechnology and genome editing

- P 315 Genetic Engineering of Oats and Lupins  
Krishna Mohan Pathi, Thorben Sprink (Quedlinburg/DE)
- P 317 Water-based solvent-casted films with UV- blocking effect produced from the Antarctic microalga *Klebsormidium* sp. ASYA19  
Baris Ballik (Rostock/DE), Onur Aras, Turgay Çakmak, Murat Kazanci (Istanbul/TR), Klaus Herburger (Rostock/DE)
- P 321 Manipulation of flowering time in carrot by gene editing  
Huidong Liu, Stephen Jackson (Coventry/GB)
- P 323 Inducible CRISPR Cas9 System to study lethal knockouts in *Arabidopsis thaliana*  
Aron Struß (Dortmund/DE)
- P 325 Enhancing Tree Resilience to Climate Change: Biotechnological Strategies and Genome Editing  
Tobias Brueggemann, Alexander Fendel, Virginia Zahn, Matthias Fladung (Großhansdorf/DE)
- P 327 An efficient CRISPR-based method for mutational analyses of redundant gene functions in winter and spring rape seed  
Kea Ille, Siegbert Melzer (Kiel/DE)
- P 329 Engineering the Wheat and Barley Spike Architecture by Targeted Mutagenesis of the Transcription Factors Branched Head and Squamosa-promotor Binding Protein-like 14 and 17  
Christian Hertig, Cornelia Marthe (Gatersleben/DE), Astrid Junker (Bad Salzungen, Gatersleben/DE), Ravi Koppolu Thorsten Schnurbusch, Jochen Kumlehn (Gatersleben/DE)
- P 331 Investigating chloroplast development in barley via functional characterization of HvLST and HvCMF genes  
Srijan Jhingan, Robert E. Hoffie, Andrea Knospe, Sabine Sommerfeld, Jochen Kumlehn (Gatersleben/DE)  
Nils Stein (Gatersleben, Halle a. d. Saale/DE)

## 17:15–18:45 Crop biology and genetics

- P 333 Establishment of cost-efficient and sustainable cultivation for yam - Characterization of primary metabolites and Identification of genetic markers for breeding-relevant properties  
Julia Drotleff, Janina Epping (Münster/DE)
- P 335 Exploring the role of H4 acetylation in shaping patterns of recombination  
Maxie Sophie Seidel, Steven Dreissig (Halle a. d. Saale/DE)
- P 337 Impact of epigenetic and post-transcriptional gene regulation on rubber biosynthesis in dandelion  
Tobias Poloczek, Jakob Wiemann, Nicole van Dam, Christian Schulze Gronover, Dirk Prüfer (Münster/DE)

- P 339 Phenotypic and transcriptional outputs of an ancestral wheat allele on spike morphogenesis  
Akshay Tawale, Ragavendran Abbai (Gatersleben/DE), Thorsten Schnurbusch (Halle a. d. Saale, Gatersleben/DE)  
 Guy Golan (Gatersleben/DE)
- P 341 A genome-wide 5-methyl cytosine reference atlas of a sugar beet genotype  
 Muriel Wulfhorst, Katharina Sielemann (Bielefeld/DE), Nicola Schmidt, Ludwig Mann (Dresden/DE)  
 Vinicius Vilperte (Einbeck/DE), Prisca Viehöver, Bernd Weisshaar (Bielefeld/DE), Britta Schulz (Einbeck/DE),  
 Tony Heitkam (Dresden/DE, Graz/AT), Daniela Holtgräwe (Bielefeld/DE)
- P 343 Elucidating gene regulatory networks in crops using DAP-seq  
 Isabel Mora-Ramirez, Kexin Liu, Keerthana Nagesh, Axel Himmelbach, Thorsten Schnurbusch  
 Jozefus Schippers (Gatersleben/DE)
- 17:15–18:45 **Applied botany for food security**
- P 345 Allelopathic activity of *Euphorbia hirta* against *Avena fatua* and *Rumex dentatus* and identification of potential allelochemicals  
 Muhammad Akbar, Taqdees Taqdees, Tayyaba Khalil (Gujrat/PK)
- P 349 Optimization of natural deep eutectic solvent-based extraction of carotenoids for diverse applications in food  
 Nithya N. Kuttu, Sudha Dhumane (Pune/IN)
- P 351 Supplemental Irrigation – A Critical Strategy for Boosting Crop Productivity and Starch Functionality  
 Wenxin Liang (Rostock/DE, Copenhagen/DK, Yangling/CN), Klaus Herburger (Rostock/DE), Yuyue Zhong (Potsdam/DE)  
 Andreas Blennow (Kopenhagen/DK), Yuncheng Liao, Xiaoxia Wen, Yang Liu (Yangling/CN)
- 17:15–18:45 **Biology of algae and cyanobacteria**
- P 353 Establishing a Minimal Endosymbiotic Metabolism in Cyanobacteria  
 Jan Hofer (Düsseldorf/DE), Tim Schulze (Bielefeld/DE), Lennart Witting, Dietrich Kohlheyer (Jülich/DE)  
 Marion Eisenhut (Bielefeld/DE), Andreas Weber (Düsseldorf/DE)
- P 355 From cyanobacteria to cell organelle - Engineering and studying a synthetic cyanobacterial endosymbiont  
 Tim Schulze (Bielefeld/DE), Jan Hofer (Düsseldorf/DE), Lennart Witting (Jülich/DE), Jeannine Volke (Düsseldorf/DE)  
 Dietrich Kohlheyer (Jülich/DE), Andreas Weber (Düsseldorf/DE), Marion Eisenhut (Bielefeld/DE)
- P 357 Heterologous Lhcx expression in the diatom *Phaeodactylum tricornutum*  
 Marie Alice Wünsch (Rostock/DE), Jochen M. Buck, Peter Kroth (Konstanz/DE), Bernard Lepetit (Rostock/DE)
- P 359 Identification of genes involved in silica biomineralization in *Stramenopile*  
 Donat Wulf, Flora Wang, Cailyn Sakurai, Setsuko Wakao, Krishna K Niyogi (Berkeley, CA/US)
- P 361 Influence of light on the interaction between *Chlamydomonas reinhardtii* and *Pseudomonas protegens*  
 Alissa Dierberger, Magdalena Rose, Raimund Nagel, Torsten Jakob, Severin Sasso (Leipzig/DE)
- P 363 Rare red bloom on a permanent snow field in Iceland: Ecophysiology of *Rosetta* sp.  
 (Chlamydomonadales, Chlorophyceae)  
 Daniel Remias (Salzburg/AT), Lenka Procházková (Praha/CZ), Sven Gindorf (Salzburg/AT)
- P 365 Cell Wall Remodeling in the Green Macroalgae *Ulva* in Response to Environmental Input  
 Yunyun Pan, Klaus Herburger (Rostock/DE)
- PL 011 Growth strategies of *Chlorella vulgaris* in seawater for the production of biomass and lipids suitable for biodiesel  
 Ralf Rautenberger, Kari Skjånes (Ås/NO), Alexandre Détain, Peter S.C. Schulze, Viswanath Kiron,  
 Daniela Morales-Sánchez (Bodø/NO)

## 17:15–18:45 Molecular evolution of plant form and function

- P 367 Evolutionary “Hotspots” in Flavonoid Biosynthesis  
Maria Fernanda Marin-Recinos, Boas Pucker (Braunschweig/DE)
- P 369 Insights into the DFG priority programme MAdLand  
Janine Fürst-Jansen (Göttingen/DE), SPP 2237 „MAdLand“ SPP 2237 „MAdLand“ (Deutschland/DE)
- P 371 Terraphos: Exploring the Evolution of Phosphate Scouting During Plant Terrestrialization  
Carolin Apel, Christin Naumann, Steffen Abel (Halle a. d. Saale/DE)
- P 373 The model organisms *Anthoceros agrestis* and *Physcomitrium patens*: Insights into their cell walls and arabinogalactan-proteins  
Kim-Kristine Mueller (Kiel/DE), Linus Wegner, Katrin Ehlers (Gießen/DE), Lukas Pfeifer, Birgit Classen (Kiel/DE)
- P 375 The role of trehalose 6-phosphate and TREHALOSE-6-PHOSPHATE SYNTASE in the evolution of land plants  
Hannah Lepper (Düsseldorf/DE), Celment Champion, Yoan Coudert (Lyon/FR), Franziska Fichtner (Düsseldorf/DE)
- P 377 Uncovering the Evolution of Anthocyanin-Related Glutathione-S-Transferases  
Milan Borchert, Boas Pucker (Braunschweig/DE)
- P 381 Developmental modifications of plasmodesmata in non-seed plants  
Katrin Ehlers, Linus Wegner (Gießen/DE)
- PL 013 *Spirogyra pratensis*: functional genomics of a filamentous algal sister to land plants  
Elisa Goldbecker, Tatyana Darienko, Spirogyra Genome Consortium, Jan de Vries (Göttingen/DE),  
Anja Holzhausen (Halle a. d. Saale/DE), Deepti Varshney, Stefan Rensing (Freiburg/DE), André Marques (Cologne/DE),  
 Klaus von Schwartzberg (Hamburg/DE)

## 17:15–18:45 Plant functional diversity in a changing world

- P 383 Plant defense against pests under Arctic light conditions  
Axel Mithöfer, Clabe Wekesa, Rayko Halitschke (Jena/DE), Laura Jaakola (Tromsø/NO)
- P 385 Gene expression profile of the herbivore-induced stress responses in *Quercus robur*  
Hilke Schroeder, Malte Mader, Franziska Orgel (Großhansdorf/DE), Tetyana Nosenko, Jörg-Peter Schnitzler (München/DE)  
 Birgit Kersten (Großhansdorf/DE)
- P 387 The adaptive potential of the leaf economics spectrum in the Brassicaceae – Role of photosynthetic carbon assimilation biochemistry in adaptation to urban environments  
Tim Blank, Urte Schlüter, Andreas Weber (Düsseldorf/DE)
- P 389 Warming and zooplankton grazing alter the composition and size-structure of a dinoflagellate dominated community of the Central Baltic Sea  
Carolin Paul, Jörg Dutz, Anke Kremp (Rostock/DE)
- P 391 Exploring the role of polysaccharide root exudates and biochar in the aggregation of coarse- and fine textured arable soils  
 O. A. Leal, M. Sader, G. Salahee, D. Drobiez, N. Siebers, N. Brüggemann, A. Kuhn, H. Klose, M. Wrona  
Silvia D. Schrey (Jülich/DE)

## 17:30–19:00 Photosynthesis and general metabolism

- P 002 How do plants overcome the saturation of sugars due to the restricted sucrose transport in leaves?  
Satoru Naganawa-Kinoshita (Münster/DE), Kyomi Taki (Nagoya/JP), Takamasa Suzuki (Kasugai/JP)  
Toshinori Kinoshita (Nagoya/JP), Iris Finkemeier (Münster/DE)
- P 004 Identification and 3D modeling of gene regulatory networks that determine leaf anatomy and physiology in C3-C4 intermediate Brassicaceae  
José Miguel Valderrama-Martín (Düsseldorf/DE), Michael Melzer (Gatersleben/DE), Urte Schlüter  
Andreas Weber (Düsseldorf/DE)
- P 006 Localization and Dynamics of the Thylakoid Ascorbate Peroxidase (tAPX) in the Thylakoid Membrane of *Arabidopsis thaliana*  
Heni Hitaj, Elena Reifschneider (Berlin/DE), Sara Stolze, Hirofumi Nakagami (Köln/DE), Margarete Baier (Berlin/DE)
- P 008 Regulation of mitochondrial malate dehydrogenase by protein modifications and interacting proteins  
Yannik Stichweh, Florian Kotnik, Jürgen Eirich, Jonas Giese, Ke Zheng, Markus Schwarzländer (Münster/DE)  
Maria del Pilar Martinez, Veronica Graciela Maurino-Larcher (Bonn/DE), Iris Finkemeier (Münster/DE)
- P 010 Shedding light on protein acetylation in chloroplasts - A new family of plastid-localized acetyltransferases  
Annika Brünje, Jürgen Eirich (Münster/DE), Jean-Baptiste Boyer (Paris/FR), Paulina Heinkow (Münster/DE)  
Ulla Neumann (Köln/DE), Minna Konert, Aiste Ivanauskaite (Turku/FI), Julian Seidel (Tübingen/DE), Shin-Ichiro Ozawa  
Wataru Sakamoto (Kurashiki/JP), Thierry Meinel (Paris/FR), Dirk Schwarzer (Tübingen/DE), Paula Mulo (Turku/FI)  
Carmela Giglione (Paris/FR), Iris Finkemeier (Münster/DE)
- P 012 The function of N-terminal acetylation of plastid precursor proteins  
Pia Möllenbeck (Bochum/DE), Julia Grimmer (Halle a. d. Saale/DE), Dominique Sebastian Stolle  
Sacha Baginsky (Bochum/DE)
- P 016 Towards fluorescent protein-based biosensing of thylakoid lumen pH  
Minh Thi Thanh Hoang, Ana Paula Cislighi, Ke Zheng, Karin Busch, Markus Schwarzländer (Münster/DE)
- P 018 GL11 is a novel chloroplastic protein involved in photoprotection  
Manuel Balparda, Jonas Matteo Böhm, Yaroslav Zaplatnikov, Jana Ellieroth, Veronica Graciela Maurino-Larcher (Bonn/DE)
- P 020 Light changes promote distinct responses of plastid protein acetylation marks in the model plant *Arabidopsis thaliana*  
Jürgen Eirich (Münster/DE)
- P 022 Light changes promote distinct responses of plastid protein acetylation marks  
Jürgen Eirich, Iris Finkemeier (Münster/DE)
- P 024 Climate change, Photosynthesis and Advanced biofuels  
Ashwani Kumar (Jaipur/IN)
- P 026 First insights into alternative trait combinations of desiccation tolerant plants  
Elisabeth Zokov, Luiz Bondi, Julius Köhler, Stefan Porembski (Rostock/DE)

## 17:30–19:00 Specialized metabolism

- P 028 *Sarcandra glabra*: characterization of BAHD hydroxycinnamoyltransferases and phenolic acids  
Paul Bömeke, Maike Petersen (Marburg/DE)
- P 030 Oil Body Lipase-type Triacylglycerol Lipases Contribute to the Production of Fatty Acid-derived Volatiles-  
 Viacheslav Lebedev, Martin Bonin (Münster/DE), Patricia Scholz, Anna Müller, Maurice Hädrich (Göttingen/DE)  
 Jandirk Sendker (Münster/DE), Dorothee Staiger (Bielefeld/DE), Till Ischebeck (Münster/DE)
- P 032 Molecular basis of phytochelatin synthase function in pathogen-triggered indole glucosinolate metabolism in the  
 Brassicaceae family  
Justyna Lalak-Kańczugowska, Mariola Piślewska-Bednarek, Sylwia Bugaj, Paweł Bednarek (Poznan/PL)
- P 034 The GAMEs cluster and specialized metabolism of the bittersweet nightshade *Solanum dulcamara* by genomic and  
 transcriptomic analyses  
Bianca Laker (Bielefeld/DE), Redouan Adam Anaia (Jena, Leipzig/DE), Donat Wulf (Bielefeld/DE)  
 Nicole van Dam (Jena, Leipzig, Großbeeren/DE), Andrea Bräutigam (Bielefeld/DE)
- P 036 The role of acetohydroxyacid synthase in the biosynthesis of pyrrolizidine alkaloids  
Marco Ebeling, Annika Engelhardt, Dietrich Ober (Kiel/DE)
- P 038 Unraveling the volatile vocabulary of maize – Characterization of two terpene synthases involved in maize volatile production  
Sarah-Maria Riedl, Claudia Schaff, Jörg Degenhardt (Halle a. d. Saale/DE)
- P 040 Cytochrome P450 dependent hydroxylation in the biosynthesis of phenolic compounds in *Anthoceros agrestis*,  
*Marchantia polymorpha*, *Physcomitrium patens* and *Chara braunii*  
Christoph Kentrath, Maike Petersen (Marburg/DE)
- P 042 Disrupted Steryl Ester Biosynthesis Alters Metabolome and Impacts Phenotypes in Tomato Seeds and Fruits  
Natalie Laibach (Kleve/DE), Joan Manel Lopez-Tubau, Alma Burciaga-Monge (Barcelona/ES)  
 Saleh Alseekh (Potsdam/DE, Plovdiv/BG), Cuiyun Deng (Barcelona/ES), Alisdair R. Fernie (Potsdam/DE, Plovdiv/BG)  
 Albert Ferrer, Teresa Altabella (Barcelona/ES)
- P 044 Functional analysis of the cytochrome P450 71B subfamily in *Arabidopsis thaliana*  
 Lauren Nicol (Straubing/DE), Teresa Müller (Freising/DE), Christoph Böttcher (Berlin/DE), Erich Glawischnig (Straubing/DE)
- P 046 Chasing histochemical and ultrastructural changes in petal tissues to provide a better understanding of scent volatiles  
 synthesis and emission in tropical flowers  
 Adinpunya Mitra (Kharagpur/IN)
- P 048 *Coffea canephora* Kaurene Hydroxylases Catalyze Key Steps to Cafestol Biosynthesis  
Maximilian Frey (Halle a. d. Saale/DE), Gaëlle Antoine (Montpellier/FR, Denton, TX/US)  
 Ulschan Bathe (Gainesville, FL/US), Rebecca Falke, Luca Meink (Halle a. d. Saale/DE), Oliver Frank  
 Corinna Dawid (Freising/DE), Ahmed Hassanin (Halle a. d. Saale/DE, Assiut/EG), Mehdi D. Davari (Halle a. d. Saale/DE)  
 Thierry Joet, Stéphane Dussert (Montpellier/FR), Alain Tissier (Halle a. d. Saale/DE)
- P 050 The 3D chromosomal organisation of plant biosynthetic gene clusters  
Hans-Wilhelm Nützmann, George Jervis (Exeter/GB), Jade Bishop, Jessica Taylor (Bath/GB), Daniel Doerr (Düsseldorf/DE)  
 Selene Fernández Valverde (Sydney/AU), Eva Wegel (Norwich/GB), Marco Di Stefano (Montpellier/FR)  
 Anne Osbourn (Norwich/GB)
- P 052 How plants eliminate an apparent toxic by-product - Identification and functional characterization of the C4-ketone products  
 of homoterpene biosynthesis in *Zea mays*  
Christina Marie Jochimsen, Claudia Schaff, Jörg Degenhardt (Halle a. d. Saale/DE)

P 054 Prediction and Validation of Terpene Synthase Functions in *Tanacetum vulgare*  
Marvin Hildebrandt, Bianca Laker, Marion Eisenhut, Sebastian Tschikin, Dominik Ziája, Ruth Jakobs, Caroline Müller  
 Andrea Bräutigam (Bielefeld/DE)

P 056 <sup>13</sup>C-Metabolic flux analysis: A powerful approach to quantify light-regulated crosstalk between Isoprenoid Pathways  
 Somnath Koley (Halle a. d. Saale/DE, Saint Louis, MO/US), Eva Grafarend Belau (Halle a. d. Saale, Gatersleben/DE)  
 Manish Raorane, Reza Chamansara, Björn H. Junker (Halle a. d. Saale/DE)

P 058 Unraveling Tropane Alkaloid Biosynthesis in *Erythroxylum coca*  
Benjamin Chavez (Gatersleben/DE), Prashanth Srinivasan, Christina Smolke (Stanford, CA/US)  
 John D'Auria (Gatersleben/DE)

P 060 A microRNA Pair Controls Colour Change in Developing Eggplant Fruit Skin  
Sayantana Panda (Halle a. d. Saale/DE), Asaph Aharoni (Rehovot/IL)

### 17:30–19:00 Gene regulation

P 062 Alternative splicing of RNA binding proteins from the RBP45 group is controlled by a structured mRNA motif  
Maren Reinhardt (Mainz/DE), Maximilian Sack, Zasha Weinberg (Leipzig/DE), Andreas Wachter (Mainz/DE)

P 064 Natural variation in gene regulatory networks  
 Arthur Korte (Würzburg/DE)

P 066 Cold acclimation of RuBisCo expression  
 Florian Rösch, Julia Legen, Benjamin Lenzen, Christian Schmitz-Linneweber (Berlin/DE)

P 068 The JA induced emission of volatiles in herbivore attacked maize is negatively influenced by a bHLH transcription factor *tf23*  
Claudia Schaff, Benedikt Athmer, Jörg Degenhardt (Halle a. d. Saale/DE)

P 070 Unraveling Maize's Defensive Arsenal: Insights into the Regulation of Volatile Terpene Biosynthesis  
Janik Telleria Marloth (Halle a. d. Saale/DE), Annett Richter (Ithaca, NY/US), Claudia Schaff  
 Jörg Degenhardt (Halle a. d. Saale/DE)

P 072 Whirly proteins regulating stress-related reprogramming of nuclear gene expression via epigenetic control  
Ivana Mladenovic, Minh Bui Manh, Klaus Humbeck, Linh Thuy Nguyen (Halle a. d. Saale/DE)

P 074 Regulation of betalain biosynthesis by a MYB transcription factor  
Susanne Katja Vollmer (Düsseldorf/DE), Tom S. Winkler (Köln/DE), Götz Hensel (Düsseldorf/DE), Markus Stetter (Köln/DE)

P 076 Activating cis regulatory elements from the histone genes of green algae and angiosperms: from characterization to application  
Yuliia Lihanova, Raimund Nagel, Torsten Jakob, Severin Sasso (Leipzig/DE)

P 078 Understanding mRNA half-lives and turnover dynamics in plants  
Sandra Schüller, Joachim Weber (Halle a. d. Saale/DE), Christian Schmitz-Linneweber (Berlin/DE)  
 Sascha Laubinger (Halle a. d. Saale/DE)

P 080 "Enhanced Plant Growth and Stress Tolerance via miR156a Overexpression from chickpea: Insights into miR156-SPL Regulatory Network in Arabidopsis"  
 Gopal Kalwan (Delhi/IN)



## POSTERS EVEN | TUESDAY, 17 SEPTEMBER

### 17:30–19:00 Plant proteins: structure to function

- P 082 Characterization of the plastidial acetyltransferase GNAT3 in *Arabidopsis thaliana*  
Marie-Christin Stenkamp, Felix Bösing, Annika Brünje, Jens Mühlenbeck, Iris Finkemeier (Münster/DE)
- P 084 *In vitro* reconstitution of the cytosolic redox regulatory network revealed dynamics of H<sub>2</sub>O<sub>2</sub> detoxification  
Lara Vogelsang, Karl-Josef Dietz (Bielefeld/DE), Jürgen Eirich, Iris Finkemeier (Münster/DE)
- P 086 Chloroplast Envelope Metabolite Channels in Lipid Remodelling and Photosynthetic Efficiency Under Cold Stress  
Wing Tung Lo (München /DE), Cecilia Tullberg (München /DE, Lund/SE), Franz Hagn, Tatjana Kleine, Martin Lehmann, Dario Leister, Serena Schwenkert (München /DE)
- P 088 Cooperative reaction kinetics of *Arabidopsis* phosphatidylinositol 4-phosphate 5-kinase 6 (PIP5K6) mediated by allosteric PtdIns4P-binding  
Johanna Carola Nordmeier, Ralph Golbik, Alexandra Schutkowski, Ingo Heilmann (Halle a. d. Saale/DE)
- P 090 Occupancy of lysine acetylation in *Arabidopsis* proteome via chemical labelling and mass spectrometry measurements  
Jonas Mussenbrock, Jürgen Eirich, Iris Finkemeier (Münster/DE)
- P 092 Prioritization of abiotic and biotic plant stress responses by a phosphatase and calcium-dependent protein kinase switch  
Heike Seybold, Jennifer Bortlik (Berlin/DE), Xiyuan Jiang, Anja Liese (Halle a. d. Saale/DE), Benjamin Conrads (Berlin/DE), Wolfgang Hoehenwarter, Susanne Matschi (Halle a. d. Saale/DE), Tina Romeis (Halle a. d. Saale, Berlin/DE)
- P 094 Revealing the Regulatory Network of KEA3 in Photosynthesis: Focus on the interactions of C-terminal Domain with pH and nucleotides  
Sarah Käbe (Düsseldorf/DE), Michał Uflewski (Potsdam/DE), Tobias Rindfleisch (Potsdam/DE; Bergen/NO), Ute Armbruster (Düsseldorf/DE, Bergen/NO)
- P 096 Squalene Cyclases in non-seed plants  
Felix Heumesser, Magdiel Lim Sheng Satha (Münster/DE), Jan de Vries (Göttingen/DE), Till Ischebeck (Münster/DE)
- P 098 The enigma of respiratory supercomplexes in plant mitochondria  
Helene Röhricht, Etienne H. Meyer (Halle a. d. Saale/DE)
- P 100 Unraveling the role of subunit e of the plant mitochondria ATP synthase  
Julianna Xavier de Brito Silva, Tim Kiesel, Etienne H. Meyer (Halle a. d. Saale/DE)
- P 102 Kinetic analysis of *Arabidopsis* PI4Kβ1 and effects of phosphorylation by mitogen-activated protein kinases  
Angela Liliana Meza Lopez, Mareike Heilmann, Ingo Heilmann (Halle a. d. Saale/DE)
- P 104 The development of untargeted peptide and FTIR approaches for identifying the geographic origins of pomelos  
Wararat Sriprapat (Pathumthani/TH), Suthathip Kittisenachai (Pathumthani/DE), Warakorn Ruankaew (Chai Nat/TH), Sittiruk Roytrakul (Pathumthani/DE)
- P 106 Barley panproteome: analyzing its nuances through in silico structural biology  
Victor Henrique Rabesquine Nogueira, Amanda Souza Câmara (Seeland / OT Gatersleben/DE)

### 17:30–19:00 Cell biology

- P 108 Evaluating the physiological role of plastid nucleus interaction: plastid to nuclear H<sub>2</sub>O<sub>2</sub> transport  
Chaima Guizani, Martin Schattat, Valeria Karsten (Halle a. d. Saale/DE)
- P 110 Modulating membrane lipid composition influences cellulose deposition in *Arabidopsis thaliana*  
Irene Stenzel, Gerd Hause, Stephanie Krüger, Ingo Heilmann (Halle a. d. Saale/DE)
- P 112 The role of regulatory lipids during the initiation of endocytosis at the plant plasma membrane  
Johanna Uhlenberg, Mareike Heilmann, Ingo Heilmann (Halle a. d. Saale/DE)



- P 114 Actin-plasma membrane-contacts mediated by the class VIII myosin ATM2 enable a self-reinforcing loop of binding and promoting  $\text{PtdIns}(4,5)\text{P}_2$ , controlling actin dynamics in pollen tubes  
Vera Wagner, Marta Fratini, Christoph Kastner, Alexandra Schutkowski, Mareike Heilmann  
 Ingo Heilmann (Halle a. d. Saale/DE)
- P 116 Meiotic temperature resilience in wild barley  
Yixuan Gao, Steven Dreissig (Halle a. d. Saale/DE)
- P 118 The mechanism of PEP7 maturation in SIRK1 signaling pathway  
Hannah Kuhn, Lin Xi, Xuna Wu, Waltraud Schulze (Stuttgart/DE)
- P 120 Calcium transporters in the endoplasmic reticulum determine plant development, fertility, and nutritional responses  
Oriana Mariani (Halle a. d. Saale/DE), Matteo Grenzi (Milan/IT), Christin Naumann, Anja Janssen  
 Steffen Abel (Halle a. d. Saale/DE), Alex Costa (Milan/IT), Bastian Meier, Edgar Peiter (Halle a. d. Saale/DE)
- P 122 Decoding the Role of Plastid  $\text{Ca}^{2+}$  Signaling in Plant Defense  
Susanne Mühlbauer, Inês Nunes, Lorenz Holzner, Carsten Völkner, Benjamin Brandt (Planegg-Martinsried/DE)  
 Dawid Jaslan, Christian Grimm (München/DE), Constance Tisserant, Silke Robatzek (Planegg-Martinsried/DE)  
 Bettina Hause (Halle a. d. Saale/DE), Sanja Zenker, Andrea Bräutigam (Bielefeld/DE)  
 Hans-Henning Kunz (Planegg-Martinsried/DE)
- 17:30–19:00 **Communication and dynamics of plant organelles**
- P 124 Molecular Characterization of the Activation Mechanism of the Small GTPase, Arabidopsis Immune- Associated Nucleotide-Binding Protein 12 (AtIAN12), and the Regulation of its Targeting to the Membrane of ER Bodies  
 Saugat Pokhrel (Hamburg/DE, San Francisco, CA/US), Christian Falter, Silja Seemann, Fatemeh Mousavimehr  
 Sigrun Reumann (Hamburg/DE)
- P 126 Conquering new grounds: Plant organellar C-to-U RNA editing factors can be functional in the plant cytosol  
 Mirjam Thielen, Béla Gärtner, Volker Knoop, Mareike Schallenberg-Rüdinger (Bonn/DE)  
Elena Lesch (Münster, Bonn/DE)
- P 128 Regulation of calcium homeostasis in the Golgi apparatus of Arabidopsis thaliana  
 Nico Rössner (Halle a. d. Saale/DE), Sara Morghen (Trondheim/NO), Franziska Daamen (Halle a. d. Saale/DE)  
 Jie He (Yangzhou/CN), Oriana Mariani, Bastian Meier, Edgar Peiter (Halle a. d. Saale/DE)
- P 130 Regulation of plant immunity through (sub)compartmentalization of the RNA granule protein TZF9  
Anindita Karkhanis, Susanne Matschi, Justin Lee (Halle a. d. Saale/DE)
- P 132 The PPR protein PPR596 is important for splicing of nad2 and assembly of Complex I in mitochondria of Arabidopsis thaliana  
Leonora Peters, Kristina Kühn (Halle a. d. Saale/DE)
- P 134 Towards the role of dual or even triple organellar localization of Whirly proteins in plant cells  
Nicolas Kistner, Ralf Bernd Klösigen, Bationa Bennewitz (Halle a. d. Saale/DE)
- P 136 Nuclear/cytoplasmic distribution and posttranslational regulation of the Arabidopsis PI4P 5-kinase PIP5K1  
Marie Lebescond, Lennart Schwalgun, Mareike Heilmann (Halle a. d. Saale/DE)

## 17:30–19:00 Molecular mechanisms of plant nutrition

- P 138 Possible involvement of a ribosomal protein ( $\mu$ L13x) in growth under low calcium conditions in *Ara bidopsis thaliana*  
Arpna Kumari, Hirofumi Fukuda, Naoyuki Sotta, Dichao Ma, Toru Fujiwara (Bunkyo/JP)
- P 140 The ancestral UPF0016 proteins Hmx1 and Hmx2 enable efficient manganese uptake in cyanobacteria  
 Mara Reis (Bielefeld/DE), Fabian Brandenburg (Leipzig/DE), Michael Knopp, Sven B. Gould (Düsseldorf/DE)  
Marion Eisenhut (Bielefeld/DE)
- P 142 The calcium-nitrogen-interaction on barley root metabolism and exudation  
Ibadete Denjali (Bielefeld/DE), Refat Abdel-Basset (Assiut/EG), Marcus Persicke, Thorsten Seidel, Vijay Kumar  
 Karl-Josef Dietz (Bielefeld/DE)
- P 144 New insights into the pH-dependent reciprocity of NRTs and AMTs during N uptake balance in *Ara bidopsis*  
Mikel Rivero-Marcos, Nicolaus von Wirén (Gatersleben/DE)
- P 146 Biofortification with Vitamin B12 in Lettuce improves light use efficiency under high light  
Katharina Huntenburg, Milan Vlaming, Charalampos Nikolopoulos, Leo Marcelis (Wageningen/NL)
- P 148 Cytochrome P450 enzyme CYP716A is a gatekeeper of bitter and hemolytic oleanolic acid biosynthesis in  
*Chenopodium quinoa*  
Pravesh Kundu, Gaurav Zinta (Palampur/IN)
- P 150 Host-dependent physiological and genetic modulations in Vampire Weed (*Rhamphicarpa fistulosa*, Orobanchaceae):  
 A Parasitic Generalist  
Olivier Dayou, Guillaume Brun, Hildah K. Kithinji, Leo Botton-Divet, Thomas Stach, Susann Wicke (Berlin/DE)
- P 152 From root to shoot: uptake, translocation, distribution and speciation of Eu(III) in hydroponically grown plants  
 Max Klotzsche, Robin Steudtner, Manja Vogel, Björn Drobot (Dresden/DE), Stefan Schymura (Grimma/DE)  
 Thorsten Stumpf (Dresden/DE)
- P 154 A causal study of the stimulating effect of phosphite ( $\text{PO}_3^{3-}$ ) on plants  
Wang Yiming, Zheng Zhou, Xu Sicheng, Klink Holger, Verreet Joseph-Alexander, Cai Daguang (Kiel/DE)
- P 156 Early nutrition strategies of parasitic *Cuscuta* spp.  
 Maleen Hartenstein, Dominik Kischka, Ruth Stadler, Markus Albert (Erlangen/DE)
- P 158 The Role of Endomembrane Potassium Transporters in Plant Abiotic Stress Tolerance: Combining Knowledge from  
 Soybean and *Arabidopsis*  
 Jennifer Hamacher, Bettina Becker (Bonn/DE), Yue Qu, Matthew Gilliam (Glen Osmond/AU), Stefanie Wege (Bonn/DE)

## 17:30–19:00 Plant development

- P 160 The potential green light receptor GRS affects plant development  
Susann Frank, Hasan Tekin, Ute Vothknecht (Bonn/DE)
- P 162 The tissue-specific functions of trehalose 6-phosphate in coordinating plant metabolism with development  
Lucas Müller (Düsseldorf/DE), Maria Grazia Annunziata, John Lunn (Potsdam-Golm/DE), Oliver Ebenhöf  
 Franziska Fichtner (Düsseldorf/DE)
- P 164 The vascular lay-out of the barley rachis: implications for transport and spikelet connection  
Twan Rutten (Seeland/OT Gatersleben/DE), Venkatasubbu Thirulogachandar (Düsseldorf/DE)  
 Yongyu Huang (Seeland/OT Gatersleben/DE), Nandhakumar Shanmugaraj (New York, NY/US), Ravi Koppolu  
 Stefan Ortleb (Seeland/OT Gatersleben/DE), Götz Hensel (Düsseldorf/DE), Jochen Kumlehn, Michael Melzer  
 Thorsten Schnurbusch (Seeland/OT Gatersleben/DE)

P 166 Tissue-specific profiling of Arabidopsis Zn<sup>2+</sup>-dependent histone deacetylases revealed that histone deacetylase 8 is a class II HDAC in roots  
Florian Kotnik, Priyadarshini Tilak, Michael Gasper, Jürgen Eirich (Münster/DE), Judit Kovacs (Vienna/AT)  
 Julian Seidel (Tübingen, Jena/DE), Claude Becker (Vienna/AT, München /DE), Dirk Schwarzer (Tübingen/DE)  
 Iris Finkemeier (Münster/DE)

P 168 Genetic and molecular mechanisms of root-mediated nitrate use efficiency in wheat and barley  
Md. Nurealam Siddiqui, Jens Léon, Agim Ballvora (Bonn/DE)

P 170 Rooting for *Lotus japonicus* WHILRY2: a new player in root development  
Thuy Linh Nguyen, Elena Roitsch, Klaus Humbeck (Halle a. d. Saale/DE)

P 172 Unveiling the role of Trehalose 6-phosphate in root development  
Moritz Göbel (Düsseldorf/DE), Jacqueline Foster (Brisbane/AU), Franziska Fichtner (Düsseldorf/DE; Brisbane/AU)

P 174 Dual function of HvSWEET11b in transporting sugars and cytokinins governs the grain filling in barley  
Volodymyr Radchuk (Gatersleben/DE), Zeinu M. Belew (Frederiksberg C/DK), Andre Gündel (Gatersleben/DE)  
 Simon Mayer (Würzburg, Gatersleben/DE), Götz Hensel (Düsseldorf/DE), Hardy Rolletschek (Gatersleben/DE)  
 Hussam H. Nour-Eldin (Frederiksberg C/DK), Ljudmilla Borisjuk (Gatersleben/DE)

P 176 Type-B response regulators (RRs) direct initial steps of barley endosperm differentiation  
 Christian Hertig, Isabel Mora-Ramirez, Twan Rutten, Jochen Kumlehn, Michael Melzer, Jozefus Schippers  
Johannes Thiel (Gatersleben/DE)

P 178 Impact of FLC genes on flowering time in spring oilseed rape  
Sarah Duveneck, Siegbert Melzer (Kiel/DE)

P 180 Plastid development and proteomics: potentials and challenges: A review  
 Ashwani Kumar (Jaipur/IN)

#### 17:30–19:00 Plant hormones

P 182 Multiple paclobutrazol and nitrogen treatments increase potato tuber formation  
Syariful Mubarak, Jajang Sauman Hamdai, Anne Nuraini, Kusumiyati Kusumiyati (Sumedang/ID)

P 184 Arabidopsis NADP-malic enzyme 1 involved in abscisic acid response during drought stress and seed development  
Ying Fu, Maximilian Klamke, Sreeranjini Rameshkumar, Veronica Graciela Maurino-Larcher (Bonn/DE)

P 188 Towards a better understanding of ethylene metabolism using a novel UPLC-MS/MS based quantification method  
Thomas Depaepe, Da Cao, Raul Sanchez-Muñoz, Hilde Janssens (Gent/BE), Filip Lemièrre, Tim Willems (Antwerp/BE)  
 Johan Winne (Gent/BE), Els Prinsen (Antwerp/BE), Dominique Van Der Straeten (Gent/BE)

P 190 ABA-signaling differs between the extremophyte *Eutrema salsugineum* and its close glycophytic relative *Arabidopsis thaliana*  
Lina Spiller, Sarah Endres, Christoph Weiste, Rainer Hedrich, Dietmar Geiger (Würzburg/DE)

## 17:30–19:00 Navigating abiotic challenges

- P 192 Unraveling Photoprotective Mechanisms: Insights from Green Algae to Vascular Plants  
Petra Redekop (Berlin/DE), Emanuel Sanz-Luque (Cordoba/ES), Arthur Grossman (Stanford, CA/US)  
 Margarete Baier (Berlin/DE; Stanford, CA/US)
- P 194 When Temperatures Rise: Exploring Root Thermomorphogenesis Through Single Cell Transcriptomics  
Tilman Jacob, Orlando Maciel Rodrigues Jr., Haiyue Ai, Bettina Hause, Lennart Eschen-Lippold, Carolin Delker  
 Marcel Quint (Halle a. d. Saale/DE)
- P 200 A New Player in Nitric Oxide Modulation and Hypoxia Response: Amidoxime Reducing Component (ARC)  
Vajiheh Safavi-Rizi, Felix Lutter, Marco Herde, Kathrin Hölscher, Christine Stöhr, Claus-Peter Witte (Leipzig/DE)
- P 202 Two liverworts from same habitats developed many similar but few distinct seasonal adaptive strategies: Insights from a transcriptomic approach  
Suvajit Basu, Sandhya Yadav, Vishal Jha, Yogesh Mishra (Varanasi/IN)
- P 204 Distinct guard cell shaped strategies for coping with repeated drought stress  
Patrick Pascal Lehr (Stuttgart/DE), Alexander Erban (Potsdam-Golm/DE), Roman Hartwig, Monika Wimmer (Stuttgart/DE)  
 Joachim Kopka (Potsdam-Golm/DE), Christian Zörb (Stuttgart/DE)
- P 206 Dynamic growth QTL action in diverse light environments - characterisation of light regime-specific and stable QTL in Arabidopsis  
Rhonda Meyer, Kathleen Weigelt-Fischer, Henning Tschiersch, Georgia Topali, Lothar Altschmied, Marc Heuermann, Dominic Knoch, Markus Kuhlmann, Yusheng Zhao, Thomas Altmann (Gatersleben/DE)
- P 208 Targeted expression of a maize NADP-Malic enzyme as a metabolic engineering tool for enhanced drought tolerance In tobacco  
Pablo Oitaven, María Fernanda Guindón (Rosario/AR), Ying Fu, Karuna Verma  
 Veronica Graciela Maurino-Larcher (Bonn/DE), María Fabiana Drincovich (Rosario/AR)
- P 210 Sediment archives as time-series of millennial phytoplankton adaption  
Sarah Bolius (Rostock/DE), Alexandra Schmidt (Konstanz/DE), Jérôme Kaiser, Olaf Dellwig, Helge Arz (Rostock/DE)  
 Laura Epp (Konstanz/DE), Anke Kremp (Rostock/DE)
- P 212 Evaluating Transpiration and Chlorophyll Dynamics alongside Stomatal Density and Size in Drought Impacted Winter Wheat Genotypes  
Eliyah Ganji (Quedlinburg/DE), Emilio Villar Alegria, Mahmoud Mohamed Mabrouk Ahmed, Tsu-Wei Chen (Berlin/DE)  
 Andrea Matros, Andreas Stahl (Quedlinburg/DE)
- P 214 Linking the drought response to the regulation of inducible crassulacean acid metabolism  
Katharina Schiller, Mara Bosse, Prisca Viehöver (Bielefeld/DE), Noé Perron (Gainesville, FL/US)  
 James Hartwell (Liverpool/GB), Andrea Bräutigam (Bielefeld/DE)
- P 216 Salt-priming induces salt tolerance in young tomato plants  
Sara Beier, Franziska Genzel, Anika Wiese-Klinkenberg (Jülich/DE)
- P 218 Abiotic conditions play a key role in the maturation of *Chlamydomonas reinhardtii* zygotes into dormant zygospores  
Martim Cardador (Leipzig/DE), Stephanie Krüger (Halle a. d. Saale/DE), Torsten Jakob, Reimund Goss  
 Severin Sasso (Leipzig/DE)
- P 220 Unraveling the link between rain stimuli and flooding-induced hypoxia acclimation in plants  
Rim Chaudhury, Aida Maric, Sjon Hartman (Freiburg i. Br./DE)

- P 222 Breeding for improved chilling tolerance in Sorghum to boost its cultivation in Germany  
Christiane Seiler (Quedlinburg/DE), Amna Eltigani (Braunschweig/DE), Amir Hajjarpoor (Kleinmachnow/DE)  
Christiane Balko (Sanitz/DE), Dragan Perovic (Quedlinburg/DE), Steffen Windpassinger (Gießen/DE)  
Andrea Matros (Quedlinburg/DE), Lorenz Kottmann (Braunschweig/DE), Til Feike (Kleinmachnow/DE), Gwendolin Wehner  
Andreas Stahl (Quedlinburg/DE)
- P 224 Remarkable phenolic iron complexes in glacier ice algae (*Ancylonema* spp., Zygnematophyceae) contribute to protection against harmful irradiation  
Lenka Procházková (Praha/CZ), Peter Mojzeš (Prague/CZ), Linda Nedbalová (Praha/CZ), Daniel Remias (Salzburg/AT)
- P 226 Root hairs are involved in stress recovery of maize plants after water limitation  
Monika Wimmer, Roman Hartwig, Lena Käsbauer, Isabelle Engel (Stuttgart/DE), Stefanie Wienkoop  
Carlos Perez-Rizquez (Vienna/AT)
- 17:30–19:00 **Resisting pathogens and pests**
- P 228 Bacteriophage based plant biocontrol strategies, understanding the mode of 'phage to seed' binding and the role of the seed-coat mucilage  
Sebastian Erdrich (Jülich, Düsseldorf, Jülich/DE), Ulrich Schurr (Jülich/DE), Julia Frunzke (Düsseldorf, Jülich/DE), Borjana Arsova (Jülich/DE)
- P 230 The emerging role of TARGET OF RAPAMYCIN in the regulation of the growth-defense tradeoff in plants  
Sarah Courbier, Andreas Hiltbrunner (Freiburg i. Br./DE)
- P 232 Root-derived N-hydroxypipicolinic acid manipulates shoot defense  
Ping Xu, Sophia Fundneider, Birgit Lange, Anton Schäffner (Neuherberg/DE)
- P 234 N-hydroxypipicolinic acid mediates root-shoot communication in response to root microbes  
Ping Xu, Sophia Fundneider, Birgit Lange, Anton Schäffner (Neuherberg/DE)
- P 236 The role of the phloem in plant defence and protection  
Alexandra Furch, Matthias Zimmermann (Jena/DE), Gundula Noll (Münster/DE), Axel Mithöfer (Jena/DE),  
Edgar Peiter (Halle a. d. Saale/DE), Karl-Heinz Kogel, Aart JE van Bel (Gießen/DE)
- P 238 Novel Disease Prevention Strategy in Arabidopsis: Reducing Apoplastic Water Availability and Water Potential via Flagellin-Mediated AQP Inhibition to Restrict Bacterial Growth  
Ahan Dalal (Düsseldorf/DE, Rehovot/IL), Ziv Attia, Menachem Moshelion (Rehovot/IL)
- P 240 Calcium-dependent protein kinases in the regulation of intrinsically disordered transcription factors in plant immunity  
Jan Oehlschläger, Sarah Lederer, Susanne Matschi, Xiyuan Jiang, Tina Romeis (Halle a. d. Saale/DE)
- P 242 The *Ustilago maydis* GATA transcription factor Nit2 controls nitrate utilization of the pathogen during biotrophy and influences major amino acid metabolism in leaf galls  
Christin Schulz, Philipp Lopinski, Nadine Schäfer, Nadja Braun, Alicia Fischer, Lars Voll (Marburg/DE)
- P 244 Molecular signatures of quantitative disease resistance against *Sclerotinia sclerotiorum* in the *Brassica napus* genome assessed by genetic, genomic and transcriptomic approaches  
Hendrik Seide, Ursel Riesterer, Wanzhi Ye (Kiel/DE), Thomas Bergmann, Steffen Rietz (Holtsee/DE), Daguang Cai (Kiel/DE)
- P 246 An untargeted proteomics method reveals plant proteins that are ADP-ribosylated by pathogen effectors to promote infection  
Simranjit Kaur (Halle a. d. Saale/DE), Thomas Colby (Köln/DE), Domenika Thieme, Carsten Proksch, Susanne Matschi (Halle a. d. Saale/DE), Ivan Matic (Köln/DE), Lennart Wirthmüller (Halle a. d. Saale/DE)
- P 248 Purification and characterization of a novel antifungal protein, CT-1 from seeds of *Clitoria ternatea*  
Manasi Mishra, Hrutuja Shirsat (Pune/IN)

- P 250      Lessons to learn from a gall-inducing fungus  
Armin Djamei (Bonn/DE)
- P 252      Degradation of plant cell wall components by type II-secreted enzymes – how phytopathogenic bacteria turn the plant apoplast into a habitat  
Samuel Goll, Jessica Erickson, Daniela Büttner (Halle a. d. Saale/DE)
- P 254      A new level of RNA-based plant protection - dsRNAs designed from functionally characterized siRNAs highly effective against variable pathogens  
Marie Knoblich, Torsten Gursinsky, Selma Gago-Zachert, Claus Weinholdt, Jan Grau, Sven-Erik Behrens (Halle a. d. Saale/DE)
- P 256      Cell wall integrity and elicitor peptide signaling modulate phytoalexin-mediated pathogen defense in Arabidopsis  
Richard N. Morton, Ahalya Rajendran, Lian Fleischberger, Timo Engelsdorf (Marburg/DE)
- P 258      Arabidopsis Shrks in the Accommodation of the Downey Mildew Pathogen Hyaloperonospora Arabidopsidis  
Athanasios Makris, Laura Caggegi, Maja Schmidt, Jörg Ziegler, Martina K. Ried-Lasi (Halle a. d. Saale/DE)
- 17:30–19:00    **Beneficial plant-microbe interactions**
- P 260      The role of the APETALA2 transcription factor ERIK in regulating arbuscular mycorrhiza in Lotus japonicus  
Haifei Zhang, Tian Zeng (Potsdam, Freising/DE), Jiuyue Pan (Freising, Potsdam/DE), Abul Khayer (Potsdam/DE) Kartikye Varshney (Potsdam, Freising/DE), Regina Hüttl (Freising/DE), Caroline Gutjahr (Potsdam, Freising/DE)
- P 262      Effect of bacterial priming on different growth and yield parameters in wheat under field condition  
Behnaz Soleimani (Quedlinburg/DE), Jennifer Thielmann (Gießen/DE), Mathias Wiegmann (Silstedt/DE) Johannes Schacht (Rosenthal, Peine/DE), Patrick Schäfer (Gießen/DE), Andrea Matros Gwendolin Wehner (Quedlinburg/DE)
- P 264      The role of microRNAs in shaping pathogenic vs symbiotic plant-fungal interactions  
Maitree Pradhan, Natalia Requena (Karlsruhe/DE)
- P 266      Treated wastewater irrigation effect on Olea europaea: is mycorrhization a game changer?  
Ameni Ben Hassena (Limoges/FR, Sfax/TN), Lina Trabelsi, Mohamed Zouari (Sfax/TN), Nacim Zouari (Medenine/TN) Pascal Labrousse (Limoges/FR)
- P 268      Unravelling the ectomycorrhizal transportome contributing to plant nutrition and adaptation  
Sabine D. Zimmermann (Montpellier/FR), Tania Ho-Plágaro (Montpellier/FR, Granada/ES) Muhammad Usman (Montpellier/FR), Carmen Guerrero-Galán (Montpellier/FR, Boadilla del Monte/ES) Gabriella Houdinet (Montpellier/FR), Monica Calvo-Polanco (Montpellier/FR, Salamanca/ES), Joske Ruytinx (Brüssel/BE) Kevin Garcia (Montpellier/FR, Raleigh, NC/US), Claude Plassard, Isabelle Gaillard (Montpellier/FR)
- P 270      Undermining the “cry for help”: how a phytopathogenic fungus undermines host recruitment of an antagonistic bacteria  
Wilko Punt, Anton Kraege, Andrea Doddi, Jinyi Zhu (Köln/DE), Kathrin Wippel (Amsterdam/NL), Natalie Schmitz Nick Snelders, Bart Thomma (Köln/DE)
- P 272      Plant immunity and leaf bacterial recruitment are parallel processes whose link shapes sensitivity to temperature stress  
Jisna Jose, Erik Teutloff, Simrat Naseem, Rayko Halitschke, Emanuel Barth, Matthew Agler (Jena/DE)
- P 274      Modeling metabolic dependencies in resource competition and cross-feeding among host-microbe communities  
Martina Feierabend, Nadine Töpfer (Köln/DE)
- P 276      Dynamic Adaptation of Rhizosphere Microbiomes to Mineral Nitrogen Forms  
Niels Maywald, Andres Hernandez-Pridyballo (Stuttgart/DE), Davide Francioli (Geisenheim/DE), Uwe Ludewig (Stuttgart/DE)

- P 278 Improved plant abiotic stress tolerance via use of beneficial microbes and alternative fertilizers, dissected via phenomics approaches  
Stefan Sanow (Jülich, Davis/DE), Olha Kapitanska (Kiev/UA), Lisa Mau, Jana Kelm, Josefine Kant  
Diana Reinecke-Levi (Jülich/DE), Pitter Huesgen (Freiburg i. Br./DE), Gabriel Schaaf (Bonn/DE)  
Michelle Watt (Melbourne/AU), Ute Roessner (Canberra/AU), Borjana Arsova (Jülich/DE)
- P 280 Algal growth and morphogenesis-promoting factors released by cold-adapted bacteria contribute to the resilience and morphogenesis of the seaweed *Ulva* (Chlorophyta) in Antarctica (Potter Cove)  
Fatemeh Ghaderiardakani, Johann F. Ulrich, Emanuel Barth (Jena/DE), Maria Liliana Quartino (Buenos Aires/AR)  
Thomas Wichard (Jena/DE)
- P 282 Osmo primed Melatonin Treatment and PGPR Inoculation Regulates Key Physio-biochemical and Molecular Pathway In *Brassica juncea* under Cd stress  
Tamanna Bhardwaj, Renu Bhardwaj (Amritsar/IN)
- 17:30–19:00 **Plant Systematics, Evolution and Biodiversity**
- P 288 Pollen and anther morphological variation was shaped by domestication in rye (*Secale cereale* L.)  
Christina Waesch, Yixuan Gao, Natalie Koch, Christin-Sophie Gaede (Halle a. d. Saale/DE), Thomas Hornick  
Christian Dusny (Leipzig/DE), Jörg Fuchs, Andreas Börner, Axel Himmelbach (Seeland / OT Gatersleben/DE)  
Martin Mascher (Leipzig, Seeland / OT Gatersleben/DE), Klaus Pillen (Halle a. d. Saale/DE), Susanne Dunker (Leipzig/DE)  
Steven Dreissig (Halle a. d. Saale, Leipzig/DE)
- P 290 Deciphering the nectar secretion physiology of evolutionarily specialized trichomatous extrafloral nectaries in *Clerodendrum chinense* (Osbeck). Mabb  
Shobhon Paul, Adinpunya Mitra (Kharagpur/IN)
- P 292 Chronological transcriptome reveals the common program for gynoecium development in eudicots  
Doudou Kong, Le-Han Nguyen, Annette Becker (Gießen/DE)
- P 294 Application of B-class genes of flowering on species-rich genus *Salvia* – Duplications and variabilities as potentials drivers for species diversity  
Sascha Wetters (Karlsruhe/DE)
- P 296 The *Crocus* panrepeatome: dynamics and dysploid  
Nomar Waminal, Frank Blattner, Dörte Harpke (Gatersleben/DE)
- P 298 Importance of Soil Silicon Availability in Drought Stress Tolerance of Annual Species in Arid and Semiarid Rangelands  
Jinyu Ouyang (Müncheberg/DE)
- P 300 Genetic basis and environmental plasticity of meiotic recombination rates  
Steven Dreissig, Christina Waesch, Maxie Sophie Seidel, Yixuan Gao (Halle a. d. Saale/DE)
- P 304 Status, changing landscape and farmers’ criteria for the continuity of paddy (*Oryza sativa*) land ecosystem of Jharkhand, India: A conservation perspective  
Neetu Kumari (Kanke/IN), Shashi Choudhary (Ranchi/IN), Basant Jha (Kanke/IN)
- P 306 Can you identify Canadian *Arabidopsis*? You can now! – A comprehensive, reliable synoptic reference races in the upland collection of *Arabidopsis thaliana* in Vancouver, BC  
Ilias Kontos, Linda P.J. Lipsen, Marco Todesco (Vancouver/CA)
- P 308 The Evolutionary History of *Ranunculus* section *Euromontani*  
Ellen Sigourney Lorberg, Salvatore Tomasello, Elvira Hörandl (Göttingen/DE)
- P 310 Addressing phylogeny and taxonomy of *Betula* L. genus using different approaches  
Andrii Tarieiev, Oliver Gailing (Göttingen/DE)

- P 312 The interplay between selection and the genomic landscape during the domestication of grain amaranth  
Corbinian Graf, Markus Stetter (Köln/DE)
- 17:30–19:00 **Biotechnology and genome editing**
- P 314 CRISPR/Cas9-induced loss of function of BnHVA22c and BnCRT1a boots plant immune re sponse and reduces the susceptibility of oilseed rape (*Brassica napus*) to fungal (*Verticillium longisporum*) infection  
Wanzhi Ye, Lingyue Han, Dirk Schenke (Kiel/DE), Steffen Rietz (Holtsee/DE), Daguang Cai (Kiel/DE)
- P 316 The Science of Blue Flowers: Phylogenetic, Genomic and Transcriptomic Analysis of Blue Flowering Plants  
Chiara Marie Dassow, Julia Ingrid Lüpkes, Vladislav Berg (Braunschweig/DE)
- P 318 Targeted cis-regulatory mutagenesis uncovers pleiotropic effects of the phototropin2 gene in tomato  
Kunnappady Princy, Narasimha Rao Nizampatnam, Neha Gupta, Valluri V. Satyavathi, Rameshwar Sharma Yellamaraju Sreelakshmi (Hyderabad/IN)
- P 320 Painting with your own colors: Using Cas9 fused exonucleases to engineer cis-genic purple tomatoes  
Alejandro Brand, Tom Schreiber, Alain Tissier (Halle a. d. Saale/DE)
- P 322 A rocky road to CRISPR - Dealing with in vitro recalcitrance in European beech (*Fagu sylvatica* L.)  
Virginia Zahn, Alice-Jeannine Sievers, Matthias Fladung, Tobias Bruegmann (Großhansdorf/DE)
- P 324 Elucidating the effect of single gene modifications on drought stress tolerance of poplars (*Populus*)  
Alexander Fendel (Großhansdorf/DE), Felix Wiedemann (Großhansdorf, Hannover/DE)  
Matthias Fladung, Tobias Bruegmann (Großhansdorf/DE)
- P 326 Functional Profiling and Regulatory Element Analysis of the Male Gametophyte-Specific Promoter Fragment in the Arabidopsis PIRL6 Gene  
Ajith T G, Jasmine M Shah (Kasaragod/IN)
- P 328 The mechanisms of heavy metal resistance in Zygnematophyceae and liverworts: first steps towards the phycore mediation of contaminated freshwater  
Jenny Woide, Annalisa Degenhardt, Henrik Buschmann (Mittweida/DE)
- P 330 CRISPR-Cas Mediated Plant Immunization: A New Frontier in Climate Challenges  
Meena Barupal (Jodhpur/IN)
- P 332 Efficient Targeted Insertion in Plant Genomes via Protoplast Regeneration  
Chen-Tran Hsu, Yu-Hsuan Yuan, Yao-Cheng Lin, Steven Lin, Qiao-Wei Cheng, Fu-Hui Wu, Jen Sheen, Ming-Che Shih Choun-Sea Lin (Taipei/TW)

17:30–19:00 **Crop biology and genetics**

- P 334 Update of the rye reference genome assembly, Lo7\_V3  
Erwang Chen, Srijan Jhingan, Axel Himmelbach (Gatersleben/DE), Martin Mascher (Gatersleben, Leipzig/DE)  
Nils Stein (Gatersleben, Halle a. d. Saale/DE)
- P 338 AGROFLUX: An innovative sensor platform to study high-frequency responses in water, carbon and greenhouse gas fluxes in a complex arable landscape  
Maren Dubbert, Adrian Dahlmann, Shrijana Vaidya, Michael Sommer, Marten Schmidt, David Dubbert, Verch Gernot Jürgen Augustin, Mathias Hoffmann (Müncheberg/DE)
- P 340 Differential drought responses in tomato landrace and commercial cultivar: implications for enhanced horticultural production  
Antonio Pompeiano, Tommaso Michele Moles, Lorenzo Mariotti, Daniela Di Baccio, Antonietta Santaniello Andrea S. cartazza, Thais Huaranca Reyes, Lorenzo Guglielminetti (Pisa/IT)
- P 342 A combined landscape genetic study and species distribution modeling (SDM) of the *Hordeum murinum* L. complex  
Maryam Keshavarzi, Raheleh Tabaripour (Tehran/IR)



- P 344 A machine-learning-approach to study the plasticity of flowering time in a worldwide field trial using an exotic barley population  
 Thomas Schmutzer (Halle a. d. Saale/DE), Cathy Westhues, Rupashree Dass, Christian Dreischer (Tübingen/DE)  
 Andrew Flavell (Dundee/GB), Klaus Pillen, Andreas Maurer (Halle a. d. Saale/DE)

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17:30–19:00 Applied botany for food security

- P 346 The effect of Electro Magnetic Fields (EMFs) on seed germination and seedlings Physiological changes OF *Coriandrum sativum* L. to achieve the optimum frequency of Electro Magnetic Field  
 Zahra Atghia (Istanbul/TR)
- P 348 Liquid in vitro culture system allows gradual intensification of osmotic stress in *Solanum tuberosum* through sorbitol  
 Katharina Wellpott, Marco Herde, Traud Winkelmann, Christin Bündig (Hannover/DE)

17:30–19:00 Biology of algae and cyanobacteria

- P 352 Rafting kelps *Macrocystis pyrifera* and *Egregia menziesii* from California: Physiological condition, ultra structure, microchemistry and sensitivity to high UV treatment  
 Andreas Holzinger, Thomas Roach, Charlotte Permann (Innsbruck/AT), Paraskevi Charalambou  
 Notburga Gierlinger (Vienna/AT), Dimitri Deheyn (La Jolla, CA/US)
- P 354 New mechanisms regulating the carbon-concentrating mechanism in cyanobacteria  
 Peter Walke, Martin Hagemann (Rostock/DE)
- P 356 Characterization of a durable biopolymer from *Chlamydomonas reinhardtii* zygospores  
 Prajwal Ranganathapura Basavaraju, Valentin Rohr, Chen Song, Raimund Nagel, Jörg Matysik, Severin Sasso (Leipzig/DE)
- P 358 DNA methylation, a regulator of keystone enzyme of chlorophyll biosynthesis in *Synechocystis* sp. PCC 6803  
 Nils Schmidt (Rostock/DE), Satoru Watanabe (Tokyo/JP), Roman Sobotka (Třebon/CZ)  
 Wolfgang R. Hess (Freiburg i. Br./DE), Martin Hagemann (Rostock/DE)
- P 360 Different Lhcx proteins in diatoms and their functional diversity  
 Nicolas Herrmann, Johannes Sattler, Claudia Büchel (Frankfurt a. M./DE)



## POSTERS EVEN | TUESDAY, 17 SEPTEMBER

- P 362 The plant xanthophyll cycle in the diatom *Phaeodactylum tricornutum*  
Chiara Giossi (Konstanz/DE), Marie Alice Wunsch (Rostock, Konstanz/DE), Peter Kroth (Konstanz/DE)  
Bernard Lepetit (Rostock, Konstanz/DE)
- P 366 Establishing a click chemistry-based toolset to visualize polysaccharide deposition in green algae  
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PL 1

**Achieving sustainable productivity in agriculture through beneficial microbial associations**

Giles E. D. Oldroyd

Crop Science Centre, University of Cambridge, 93 Lawrence Weaver Way, Cambridge, CB3 0LE, UK

The availability of nitrogen and phosphorus is a major limitation to crop productivity and this is currently addressed primarily through application of inorganic fertilisers to augment these limiting nutrients. Use of such fertilisers contributes the greatest cause of pollution from agriculture in high and middle-income countries, while access to inorganic fertilisers is extremely limited for farmers in low-income countries. In natural ecosystems many species of plants acquire nitrogen and phosphorus through associations with beneficial fungi and bacteria, but the use of these beneficial microbial associations is currently very limited in agriculture. Through a detailed understanding of how plants associate with beneficial microorganisms, we are attempting to broaden their use in agriculture to facilitate sustainable productivity, accessible to all of the world's farmers.

PL 2

**Mapping and managing causes and consequences of plant biodiversity**Meredith C. Schuman and the Spatial Genetics team

Department of Geography and Department of Chemistry, University of Zurich, Zurich, Switzerland

Plants feed much of life on earth, cultivate and secure soil, fix carbon and exhale oxygen. Volatile organic compounds in Earth's atmosphere originate primarily from plants, with each plant having its own fluctuating body odor as a result of its particular genetic make-up interacting with its environment over time. Trees can be smelled from a distance and seen or even counted from the air or from space. Differences in the spectral reflectance of sunlight off tree canopies can reveal important information about their functional diversity and performance, as well as their genetic differentiation. Scalable tools, such as those that allow us to sample chemistry over forest canopies and to record their spectral reflectance of sunlight, allow us to map different aspects of forest biodiversity and functioning, and to study changes over time in response to human interventions and other factors. I will discuss work by the Spatial Genetics group at the University of Zurich aimed at mapping molecular differences that matter for the responses of plant populations, species, and their ecosystems to the environment, including the variation within and among plants that is critical for acclimation and adaptation. I will present some foundations of our approach, and recent work with European and Caucasian beech, a system in which we are developing approaches to scale the mapping and monitoring of genetic and functional diversity, and relate it to past, current and future states of ecosystems.

PL 3

**How long can plants prevent more severe climate change?**Josep Peñuelas<sup>1,2</sup><sup>1</sup> CSIC, Global Ecology Unit CREAM-CSIC-UAB, Cerdanyola del Valles 08193, Catalonia, Spain<sup>2</sup> CREAM, Cerdanyola del Valles 08193, Catalonia, Spain

I will address the decreasing efficiency and slowdown in the increase of terrestrial carbon-sink activity. Anthropogenic activities, such as elevated atmospheric CO<sub>2</sub> and nitrogen inputs, have historically enhanced plant photosynthesis and the carbon sequestration capabilities of vegetation. However, recent evidence suggests that the effectiveness of these carbon sinks is diminishing due to limitations in nutrients, water, and heat, as well as other factors like fires, pollution, and reduced vegetation carbon residence time, as it would be expected from the five laws of life proposed by Peñuelas and Baldocchi (2019): 1) the law of the conservation of mass, 2) energy cannot be created or destroyed in an isolated system, 3) the entropy of any isolated system always increases, 4) the information content is a power of the material size of its store with an exponent larger than one, and 5) Basic mechanisms such as natural selection, self-organization, and random processes (not driven by selection) drive evolution, generating the huge complexity of organisms and ecosystems.

I will emphasize the critical role terrestrial plants have played in mitigating climate change by assimilating a significant portion of CO<sub>2</sub> emissions, thus buffering against more severe warming. I will raise concerns about how long this mitigation effect can continue as the efficiency of carbon sinks appears to be declining. The potential deceleration in carbon fixation and its implications for climate change mitigation are not well studied, and current models may overestimate the capacity of carbon sinks and underestimate the severity of future climate warming if these factors are not accounted for. I will thus call for more comprehensive studies and updated models that consider plants and their structure and functioning to better predict and manage the planet's carbon balance and climate change mitigation efforts.

Reference

Peñuelas, J., Baldocchi, D. 2019. Life and the five biological laws. Lessons for global change models and sustainability. *Ecological Complexity* 38 (2019), 11-14. doi: 10.1016/j.ecocom.2019.02.001.

PL 4

Understanding how plant roots sense soil compaction

Bipin Pandey

School of Biosciences, University of Nottingham, UK

Compaction disrupts soil structure, reducing root growth, nutrient and water uptake, gas exchange and microbial growth. Root growth inhibition by soil compaction was originally thought to reflect the impact of mechanical impedance and reduced water availability. However, recent research has revealed plant roots sense soil compaction using a novel gas diffusion-based mechanism employing the hormone ethylene. Non-compacted soil features highly inter-connected pore spaces that facilitate diffusion of gases like ethylene which are released by root tips. In contrast, soil compaction stress disrupts the pore network, causing ethylene to accumulate around root tips and trigger growth arrest. Genetically disrupting ethylene signalling causes roots to become much less sensitive to compaction stress. Ethylene also regulates auxin and ABA to monitor root elongation and root swelling responses in compacted soil conditions.

PL 5

Tales of many algae

Sabeeha Merchant

University of California, Berkeley, Berkeley, CA 94720

The green algae share an evolutionary history with land plants, which means that discoveries in one system inform our understanding of the other, especially in the context of photosynthesis and chloroplast biology. Although *Chlamydomonas* is the most well-known “model” organism, other green algae present interesting physiologies for investigation, or offer experimental advantages for laboratory-based research. The Merchant group has assembled genomes for *Dunaliella* spp., *Chromochloris zofingiensis* and *Auxenochlorella protothecoides* in addition to our work on the *Chlamydomonas reinhardtii* genome.

*Dunaliella* spp. are halotolerant extremophiles and have long been cultivated for valuable bioproducts. Systems analysis of Fe-deficiency in two divergent species from the Sinai Peninsula (*D. salina*) or Oslofjord (*D. tertiolecta*) suggested compositional variation in Photosystem I, which motivated structural analysis and revealed a novel pattern of light-harvesting antenna organization. This work was contributed by Helen Liu, Radhika Khera and Masa Iwai.

Homologous recombination is facile in *A. protothecoides*, which opens the door to classic reverse genetic approaches for deducing and testing specific hypotheses. The organism is a prolific accumulator of triacylglycerols, suggesting that it can be engineered to produce fats for food applications. We have tailored the fatty acid and TAG profile of *A. protothecoides* to mimic the unique composition of human milk fat, including regioisomeric distribution of the major TAG species. This work is contributed by Jon Lin and Jeff Moseley.

Polycistronic mRNAs were discovered in each of these algal genomes, which is rare for eukaryotic genes. Bioinformatics and mutational analysis suggest that leaky ribosome scanning provides the main mechanism for translating two open reading frames from a single mRNA molecule. This work is contributed by Marco Dueñas and Jeff Moseley.

These examples validate the contributions to basic and applied research of studies using simple unicellular organisms.

PL 6

Deciphering the molecular basis of plant receptor kinase-mediated immune signaling

Cyril Zipfel<sup>1,2</sup>

<sup>1</sup>Institute of Plant and Microbial Biology, Zürich-Basel Plant Science Center, University of Zürich, 8008 Zürich, Switzerland.

<sup>2</sup>The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, United Kingdom.

Plant immunity relies on both cell-surface and intracellular immune receptors. Recent studies showed that signaling triggered upon activation of these receptors is more connected than previously thought, and that both types of immune receptors can induce congruent cellular immune signaling outputs. Yet, the exact molecular mechanisms and components involved in the generation and regulation of such outputs are not fully understood. I will present our recent efforts to gain a better understanding of the molecular basis of early immune signaling, particularly that mediated by cell-surface receptor kinases acting as pattern-recognition receptors – which also provide a blueprint for similar deciphering of other receptor kinase-based pathways in plants.

PL 7

## Regulation of carbon allocation and cell wall synthesis

Staffan Persson

Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

All plant cells are surrounded by polysaccharide-based extracellular matrices, called cell walls. These walls underpin cell morphology, thus providing the driving force for plant architecture and protecting plants against their environment. The cell wall polysaccharides are divided into three main classes; pectins, hemicelluloses and cellulose, that together with proteins, ions and sometimes hydrophobic polymers form the cell walls. Nevertheless, the cell wall content and organization can vary substantially across different species, and also across tissues and cell types within a single species. Altering cell wall content and organization may therefore change what a plant looks like and the functional aspects of plant cells. In this talk I will outline efforts done to increase our knowledge of cellulose synthesis, especially how this process may be aided during salt stress exposure. I will also summarize new ways to rapidly identify protein-protein interactions in plant cells to decipher protein complexes involved in cell wall synthesis, sugar sensing and allocation, as well as other processes that drive plant growth.

PL 8

## Environmental control of stomatal development - From intricate modulation to innovation

Keiko U. Torii

Howard Hughes Medical Institute and Department of Molecular Biosciences, The University of Texas at Austin, USA  
The Institute of Transformative Biomolecules, Nagoya University, Japan

Stomata, cellular valves on the plant epidermis, serve as critical interface between plant and atmosphere. The presence of stomata is critical for plant growth, survival and water-use efficiency, and it further impacts global carbon and water cycles. In the past two decades, molecular genetic studies in the model plant *Arabidopsis* unraveled the key regulators of stomata differentiation and the mechanism that ensures proper differentiation and patterning of stomata. The development of stomata occurs through a consecutive action of 'master-regulatory' basic-helix-loop-helix (bHLH) transcription factors, *SPEECHLESS* (SPCH), *MUTE*, and *FAMA*, all of which form a heterodimer with a partner bHLH, *SCREAM* (SCRM). Plants respond to diverse environmental cues and adjust the numbers and density of stomata in order to optimize their growth and survival. Therefore, understanding exactly how environmental stress signaling pathways impact the expression, activity, and function of these transcription factor modules is critical. Our genetics and chemical biology studies revealed the importance of bHLH protein heterodimerization. Furthermore, we discovered that certain environmental stress suppresses stomatal differentiation through interfering with the bHLH heterodimerization. While we are learning how environmental and hormonal signal transduction pathways feed into the regulation of stomatal master regulators using *Arabidopsis* as a model, new insights are coming from the study of diverse plant species. For example, we are expanding our study into Brassicaceae amphibious plant, *Rorippa aquatica*, to understand how this extremophyte has invented a new way to re-wire environmental/hormonal signal transduction pathways to swiftly switch to astomatous life style under water.

IT 1

## Macroevolutionary and genomic investigations of sex chromosome evolution in seed plants

Jim Leebens-Mack

University of Georgia, United States

A growing number of genome sequence assemblies are becoming available for dioecious seed plant species, enabling investigation of factors that might be influencing plant sex chromosome evolution. However, sex-linked sex determination genes have been identified in just a few dioecious plant species. Nonetheless, we can characterize the gene content of sex determination regions through analyses of phased assemblies of X and Y or Z and W sex chromosomes to assess predictions of current models for sex chromosome evolution. At the same time, future work on plant sex chromosome evolution should focus on functional characterization of sex determination genes. I will discuss work we are doing in the genus *Asparagus* and *Amborella trichopoda*.

IT 2

Spatiotemporal analysis of brassinosteroid receptors unveils novel mechanisms to unlock drought adaptation in crops

Ana I. Caño-Delgado

Molecular Genetics, Centre for Research in Agricultural Genomics, Campus UAB, Barcelona, Spain

Despite the massive amount of information gathered around the functions and mechanisms of Brassinosteroids (BRs) in plants, an important limitation persists in our knowledge of this signaling pathway: almost all we know comes from observations on the BRI1 receptor pathway, that is essential for growth and development, and for which mutants are highly pleiotropic and typically dwarf.

Since the discovery of BRI1-like receptors (BRL1/3) twenty years ago<sup>1</sup>, the fundamental functions of BRI1-like receptors (BRL1/3) remain elusive, largely viewed as redundant with limited vascular expression and minimal mutant phenotypes. However, our lab has taken a novel perspective to explore the function of BRLs in Arabidopsis, to understand the inner working of this pathway and we have now deciphered the BRL3 pathway in Arabidopsis, including novel cell-specific components in the pathway essential to plant adaptation to elevated temperatures and drought resistance<sup>2-3</sup>. Our data not only change the paradigm for our present understanding of BR signaling in plants<sup>4</sup>, but also open new and exciting avenues for engineering climate resilient crops<sup>5</sup>. Our latest results and our effort for transferring those findings into agricultural valuable crops will be presented at the seminar.

References

1. Planas-Riverola, A.; Gupta, A.; Betegón, I.; Bosch, N., Caño-delgado, A. I.. Brassinosteroid Signaling in Plant Development and Adaptation to Stress. *Development* 2019, 1, 1–11. <https://doi.org/10.1242/dev.151894>
2. Fàbregas, N.; Lozano-elena, F.; Blasco-escámez, D.; Tohge, T.; Martínez-andújar, C.; Albacete, A.; Osorio, S.; Bustamante, M.; Riechmann, J. L.; Nomura, T.; Yokota, T.; Conesa, A.; Alfocea, F. P.; Fernie, A. R.; Caño-Delgado, A. I. Overexpression of the Vascular Brassinosteroid Receptor BRL3 Confers Drought Resistance without Penalizing Plant Growth. *Nat. Commun.* 2018, 9, 1–13. <https://doi.org/10.1038/s41467-018-06861-3>.
3. Lozano-Elena, F.; Fàbregas, N.; Coletto-Alcudia, V.; Caño-Delgado, A. I. Analysis of Metabolic Dynamics during Drought Stress in Arabidopsis Plants. *Sci. Data* 2022, 9 (1), 1–12. <https://doi.org/10.1038/s41597-022-01161-4>.
4. Gupta, A.; Rico-Medina, A.; Lozano-Elena, F.; Marqués-Bueno, M.; Fontanet, J.B.; Fàbregas, N.; Alseekh, S.; Fernie, A. R.; Caño-Delgado, A. I. Brassinosteroid receptor BRL3 triggers systemic plant adaptation to elevated temperature from the phloem cells. *Biorxiv* 2023. <https://doi.org/10.1101/2023.03.07.531487>.
5. Gupta, A.; Rico Medina A.; Caño-Delgado, A. I. The Physiology of Plant Responses to Drought. *Science* 2020, 368 (April), 266–269. <https://doi.org/10.1126/science.aaz7614>.

T 3

PSII Assemble! About some of the many hands that make LIGHT work

U. Armbruster

Heinrich Heine University Düsseldorf, Düsseldorf, Germany

The initial step of oxygenic photosynthesis is the thermodynamically challenging extraction of electrons from water and the release of molecular oxygen. This light-driven process, which is the basis for most life on Earth, is catalyzed by photosystem II (PSII) within the thylakoid membrane of photosynthetic organisms. The functional PSII dimers of cyanobacteria and the chloroplasts of land plants are highly conserved. Moreover, their assembly process follows a very similar sequence: first the reaction center (RC), which already contains most of redox-active cofactors of PSII, is assembled from D1, D2, Cyt *b559* and Psbl, then the intrinsic antenna protein CP47 together with additional subunits is assembled with the RC to form RC47, followed by the integration of the oxygen evolving complex and CP43 to form the monomer before the final dimerization. Thus, it is not surprising that previously identified factors involved in the assembly of plant PSII have orthologues in cyanobacteria with conserved function. In the past years, our group has identified a number proteins in *Arabidopsis thaliana* with a likely function in PSII assembly, which present eukaryotic inventions. In this talk, I will present some of our results on the molecular characterization of these assembly factors and speculate on potential evolutionary and/or environmental factors that drove their establishment in the plant lineage.

IT 4

**Some answers are hidden underfoot – belowground traits and their power to predict plant functioning**

Joana Bergmann

Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany

ecosystem functioning. Traditionally many of these scientific endeavors have been carried out using life-history traits and aboveground plant traits, mainly leaf traits. During the last decade though, root traits have come into focus. A growing body of literature now suggests that fine root trait variation is multidimensional, displaying independent aspects of plant functioning. The conservation gradient thereby depicts plant functional variation ranging from 'fast' to 'slow', mirroring the aboveground leaf economics spectrum. The collaboration gradient, ranging from 'outsourcing' to 'do-it-yourself' depicts plant variation in collaboration with mycorrhizal fungi. Due to the intricate relationship of belowground organs and the soil environment, abiotic as well as biotic soil characteristics act as strong filters for fine root functional traits. But neither do plants separate their belowground from their aboveground functioning, nor are roots the only belowground organs. A more holistic view on plant functioning seems to be the logical next step in order to fully understand species occurrences and ecosystem functioning. Specifically in the light of global change, we need to combine knowledge of different scientific realms that have been rarely synthesized in the past. Starting from belowground, the integration of fine root traits with root system traits like rooting depth and width but also clonal and bud-bank traits holds the potential to shed light on holistic plant functioning.

IT 5

**Unraveling the Secret of Root Architecture: From Cell Division to Organ Formation**Viola Willemsen, Merijn Kerstens, Yvet Boele, Kavya Yalamanchili, Zhuang Yang, Vera Heslen, Andrea Bimbo, Max Goessens  
Roman Lakerveld, Wu Liu, Jordi Floriach-Clark, Jiawei Yao

Cell and Developmental Biology, Wageningen University and Research, Droevendaalsesteeg 1, Wageningen, The Netherlands.

Plant shape, or architecture, is a primary determinant of productivity and yield. The shape of the above-ground part of the plant determines light interception and photosynthesis, whereas the below-ground root system determines the interaction with the soil, including uptake of water and nutrients and anchoring. As plant cells are bound by a cell wall and cannot move, shape is an outcome of the reorientation of the cell division plane and subsequent cell growth. In the model plant *Arabidopsis*, distribution patterns of the plant growth regulator auxin have been linked with altered cell division planes during lateral root initiation and in primary roots. The importance of orienting cell planes also becomes apparent in asymmetric cell divisions associated with the *Arabidopsis* root stem cell niche that are sustained by the activity of PLETHORA (PLT) proteins. These members of the AP2 transcription factor family are the main regulators of primary root meristem maintenance and the position of the meristematic boundary, regeneration, lateral root formation and have an intimate relationship with auxin. Studying orientation of cell division planes in higher plants is difficult due to tissue complexity while mosses like *Physcomitrium patens* have a more simple tissue organization and core genes are highly conserved, but lack the complexity. This makes *Physcomitrium patens* a valuable model organism for studying the basic mechanisms of oriented cell divisions. Taken together, these combined efforts will continue to elucidate the molecular mechanisms that drive the key processes in root architecture.

IT 6

**Form and function of a wide-spread plant-fungal symbiosis**

Caroline Gutjahr

Max-Planck-Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

Most land plants form symbioses with Glomeromycotina fungi to acquire mineral nutrients from the soil. This so-called arbuscular mycorrhiza (AM) symbiosis is evolutionarily ancient and found in the oldest fossils of land plants, sparking speculations about its importance in the colonization of the land by originally aquatic plants during evolution, at a time when plants had not yet evolved complex root systems for nutrient uptake. The fungi form extended hyphal networks in the soil to scavenge mineral nutrients. These are transported into the root and released via beautifully-shaped, highly branched hyphal structures, the arbuscules inside inner root cells. In return they receive up to 20% of photosynthetically fixed carbon from their host in the form of sugars and lipids. As a consequence, AM contributes significantly to plant nutrition and to global carbon cycles.

For symbiosis establishment, AM fungi colonize the root interior and the inside of plant cells. Symbiotic infection of single, already differentiated cells within the tissue context requires a poorly understood cellular remodeling program that is intertwined with mechanisms that control plant development and physiology. In my presentation, I will provide examples of how we investigate the molecular mechanisms underlying development and functioning of this fascinating symbiosis.



IT 7

**Telling left from right - the genetic and molecular basis of enantiostyly in *Wachendorfia* and *Barberetta***

Haoran Xue<sup>1</sup>, Kelly Shepherd<sup>2</sup>, Oliver Marketos<sup>2</sup>, Olivia Page-Macdonald<sup>2</sup>, Caroline Robertson<sup>2</sup>, Christian Kappel<sup>1</sup>, Robert Ingle<sup>2</sup>, Nicola Illing<sup>2</sup>, Michael Lenhard<sup>1</sup>

<sup>1</sup> University of Potsdam, Potsdam, Germany

<sup>2</sup> University of Cape Town, Cape Town, South Africa

Telling left from right is a difficult task for organisms, as the two sides can only be defined by reference to two other axes. While genetically encoded left-right asymmetry is found in many animals, it is very rare in plants. One example is the mirror-image flowers of *Wachendorfia* and *Barberetta*. Species of these two closely related genera show enantiostyly, i.e. they form flowers with the style deflected to the left of the midline and one of the three stamens to the right, or with the opposite arrangement. All flowers on one individual have the same handedness, indicating genetic control. Functionally, the reciprocal placement of the stigma and the opposing anther promotes pollen placement on segregated sites of the pollinators' bodies and efficient transfer to stigmas of the opposite morph. We are investigating the genetic, molecular and developmental basis of mirror-image style and stamen deflection in *Wachendorfia* and *Barberetta*. Genome assemblies and pooled sequencing of left- and right-morph individuals from natural populations have identified a hemizygous region whose presence causes right-styled flowers, whereas plants lacking this region form left-styled flowers. This region contains two conserved genes throughout all the species tested, one of which is expressed in the developing stamen, the other in the style around the time when the organs begin to deflect from the midline. Efforts to assign functions to these genes are ongoing. Identification of the hemizygous region opens up the possibility of assaying realized mating patterns in natural populations and testing the efficiency of enantiostyly in promoting disassortative mating. Thus, our results pave the way for a mechanistic understanding of left-right asymmetry in plants and its impact on plant reproduction.

IT 8

**Kill the messenger - spatial regulation of iron acquisition in plant-microbe interactions**

Wolfgang Busch

Plant Molecular and Cellular Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA, United States

Iron is an essential nutrient for organismal growth. While iron is among the most common elements on our planet, its bioavailability in most environments is low. It is therefore a limiting factor for the growth of almost all organisms including microbes and plants. Accordingly, iron plays an important but complex role in regulating plant-microbe interactions. Restriction of available iron by the host during infection is an important defense strategy, coined nutritional immunity. In the soil, however, the multipartite interactions of iron and roots along with beneficial, commensal, and pathogenic microbes in the rhizosphere are more complex. Plant roots acquire iron mostly from the soil. When there is an iron deficiency, roots actively increase the availability of iron through mechanisms that include rhizosphere acidification and secretion of iron chelators. Yet if under microbial attack, the elevated iron bioavailability generated by plants is also beneficial for the growth of bacteria that threaten plant health. During our investigations of plant responses to microbe-associated molecular patterns (MAMPs) under different iron availability conditions, we found that root responses to MAMPs or invading bacteria abolish an important subset of iron deficiency responses in a cell-type specific manner. Through several lines of inquiries, we found that this cell-type specific termination of the iron deficiency response is facilitated through the spatial regulation of a systemic iron level signaling relay. This modulation of the iron signaling relay coordinates iron homeostasis and immunity responses in the root and leads to the cessation of rhizosphere acidification that would otherwise lead to bioavailable iron for the microbes and a dampening of plant defense responses. The molecular mechanism that we have elucidated allows for the integration of the whole-plant iron status and local defense signals that are perceived in the root and consequently for a finely tuned response to iron and microbial conditions.

IT 9

**Fine-tuning vernalization in hexaploid bread wheat**

Dominique Hirsz, India Lacey, Kathryn O'Connor, Yanjie Song, Laura Dixon

School of Biology, University of Leeds, UK

The yield potential of cereal crops is influenced by their ability to optimally coordinate development with the changing seasons and weather. In hexaploid bread wheat (*Triticum aestivum*) the perception of winter via a process known as vernalization enables a longer growing season whilst maintaining the best seasonal flowering, therefore supporting high yield potential. The key genes in this process have been characterised, as VERNALIZATION 1-3, however, much remains unknown regarding how the vernalization network forms and responds to environmental signals, especially under field conditions. I will present molecular genetic characterization of the vernalization and the wider temperature adaptation network. This understanding will enable us to place the vernalization response within broader seasonal regulation of wheat growth. Through an improved understanding of the vernalization network we aim to support the development of climate-robust cultivars.

IT 10

## Diatom genome evolution in real time

T. Mock

University of East Anglia, Norwich, United Kingdom

Oceanic algae such as diatoms contribute approximately 50% of global primary production. Yet, how their genomes evolve in real time to cope with environmental conditions of the upper ocean has barely been investigated. However, this fundamental knowledge is not only essential to identify mechanisms of how they adapt, but it may also be used to investigate and to predict the evolutionary response of important primary producers to global climate change. To address this knowledge gap, this presentation will provide insights into the real-time genome evolution of the model diatom *Thalassiosira pseudonana*. Experimental evolution with wild-type and genetically modified strains was conducted in combination with genome, transcriptome and DNA methylome sequencing to provide insights into how individual chromosomes evolve in real time under natural (temperature change) and artificial (DNA mutagens) selection pressure. Our results not only revealed mechanisms of adaptive genome evolution in diatoms, but they have also shown that there are hotspots of evolution and that the structure of chromosomes impacts the rise of genetic variants involved in adaptation and population differentiation.

IT 11

## Probing organelle interactions using optical tweezers: Trap it, pull it, set it 'free'?

Imogen Sparkes

School of Biological Sciences, University of Bristol, Bristol Life Sciences Building, 24 Tyndall Avenue, Bristol, UK

Plant cells are extremely dynamic at the subcellular level with organelles moving and changing position in the cell in response to biotic and abiotic factors. The classic textbook image of organelles acting in isolation is being challenged by our understanding that organelles move, physically interact at membrane contact sites and communicate with one another. Our group studies the molecular mechanisms controlling organelle movement (actomyosin) and interaction (membrane contact sites). Here, I will discuss our pioneering application of optical tweezers which enables the user to physically trap and move organelles in vivo. In a densely packed cytoplasm, it is difficult to distinguish between organelles physically interacting or just sitting next to one another. By physically trapping and moving organelles, we have discovered interactions between the ER-Golgi, peroxisome-chloroplast and ER-mitochondria and started to characterise the molecular components and functional role of the interactions.

IT 12

## Applications of phytohormone signalling: from deficit irrigation to microbial inoculants

Ian C. Dodd

Lancaster Environment Centre, Lancaster University, United Kingdom

Climate change and/or supermarkets requiring increased efficiency from their suppliers are likely to stimulate grower adoption of deficit irrigation, which supplies less water to the crop than considered optimal. One form of deficit irrigation, known as partial rootzone drying (PRD), irrigates only part of the rootzone and allows the other to dry the soil. Soil drying stimulates phytohormone accumulation (ABA, ACC) or depletion (cytokinins) in the roots, with some of these hormones transported in the transpiration stream to the shoot to initiate partial stomatal closure that enhances leaf water use efficiency. One of the paradoxes of PRD is that soil drying stimulates root phytohormone accumulation while diminishing hormone transport as the roots in dry soil contribute proportionally less sap flow to the transpiration stream. A practical solution is to alternate the wet and dry parts of the root system to maintain root-to-shoot phytohormone transport, but this generates experimental empiricism (when? how often?) and additional complexity for the grower. Nevertheless, altered soil moisture dynamics during PRD offers unique phytohormonal outcomes compared to conventional deficit irrigation when the soil is allowed to dry homogeneously. Additional benefits of repeated drying and re-wetting cycles include enhanced nutrient cycling, stimulation of root growth and microbiome dynamics.

A complementary or alternative way to manipulate phytohormone dynamics as the soil dries is to apply microbial inoculants. Soilborne bacteria have been isolated that either synthesise or metabolise the major phytohormones. Most attention has been devoted to auxin-producing bacteria that stimulate lateral root growth and ACC-deaminase containing rhizobacteria (that break down the ethylene precursor ACC) that enhance primary root elongation. Bacteria have been isolated that utilise ABA as a carbon source, but this seems to be quite a rare trait. These phytohormonal impacts modulate root hair length and density that enhance soil adherence to the root (rhizosheath development) thereby providing a niche from microbial proliferation. Microbial enhancement of rhizosheath development might also enhance crop water use efficiency.

IT 13

**Hormonal regulation of plant development: auxin and cytokinin cross-talk and beyond**

Eva Benkova

Institute of Science and Technology Austria (ISTA), Am Campus 1, Klosterneuburg, Austria

Auxin and cytokinin are key hormonal orchestrators of root system architecture and its developmental plasticity. In the past, we have identified several convergence points and pathways that might integrate auxin and cytokinin hormonal inputs to coordinate root organ development. Intriguingly, some of these recently identified molecular components seem to exceed their simple function in the auxin-cytokinin cross-talk, and they provide functional links with other regulatory pathways, for instance, by a mediating perception of environmental stimuli, such as abiotic stress, nitrate availability, or by controlling the subcellular trafficking. Our insights into mechanisms integrating these auxin and cytokinin regulatory pathways into complex molecular networks that coordinate plant growth and its flexibility to varying external inputs will be discussed.

IT 14

**Maize domestication resolves root formation and drought stress resilience**

Peng Yu

Emmy Noether Group Root Functional Biology, Institute of Crop Science and Resource Conservation (INRES),  
University of Bonn, Germany

The spread of crops and expansion of cultivation from their ancestral habitats were accompanied by substantial phenotypic changes driven by a combination of direct farmer selection and environmental adaptation. Root system function is instrumental in colonizing new habitats and acquiring resources, in particular water and nutrients in natural soils of different geographical origin. During domestication and diversification, the maize root system has become more complex by acquiring the capacity to form seminal roots, a feature largely absent in the maize progenitor teosinte. Nevertheless, the question of how the maize root system adapted its form and function during domestication and global expansion remains elusive. However, understanding the genetic basis, environmental drivers and the potential adaptive value of root system variation to changing environments is essential to develop crops resilient to future climatic challenges.

We characterized the root systems of >9,000 global maize accessions and its wild relatives, defining the geographical signature and genomic basis of variation in seminal root number (Yu et al., 2024). We demonstrated that seminal root number has increased during maize domestication followed by a decrease to limited water availability in locally adapted varieties. By combining environmental and phenotypic association analyses with linkage mapping, we identified genes linking environmental variation and seminal root number. Functional characterization of the transcription factor ZmHb77 and in silico root modelling provides evidence that reshaping root system architecture by reducing the number of seminal roots and promoting lateral root density is beneficial for the resilience of maize seedlings to drought. Our results not only reveal the past signature of domestication and adaptation of maize roots but highlight the genetic potential to improve climate resilience in future crops.

Reference

Yu P, Li C, Li M, He X, Wang D, Li H, Marcon C, Tegethof H, Klaus A, Perez-Limón S, Chen X, Delgado-Baquerizo M, Koller R, Metzner R, van Dusschoten D, Borisjuk L, Plutenko I, Mahon A, Resende Jr MFR, Salvi S, Akale A, Abdalla M, Ahmed MA, Bauer FM, Schnepf A, Lobet G, Heymans A, Suresh K, Schreiber L, McLaughlin CM, Li C, Mayer M, Schön C-C, Bernau V, von Wirén N, Sawers RJH, Wang T, Hochholdinger F (2024) Root system adaptation to water availability during maize domestication and global expansion. *Nature Genetics* 56: 1245–1256.

IT 15

**Applying CRISPR/Cas to plants: From gene editing to chromosome engineering**

Holger Puchta

Joseph Gottlieb Kölreuter Institute for Plant Sciences, Karlsruhe Institute of Technology

Till today programmable nucleases as CRISPR/Cas have been applied to plants mainly on genes for the improvement of traits. However, breeding also requires the breaking or establishing genetic linkages on the chromosome level. Using Cas9, we were able to change genetic linkages by inducing heritable translocations in the Mb range between heterologous chromosomes in *Arabidopsis thaliana*. Recent improvements in sequence analysis of crop plants reveal that multi Mb long inversions occur with high frequency between different genotypes, leading to crossover suppression. We were not only able to demonstrate that inversions up to almost chromosome size can be achieved in *Arabidopsis*, but also meiotic recombination can be redirected this way. Thus, on one side a recombination dead region could be reactivated after 5000 years and on the other almost a complete chromosome could be excluded from genetic exchange. In the future, CRISPR/Cas-mediated chromosomal engineering will allow us to restructure plant genomes according to our needs for breeding. Finally, we developed a new technology based on DSB-induced genome elimination for tissue engineering named CRISPR-Kill, allowing us to induce targeted cell death in different organs at select developmental stages. Recently we were able to set up an inducible CRISPR-Kill system, allowing also the temporal control of cell death.

IT 16

### Growth coordination from subcellular to organ scale

Jürgen Kleine-Vehn

Molecular Plant Physiology (MoPP), Faculty of Biology II, University Freiburg, Schänzlestraße 1, 79104 Freiburg, Germany

Plants display a remarkable phenotypic plasticity. Our research aims to uncover how molecular mechanisms implement environmental cues into growth regulation at various scales, from subcellular to organ level. The phytohormone auxin is central to integrating intrinsic cues with environmental information, translating this information into growth regulation. Auxin's dynamic transport within and between cells leads to spatial and temporal variations in its concentration, defining spatiotemporal auxin signalling processes essential for the acclimation of growth responses. I will present an update on recent findings of our lab.

IT 17

### Long Non-Coding RNAs in the Regulation of plant Gene Expression

M. Heidecker<sup>1</sup>, A. Christ<sup>1</sup>, R. Rigo<sup>1</sup>, T. Roulé<sup>1</sup>, M. Benhamed<sup>1</sup>, C. Charon<sup>1</sup>, F. Ariel<sup>2</sup>, T. Blein<sup>1</sup>, J. Bazin<sup>1</sup>, M. Crespi<sup>1</sup>

<sup>1</sup>Institute of Plant Sciences Paris-Saclay (IPS2), CNRS, INRA, Universities Paris-Saclay, Evry and Paris- Cité, Gif sur Yvette, France

<sup>2</sup>Instituto de Agrobiotecnología del Litoral, CONICET, FBCB, Universidad Nacional del Litoral, Santa Fe, Argentina

Many long noncoding RNAs, or lncRNAs, were shown to act as positive or negative quantitative regulators of gene expression through multiple mechanisms involving chromatin, silencing, splicing, epigenetics, translation and the modulation of small si/miRNA action. In our laboratory, we have identified lncRNAs genome-wide acting through the regulation of chromatin conformation in space or by modulating alternative splicing (AS) of specific targets. The plant lncRNA APOLO regulates multiple targets through the formation of R-loops whereas the lncRNA MARS (for MARNeral Silencing) affected chromatin condensation of neighboring loci in response to ABA to control the interaction between a distant ABA-related enhancer and its target, the MARNERAL SYNTHASE 1 gene promoter, through a chromatin looping.

On the other hand, we previously showed that another lncRNAs, called ASCO, interact with alternative splicing regulators called NSRs (for Nuclear Speckle RNA-binding proteins). This affected alternative splicing (AS) of specific NSR-dependent mRNA targets involved in lateral root organogenesis. More recently, we showed that the ASCO lncRNA also binds to the highly conserved spliceosome components PRP8a and SMD1. Furthermore, we developed a transient assay screen to search for lncRNAs able to modulate AS genome-wide and found 4 new lncRNAs having this activity. Hence, lncRNAs may integrate a dynamic network including conserved spliceosome core proteins or epigenetic chromatin regulators to affect the transcriptional output of the transcriptome. lncRNAs may reveal then novel mechanisms for transcriptome reprogramming in eukaryotes.

IT 18

### Harnessing plant transporters for sustainance agriculture

Deyang Xu, Barbara Ann Halkier, Niels Christian Sanden

University of Copenhagen, Copenhagen, Denmark

The green transition dictates an urgent need to change from animal-based towards plant-based protein sources in our diet. Rapeseed is the world's third-largest oilseed crop, but the rapeseed press cake that contains 30-40% protein with an excellent amino acid composition is unexploited as plant-based protein food for human consumption due to the presence of anti-nutritional glucosinolates.

In the model plant *Arabidopsis thaliana*, we recently discovered that the funiculus, which connects the silique septum in the mother plant with the seed, is a highly active production site for seed-bound glucosinolates. Additionally, we identified three funiculus-localized transporters UMAMIT29, -30 and -31, as glucosinolate exporters in *Arabidopsis* (Xu et al., *Nature*, 2023). UMAMIT stands for Usually Multiple Amino acids Move In and out Transporter and was believed to be a family of only amino acid transporters. We found that UMAMIT exporters and previously identified GLUCOSINOLATE TRANSPORTERS (GTRs) importers (Nour-Eldin et al., *Nature*, 2012) form a transporter cascade that is both essential and sufficient for moving glucosinolates across at least four plasma membrane barriers along the seed loading route (Sanden et al., *Nature Plants*, 2024). Mutating both importer and exporter genes eliminates seed glucosinolates, while maintaining the defense compounds in the rest of the plant. We are currently translating this transport engineering technology to rapeseed.

Successful development of a rapeseed with low glucosinolate level in the seeds without altering the glucosinolate level in the remaining part of the plant, could enable breeding towards increased disease and pest resistance and potentially open the gene pool beyond the Bronowski that is the genetic background of all elite lines. By addressing today's farming challenge in growing rapeseed after EU's ban on selected neonicotinoids in 2013, this crop has great potential for becoming an attractive protein crop for human consumption. Development of the already-existing, locally-grown rapeseed into a novel protein crop will contribute to sustainable agriculture in the green transition.

#### References

Nour-Eldin et al., *Nature* 488, pages531–534 (2012)

Xu et al., *Nature* 617, pages132–138 (2023)

*Nature Plants* volume 10, pages172–179 (2024)

IT 19

The presence of arbuscular mycorrhizal fungi in plant rhizospheres drives changes in plant and herbivore microbiome composition

Alison E. Bennett, Antonino Malacrino, Alison J. Karley

Arbuscular mycorrhizal (AM) fungi are often considered keystone rhizosphere taxa due to their associations with host plants and soil. In two systems (potato and tomato) we explored whether AM fungi influence plant microbiome composition, and the mechanism by which such an influence might occur. We grew tomatoes and potatoes with microbial communities containing AM fungi or not containing AM fungi, and exposed them to common herbivores. *Macrosiphum euphorbiae* (potato aphid) fed on potatoes, and *Manduca sexta* (tobacco hornworm), a chewing herbivore, fed on tomatoes. *M. euphorbiae* is a sap sucking aphid with a defined microbiome hosting primary and secondary symbionts, while *M. sexta* is a lepidopteran chewing herbivore with no known core microbiome. Thus, we expected these herbivores to respond to changes in the rhizosphere and host plants differently. However, in both systems we found that AM fungi in the rhizosphere altered the root microbiome and the herbivore microbiome, but not the leaf microbiome. This was surprising as the herbivore microbiome of *M. euphorbiae* and *M. sexta* are known to be assembled differently. In the tomato system we also explored whether the starting microbial community composition played a role in our results, and found that the starting microbial community composition drove larger effect sizes for shifts in microbiome composition in roots and herbivores. We then explored the mechanism for how AM fungi in the rhizosphere altered the microbiome composition of the herbivore. A previous study suggested that soil splashed on leaves, and herbivores consumed soil microbes. We tested this hypothesis by covering soil in the tomato system, and found no influence of soil coverage on herbivore microbiome composition. Thus, the influence of the rhizosphere microbiome on the herbivore microbiome occurs via the host plant. These results provide multiple opportunities for manipulating rhizosphere microbiomes in ways that could alter plant-herbivore interactions.

IT 20

Connecting hormone biosynthesis and signal transduction – the molecular basis for brassinosteroid bioactivity

Alberto Caregnato<sup>1</sup>, Houming Chen<sup>1</sup>, Miroslav Kvasnica<sup>2</sup>, Jana Oklestkova<sup>2</sup>, Miroslav Strnad<sup>2</sup>, Michael Hothorn<sup>1</sup>

<sup>1</sup>Department of Plant Sciences, University of Geneva, CH

<sup>2</sup>Palacky University, Olomouc, CZ

Brassinosteroids are vital plant steroid hormones sensed at the cell surface by a membrane signaling complex comprising the receptor kinase BRI1 and a shape-complementary co-receptor kinase. Brassinolide is considered the physiological ligand for BRI1 in the model plant *Arabidopsis*. However, chemically diverse brassinosteroids are synthesised by a complex and non-linear biosynthetic pathway in *Arabidopsis* and in other plant species. Combining structural biology, quantitative biochemistry, reverse genetics and brassinosteroid metabolomics, we have characterized the ligand binding preference of BRI1 and of its homologs BRL1, BRL2 and BRL3. We find that chemically diverse brassinosteroids accumulate in cells and can be detected with high affinity by BRI1, BRL1 and BRL3. A complete structural and mutational analysis of the BRI1 steroid binding pocket defines key determinants of ligand selectivity, uncovers the chemical features rendering a brassinosteroid bioactive, and enables the design of novel receptor agonists and antagonists. Our work highlights that three biochemically redundant but functionally unique steroid receptors in *Arabidopsis* have evolved to sense a broad spectrum of bioactive brassinosteroids, adding another layer of regulatory complexity to brassinosteroid signaling.

IT 21

Evolution and development of parasitism in the Broomrape family (Orobanchaceae)

Susann Wicke

Department for Systematic Botany and Biodiversity, Humboldt-University, Berlin

Parasitism represents the most extreme and extraordinary interaction between two plants, occurring when one plant extracts both water and nutrients from another. Parasitic plants develop a highly specialized organ called a haustorium, which allows them to directly connect with the vascular tissue of a host plant. This transition to parasitism, and thereby the abandonment of a self-sustaining lifestyle typical of plants, results in massive alterations to the genome, morphology, and physiology—or are some of these alterations foundational? This talk will explore the uniqueness of the Orobanchaceae family, known for its multiple transitions to obligate parasitism and shifts to a non-photosynthetic lifestyle. It will provide a general overview of the evolution of parasitism and the emergence, as well as potential bases, of weediness from an evolutionary, ecological, and developmental perspective, through all stages of a parasitic plant's life. In this context, insights gained from combining high-throughput sequencing, advanced bioinformatic procedures, high-performance imaging, and biotechnological applications will illustrate the developmental shifts as the parasitic lifestyle unfolds. Specifically, we will discuss the contributions of soil seed banks, extensive haustorial networks, and selected genic interactions involving Orobanchaceae-specific de novo proteins that mark the parasites' ecological-evolutionary success.

S1 T1

## Transcription factor binding motifs have been conserved for 500 million years

S. Zenker<sup>1,2</sup>, A. Meierhenrich<sup>1,2</sup>, A. Bräutigam<sup>1,2</sup><sup>1</sup>Bielefeld University, Computational Biology, Bielefeld, Germany<sup>2</sup>CeBiTec, Bielefeld, Germany

The water-to-land transition was a major event during evolution of today's plant kingdom diversity, as it required mechanisms to cope with living outside of the water, e.g. balancing water intake and loss. These mechanisms need to be regulated in response to changes in the environment. Transcriptional regulation is mediated by transcription factors (TFs), which directly bind DNA.

*Arabidopsis thaliana* has more than 1,700 annotated transcription factors belonging to 70 TF families, which have expanded through ancient whole genome and local duplication events. It is already known that some TF families bind a conserved core TF binding motif (TFBM), e.g. the WRKY TFs all recognize the DNA sequence TTGAC. We hypothesize that TFBMs have a high degree of conservation within and across most families and that the majority of the current TFBM vocabulary already existed in the last common ancestor of *A. thaliana* and *Marchantia polymorpha*, since most TF families are present in the bryophyte. Comparative analyses of more than 700 TFs from the public domain revealed a single conserved motif in 21 families and 5 families with conserved phylogenetic subgroups. Some families like C2H2 were found to be more diverse. To investigate the conservation across evolutionary timescales, we mapped all *M. polymorpha* TFs on orthogroups and we experimentally characterized binding motifs using ampDAP-seq data for 20 TFs across 14 families, including highly conserved WRKY TFs and the partially conserved MYB-related family. Our results show that in the analyzed families, TFBMs of phylogenetically similar TFs are conserved since at least 500 million years. Therefore, neofunctionalization of TFs after duplication is likely to occur on other levels like expression patterns, DNA-shape preferences, or protein:protein interactions.

S1 T2

Hornworts reveal a spatial model for pyrenoid-based CO<sub>2</sub>-concentrating mechanisms in land plantsF. W. Li<sup>1,2</sup><sup>1</sup>Boyce Thomason Institute, Ithaca, NY, United States<sup>2</sup>Cornell University, Ithaca, NY, United States

Rubisco is central to photosynthesis and therefore global primary productivity. To improve the efficiency of photosynthesis, certain organisms have evolved specialized Rubisco compartments called pyrenoids. Through the strategic placement of inorganic carbon channels and carbonic anhydrases, CO<sub>2</sub> is concentrated within pyrenoids to increase carbon fixation. The pyrenoid-based CO<sub>2</sub>-concentrating mechanism (CCM) of the green alga *Chlamydomonas reinhardtii* has been extensively studied with the hope to install it into crops. We believe that hornworts, a lineage of bryophytes and the only land plants that operate a pyrenoid-based CCM, could provide critical translational insights. Here we report the first thorough investigation of hornwort CCM using the model *Anthoceros agrestis*. We demonstrated that *A. agrestis* pyrenoids exhibit liquid-like properties, similar to *Chlamydomonas* pyrenoids. In contrast to *Chlamydomonas*, *A. agrestis* pyrenoids lack starch sheath but are instead enclosed by multiple thylakoid membranes, which could serve as an alternative CO<sub>2</sub> diffusion barrier. We found that orthologs of many core CCM components in *Chlamydomonas* are conserved in *A. agrestis* and likely play similar functions based on their subcellular localizations. Therefore, the underlying chassis for concentrating CO<sub>2</sub> is probably shared between hornworts and *Chlamydomonas*, and might have been present in the first land plants. On the other hand, we could not identify any canonical Rubisco linker protein in *A. agrestis*, implying that hornworts might adopt a different approach for pyrenoid formation. Altogether, our study provides the first spatial model for pyrenoid-based CCM in a land plant, setting the stage for future biochemical and genetic interrogations.

S1 T3

Evolution of arabinogalactan-proteins: Independent evolutionary history of protein backbone and glycosylation

L. Pfeifer<sup>1</sup>, B. Classen<sup>1</sup>

<sup>1</sup>CAU Kiel University, Pharmaceutical Biology, Kiel, Germany

Charophyte green algae (CGA) are assigned to be the closest relatives of land plants<sup>1</sup> and can therefore help to enlighten crucial processes in colonization of terrestrial habitats. As arabinogalactan proteins (AGPs) are considered common for all land plant cell walls, we were interested when these special glycoproteins evolved in plant kingdom.

With an analysis of available genomic and transcriptomic data from several plant species within the green lineage, we were able to show that AGP protein backbones seem to have evolved prior to characteristic AGP glycosylation. Carbohydrate attachment seem to have occurred firstly within the group of CGA. Our investigation therefore focussed on a number of algae from the Charales order, as well as on *Spirogyra pratensis* (Spirogyrales). AGPs were isolated *via* the use of  $\beta$ -Glc-Yariv reagent and their composition and fine-structure analysed by GC-FID/MS and AGP antibodies. Interestingly, no AGPs precipitated and no hydroxyproline was detected in all investigated members of the Charales. Within the Spirogyrales, the absence of arabinose and the presence of rhamnose side-chains (= RGP), together with occurrence of an AGP-like galactan backbone in *Spirogyra* Yariv-precipitates<sup>2</sup> led to the concept of a conserved galactan backbone structure with more flexibility in the decorating sugars.

References:

<sup>1</sup> de Vries and Archibald. 2018. *New Phytologist* 217: 1428–1434.

<sup>2</sup> Pfeifer, *et al.* 2022. *The Plant Journal* 109: 568–584

S1 T4

Investigating contributions of GIPCs to fundamental cell functions using mutants of the moss *Physcomitrium patens*

T. Haslam<sup>1</sup>, L. Wegner<sup>2</sup>, K. Ehlers<sup>2</sup>, C. Herrfurth<sup>1,3</sup>, I. Feussner<sup>1,4</sup>

<sup>1</sup>Georg-August University Goettingen, Department of Plant Biochemistry, Göttingen, Germany

<sup>2</sup>Justus-Liebig University, Institute of Botany, Gießen, Germany

<sup>3</sup>Georg-August University Goettingen, Service Unit for Metabolomics and Lipidomics, Göttingen, Germany

<sup>4</sup>Georg-August University Goettingen, Goettingen Center for Molecular Biosciences, Göttingen, Germany

Essential membrane lipids, such as complex phosphosphingolipids (in plants, glycosyl inositol phosphorylceramides, GIPCs), are difficult to functionally characterize due to non-viable phenotypes of mutants affected in their synthesis. We previously developed a CRISPR/Cas9-based method for generating knock-down mutants of essential genes, which retain sufficient activity to be viable, using the moss *Physcomitrium patens*. Following isolation and biochemical characterization of our mutants, we now report some of the developmental phenotypes observed in the most interesting alleles. Helpfully, some of these mutants displayed altered GIPC levels without corresponding increases in their free ceramide precursors, allowing for uncoupling of phenotypes associated with product deficit versus substrate accumulation, which in this case is a potent cell death signal. Additionally, the relatively uncomplicated development and organ structure of the moss simplified observation of morphological and anatomical defects. In our mutants, we observed alteration in cell division, expansion, and differentiation, as well as changes to intercellular cytosolic connectivity. Specific aspects of these phenotypes and their implications for our understanding of GIPC functions and localization will be discussed.

S1 T5

Gene space travels in the Arabideae – approaches for detecting genomic footprints of life history evolution

C. Kiefer<sup>1</sup>, N. Walden<sup>1</sup>, M. Koch<sup>1</sup>

<sup>1</sup>COS, Heidelberg University, Biodiversity and Plant Systematics, Heidelberg, Germany

Parallel evolution – the evolution of a specific trait value by the same genetic basis in distinct and unrelated species – is a common phenomenon in the Brassicaceae. It does not only affect simple characters like flower colour or trichome types but also suites of characters summarized e.g. as life-history trait. Perennial and polycarpic *Arabis alpina* and its annual sister species *Arabis montbretiana* have been established as a model system for studying life history evolution in Brassicaceae. In the phylogenetic clade which contains these two species, switches between perennial and annual life history are common. Thus, they and other species pairs in neighboring branches represent evolutionary replicates. We have sequenced 26 accessions of Arabideae representing 13 species, including three species pairs that switched from perennial to annual life history, as well as additional annuals and perennials from the same evolutionary lineage but not in a sistergroup relationship. We have *de-novo* assembled and annotated four gene space assemblies (e.g. *Draba nemorosa* with a completeness of 81% completeness based on BUSCO score) and defined orthologues and paralogues for these four species, in addition to *A. alpina* and *A. montbretiana* from published data. Furthermore, we used a mapping approach for all 26 accessions to obtain a gene space as complete as possible for all included taxa. We are comparing the results from both approaches using the *de-novo* assemblies to filter paralogues from our reference-based assemblies. These “clean” datasets are then used in multiple tests for selection to detect genomic regions that may have played a role in the multiple switches in life history modes.

S2 T1

**Feeling the warmth in spring: Elucidating molecular mechanisms underlying the morphogenetic response of spring barley to warmer temperatures**

J. Zhu<sup>1</sup>, M. Richter<sup>1</sup>, J. Lourantos<sup>1</sup>, R. George<sup>1</sup>, A. Hartmann<sup>1</sup>, Y. Zhao<sup>1</sup>, Y. Huang<sup>1</sup>, T. Hartwig<sup>2</sup>, N. Bajaczký<sup>2</sup>

<sup>1</sup>Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Department of Physiology and Cell Biology, Seeland/OT Gatersleben, Germany

<sup>2</sup>Max Planck Institute for Plant Breeding Research, Köln, Germany

Temperature is a major environmental factor governing the distribution and seasonal behavior of plants. The mild elevated ambient temperatures below heat stress profoundly affect the growth rate and morphology of plants. Global warming makes it imperative to decipher how cereal crops sense and transduce the warm temperature signals to physiological acclimation and developmental programs. Here, we phenotyped thermo-responsive traits in a spring barley collection consisting of 400 accessions grown until tillering at average spring time and mildly warmer day-time temperatures. Genome-wide association studies identified SNPs and their proximal genes associated with leaf length and tillering. We examined the organ-specific temporal dynamics of the transcriptome in three barley genotypes under both temperature conditions. The time-courses of differentially expressed genes regulated by warmer temperatures reflect the variation in warm-temperature sensitivities and thermo-morphogenetic changes. We performed MNase-defined cistrome occupancy analysis (MOA-seq) to identify genomic regions with altered chromatin accessibility and transcription factor occupancy induced by warmer temperatures. Integrating GWAS, transcriptome and cistrome, we found that i) thermomorphogenetic responses differ in a genotype- and organ-specific manner, and ii) identified certain TCP-type transcription factors as candidate genes for mediating in thermo-morphogenetic changes.

S2 T2

**Identification of core, conditional, and crosstalk components of tomato heat stress response using integrative transcriptomics and orthology**

D. Psaroudakis<sup>1</sup>, A. Khayer<sup>1,2</sup>, L. V. Aiyesa<sup>1,3</sup>, N. Bergau<sup>4</sup>, A. Tissier<sup>4</sup>, Y. Lu<sup>5</sup>, P. A. Wigge<sup>5</sup>, A. Israeli<sup>6</sup>, N. Teboul<sup>6</sup>, A. Bräutigam<sup>7</sup>  
J. Szymanski<sup>1,8,9</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

<sup>2</sup>University of Göttingen, Faculty of Agricultural Sciences, Göttingen, Germany

<sup>3</sup>University of Göttingen, Department of Crop Sciences, Göttingen, Germany

<sup>4</sup>Leibniz Institute of Plant Biochemistry (IPB), Halle a. d. Saale, Germany

<sup>5</sup>Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany

<sup>6</sup>The Hebrew University of Jerusalem, Institute of Plant Sciences and Genetics in Agriculture, Jerusalem, Israel

<sup>7</sup>Bielefeld University, Faculty of Biology, Bielefeld, Germany

<sup>8</sup>Forschungszentrum Jülich, IBG-4: Bioinformatik, Jülich, Germany

<sup>9</sup>Heinrich-Heine-Universität, Cluster of Excellence on Plant Sciences (CEPLAS), Düsseldorf, Germany

Heat stress significantly affects agricultural yield and since the frequency and severity of heatwaves is expected to increase due to climate change, this is a growing challenge for global food security. Tomato plants are particularly prone to heat exposure in both the field and the greenhouse which makes increased heat stress resilience a key trait for breeding. Several heat-associated genes have been identified in individual studies but to fully characterize the complex network of actors involved in the heat stress response, quantitatively integrating data from multiple experiments can provide a more complete answer. We have therefore compiled a comprehensive data resource containing both novel and publicly available RNA-seq data on tomato in heat stress spanning multiple tissues, genotypes, and levels and durations of stress exposure. We show that the large majority of the response is specific to the individual study but by intersecting differentially expressed genes across experiments, we can identify a robust core response of 57 genes which encode heat shock proteins, transcriptional regulators, enzymes, transporters and several uncharacterized proteins. 17 of these genes lie within previously identified genetic loci associated with heat tolerance traits. To understand the relationship of heat stress response to other abiotic stresses, we applied the same approach to all publicly available tomato RNA-seq data on drought and salt stress. Even though many genes were responsive in individual studies across multiple stresses, the core responses derived in the above manner were mostly stress-specific. Finally, we show that the core responses to these stresses are enriched with evolutionarily ancient genes with orthologs across all domains of life and that the heat core response genes form identifiable co-evolving clusters within the Streptophyta. Our study exemplifies the importance and advantage of using FAIR public data to interpret results of new stress experiments, and provides tools to perform such analyses in a relatively short time. This work is available as a preprint at <https://doi.org/10.21203/rs.3.rs-4337825/v1>.



S2 T3

**Metabolic acclimation to low temperature: a central role for C1 transfer**

T. Naegele<sup>1</sup>, A. Kitashova<sup>1</sup>, M. Lehmann<sup>2</sup>, S. Schwenkert<sup>2</sup>

<sup>1</sup>LMU München, Plant Evolutionary Cell Biology, Planegg-Martinsried, Germany

<sup>2</sup>LMU München, Plant Molecular Biology, Planegg-Martinsried, Germany

Exposing plants to a changing environment induces a large array of molecular processes which finally constitute stress and acclimation responses. While many studies have shown that regulation and adjustment of both primary and secondary metabolism as well as their metabolic interface play a crucial role for plant acclimation and survival in diverse environments, a quantitative understanding of the underlying metabolic network is still elusive. A prominent example is cold acclimation during which plants stabilize photosynthesis and increase their capacity to survive freezing temperatures. Cold acclimation affects numerous molecular processes in primary and secondary metabolism ranging from the transcriptome to the metabolome. Here, we present a study in which we analysed the regulatory interface between primary and flavonoid metabolism during cold acclimation of *Arabidopsis thaliana*. Application of an experimental protocol which combined enzyme kinetic modelling, subcellular fractionation, proteomics, and metabolomics analyses revealed a central role of sucrose biosynthesis for stabilization of photosynthetic CO<sub>2</sub> uptake at low temperature. Additionally, sucrose biosynthesis was found to regulate carbon allocation between primary and secondary metabolism under stress conditions (Kitashova et al. 2023). Subcellular analysis of the proteome and metabolome showed a compartment-specific effect of flavonoid deficiency on amino acid metabolism which was connected to a deregulated C1 metabolism. Further, deficiencies in flavonoid accumulation were found to affect the cellular protein homeostasis, most probably due to dysfunctional ribosomal and/or proteasomal complexes. In summary, these findings suggest a regulatory network which connects photosynthetic and metabolic acclimation to low temperature.

Kitashova A, Adler SO, Richter AS, Eberlein S, Dziubek D, Klipp E, Nägele T (2023) Limitation of sucrose biosynthesis shapes carbon partitioning during plant cold acclimation. *Plant, Cell and Environment* 46 (2):464-478. doi:10.1111/pce.14483

S2 T4

**Cytosolic class I glutaredoxins integrate glutathione homeostasis and stress-related redox signalling**

M. SchlöBer<sup>1</sup>, J. M. Ugalde<sup>1</sup>, A. Meyer<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, INRES - Chemical Signalling, Bonn, Germany

Reactive oxygen species (ROS) are generated in plants in response to a variety of abiotic and biotic stresses. Highly reactive superoxide, which is initially formed, gets efficiently converted into hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) that can serve as a signaling molecule due to its comparatively long half-life. In plants, H<sub>2</sub>O<sub>2</sub> is detoxified through reduction to water that relies on electrons derived from various cellular redox-active metabolites and cofactors. Detoxification of H<sub>2</sub>O<sub>2</sub> through the ascorbate-glutathione pathway results in a drain of electrons from reduced glutathione (GSH) and NADPH and transiently oxidises both redox pools. While the redox state of the NADP pools is linked to thioredoxins (TRXs) via NADPH-dependent TRX reductase (NTR), a shift in the local glutathione redox potential ( $E_{\text{GSH}}$ ) is considered to impact the redox state of certain protein through redox equilibration facilitated by glutaredoxins (GRXs). Glutaredoxins are catalytically active oxidoreductases that mediate reversible glutathionylation and deglutathionylation of protein thiols. This mechanism has been exploited to construct an  $E_{\text{GSH}}$ -biosensor where a reduction-oxidation-sensitive GFP2 (roGFP2) acts as artificial GRX target and reports changes in  $E_{\text{GSH}}$  through changes of the roGFP2 fluorescence. In the cytosol of *Arabidopsis*, two class I glutaredoxins, GRXC1 and GRXC2, have been identified. Subcellular localization studies identified GRXC1 as membrane-bound and GRXC2 as a soluble protein. Functional biochemical redundancy between both oxidoreductases explains why single deletion mutants show no distinct phenotype under a range of different growth conditions tested. While even double mutants show no detectable growth defects under non-stress conditions, they are more sensitive to the oxidant DPS (2,2'-dipyridyl disulfide) during germination. The fully reversible dynamic properties of roGFP2 in conjunction with a class I GRX enables direct *in vivo* monitoring of  $E_{\text{GSH}}$  and potential alterations in the redox state of the cytosolic glutathione pool under stress situations. We show that the presence of cytosolic class I GRXs is critical for altering the redox state of roGFP2 in response to different stresses like external injection of H<sub>2</sub>O<sub>2</sub>, hypoxia, high light, or bacterial elicitors. In this context, roGFP2 serves as a proxy for endogenous target proteins of the cytosolic GRXs that are yet to be identified.

S2 T5

### Understanding the antagonistic regulation of microRNA858a in response to biotic and abiotic stress factors

Z. Zhou<sup>1</sup>, D. Cai<sup>1</sup>

<sup>1</sup>Institute of Phytopathology, Christian-Albrechts University of Kiel, Department Molecular Phytopathology, Kiel, Germany

Plants face various environmental stresses and have developed sophisticated adaptive mechanisms to cope. Our previous research demonstrated that miR858a is a key regulator in modulating plant responses to biotic and abiotic stresses via its target, MYB111. We also demonstrated that the miR858a is antagonistically regulated by bacterial infection, mimicked by flg22 treatment (up) and by UV-B irradiation (down), respectively. A dominant role of flg22 compared with UV-B treatment by inducing miR858a expression is believed to be a mechanism how plants direct the limited resource and energy to cope with multiple concurrent threats. The mode of action remains undissolved, so far. To understand the underlying mechanisms, we performed yeast one-hybrid (Y1H) assays by screening an Arabidopsis transcription factor (TF) library with 1589 TFs from 62 TF families. A 1778 bp upstream sequence of miR858a served as bait. Initially, we identified 87 candidate interacting TFs. Subsequent validation confirmed 32 TFs specifically interact with the miR858a promoter *in vitro*. This meets the multi-function of miR858a being involved in regulating plant responses to various stress factors. In the next step, we examined the expression profiles of candidate TFs under different stress conditions, mimicked by flg22, UV-B, and co-treatment of flg22/UV-B treatments, respectively. In this way, we identified five flg22-responsive TFs (ERF73, bZIP63, NF-YC6, AL7 and AIN1) with a positive correlation with miR858a and one UV-B-responsive TF (MYBD) that correlated negatively with miR858a. The expression of MYBD was however suppressed by flg22 or by co-treatment. These data demonstrate for the first time how miR858a is antagonistically regulated by distinct stress-specific TFs, and a possible mechanism of how plants prioritize their response to multiple simultaneous stress challenges. Furthermore, these results underline the role of miRNAs in regulating plant stress response and adaptations to changing environment, offering valuable targets for breeding crops resilient to multiple stresses. Further characterization of the TFs and their interplay in regulating miR858a expression in plants currently in progress.

#### Reference:

Zhou Z, Schenke D, Shen E, Fan L, Cai D (2024). MicroRNAs constitute an additional layer in plant response to simultaneous bio- and abiotic stresses as exemplified by UV-B radiation and flg22-treatment on *Arabidopsis thaliana*, *Plant Cell & Environment*, 47(3), pp.765-781.

S3 T1

### Metabolic maintenance in photosynthesis

D. Leister<sup>1</sup>, A. Sharma<sup>2,3</sup>, N. Kerber<sup>1</sup>, T. Nägele<sup>1</sup>, B. Reiter<sup>1</sup>, V. Pasch<sup>1</sup>, S. Beeh<sup>1,4</sup>, P. Jahns<sup>5</sup>, R. Barbato<sup>6</sup>, M. Pribil<sup>2</sup>, T. Rühle<sup>1</sup>

<sup>1</sup>LMU Munich, Biology, Planegg-Martinsried, Germany

<sup>2</sup>University of Copenhagen, Department of Plant and Environmental Sciences, Copenhagen, Denmark

<sup>3</sup>The Rockefeller University, New York, NY, United States

<sup>4</sup>University of Tübingen, Department of Plant Physiology, Tübingen, Germany

<sup>5</sup>Heinrich-Heine-University Düsseldorf, Plant Biochemistry, Düsseldorf, Germany

<sup>6</sup>Università del Piemonte Orientale, Dipartimento per lo Sviluppo Sostenibile e la Transizione Ecologica, Vercelli, Italy

The enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is responsible for catalyzing the conversion of atmospheric CO<sub>2</sub> into reduced carbon compounds within the Calvin-Benson-Bassham (CBB) cycle. RuBisCO exhibits substrate promiscuity, accepting O<sub>2</sub> in addition to CO<sub>2</sub>, which leads to both carboxylation and oxygenation reactions that are prone to processing errors. These errors result in the formation of inhibitory sugar phosphates, such as xylulose-1,5-bisphosphate (XuBP), which binds to RuBisCO's active site and locks the enzyme into a catalytically impaired state. RuBisCO activase (Rca) facilitates the release of these inhibitors, including XuBP, through enzymatic remodeling. In this study, we investigate the negative impacts on plant growth and photosynthesis resulting from the disruption of the XuBP recycling system in *Arabidopsis thaliana*. This system involves Rca and two phosphatases, CbbYA and CbbYB. Our biochemical analyses demonstrate the specific dephosphorylation of XuBP by CbbYA and CbbYB, which enables the subsequent entry of xylulose-5-phosphate into the CBB cycle. Furthermore, we show the functional conservation of the XuBP phosphatase from the purple bacterium *Rhodobacter sphaeroides* through cross-species complementation assays.

Our findings underscore the physiological significance of an ancient metabolite damage-repair system in mitigating the adverse effects of RuBisCO by-products. These insights have implications for enhancing the efficiency of carbon fixation in photosynthetic organisms.

S3 T2

Spliceosomal complex components are critical for adjusting the C:N balance during high-light acclimation

G. E. Araguirang<sup>1</sup>, B. Venn<sup>2</sup>, N. M. Kelber<sup>1</sup>, R. Feil<sup>3</sup>, J. Lunn<sup>3</sup>, T. Kleine<sup>4</sup>, D. Leister<sup>4</sup>, T. Mühlhaus<sup>2</sup>, A. S. Richter<sup>1</sup>

<sup>1</sup>University of Rostock, Physiology of Plant Metabolism, Rostock, Germany

<sup>2</sup>RPTU Kaiserslautern-Landau, Kaiserslautern, Germany

<sup>3</sup>MPI Molecular Plant Physiology, Potsdam-Golm, Germany

<sup>4</sup>LMU München, München, Germany

Plant acclimation to an ever-changing environment is decisive for growth, reproduction, and survival. Light availability limits biomass production on both ends of the intensity spectrum. Therefore, the adjustment of plant metabolism is central to high-light (HL) acclimation, and the accumulation of photoprotective anthocyanins is commonly observed. However, mechanisms and factors regulating the HL acclimation response are less clear. Two *Arabidopsis* mutants of spliceosome components exhibiting a pronounced anthocyanin overaccumulation in HL were isolated from a forward genetic screen for new factors crucial for plant acclimation. Time-resolved physiological, transcriptome, and metabolome analysis revealed a vital function of the spliceosome components for rapidly adjusting gene expression and metabolism. Deficiency of INCREASED LEVEL OF POLYPLOIDY1 (ILP1), NTC-RELATED PROTEIN1 (NTR1), and PLEIOTROPIC REGULATORY LOCUS1 (PRL1) resulted in a marked overaccumulation of carbohydrates and strongly diminished amino acid biosynthesis in HL. While not generally limited in N-assimilation, *ilp1*, *ntr1*, and *prl1* showed higher glutamate levels and reduced amino acid biosynthesis in HL. The comprehensive analysis reveals a function of the spliceosome components in the conditional regulation of the carbon:nitrogen balance and the accumulation of anthocyanins during HL acclimation. The importance of gene expression, metabolic regulation, and re-direction of carbon towards anthocyanin biosynthesis for HL acclimation are discussed.

S3 T3

Integrating spatial constraints with metabolic models predicts C<sub>3</sub>, C<sub>4</sub> and intermediate photosynthesis

T. Moreira Machado<sup>1</sup>, N. Töpfer<sup>1</sup>

<sup>1</sup>University of Cologne, Institute for Plant Sciences, Köln, Germany

Enhancing photosynthesis holds promise for increased crop productivity. Most plants use C<sub>3</sub> photosynthesis, limited by Rubisco's oxygenase activity and subsequent photorespiration under high temperatures and low CO<sub>2</sub> concentrations. High photorespiration drives the expression of to C<sub>3</sub>-C<sub>4</sub> photosynthesis and eventually the evolution of the C<sub>4</sub> carbon concentration mechanism, which reduces photorespiration and improves growth through regulatory, metabolic and anatomical changes. This study examines how spatial constraints affect metabolic flux in mesophyll and bundle sheath cells. We developed a mathematical model of mesophyll and bundle sheath metabolism, incorporating changes in tissue volume and photosynthetic capacity. The model includes plant primary metabolism, with two networks connected by cytosolic metabolite exchange. Photorespiratory conditions were simulated by adjusting Rubisco's carboxylation:oxygenation ratio in both cell types. Unlike previous models, ours includes spatial constraints accompanying photosynthetic biochemical changes. Using flux balance analysis, we explored metabolic shifts for efficient photosynthesis under various conditions, incorporating parameters like relative cell sizes, chloroplast abundances, light intensities, leaf biomass compositions, and carbon and nitrogen assimilation rates. Our findings show that transitioning from C<sub>3</sub> to C<sub>3</sub>-C<sub>4</sub> and C<sub>4</sub> states correlates with increased bundle sheath volume under carbon and nitrogen-limited conditions. First, we predict a transition between C<sub>3</sub>-C<sub>4</sub> stages, involving initial activation of bundle sheath glycine decarboxylase, followed by engagement of C<sub>4</sub> decarboxylation enzymes. Second, initial glycine shuttle activity was preceded by decarboxylation of TCA cycle intermediate isocitrate, providing CO<sub>2</sub> to the bundle sheath plastid. Third, comparing *Arabidopsis thaliana* and maize (*Zea mays*) biomass compositions revealed more frequent C<sub>4</sub>-like flux solutions with maize composition. Fourth, inactivating bundle sheath photosystem II triggered C<sub>4</sub> decarboxylation enzyme activity at smaller bundle sheath sizes. Lastly, spatial constraint changes impacted energy metabolism, shifting electron transport rates, ATP production ratios, and the ATP/NADPH ratio between tissues. These insights enhance understanding of leaf anatomy and photosynthetic metabolism, guiding metabolic engineering for improved crop productivity and quality.

S3 T4

**Synthetic redesign of plant photorespiration: photorespiratory bypasses towards more efficient carbon fixation**

L. Segura Broncano<sup>1</sup>, P. Westhoff<sup>1</sup>, T. Erb<sup>2</sup>, A. P.M. Weber<sup>1</sup>

<sup>1</sup>Heinrich-Heine Universität, Plant Biochemistry, Düsseldorf, Germany

<sup>2</sup>Max Planck Institute for Terrestrial Microbiology, Biochemistry and Synthetic Metabolism, Marburg, Germany

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) is responsible for the conversion of carbon dioxide into biomass in the majority of plant species, yet is still prone to errors. The oxygenation reaction of RubisCO leads to the formation of 2-phosphoglycolate (2-PG), a harmful metabolite which is neutralized through the process of photorespiration. The photorespiratory pathway is an energetically expensive reaction that ultimately leads to the loss of 25% of the previously fixed carbon, decreasing the photosynthetic efficiency of C3 crops by 30%. Using a synthetic biology approach, our work aims to assemble, implement, and test both natural and synthetic photorespiratory bypasses in plants. To that end, we established the recently discovered  $\beta$ -hydroxyaspartate cycle (BHAC) and the new-to-nature tartronyl-CoA (TaCo) pathway in *Arabidopsis thaliana* chloroplasts, to redirect the carbon flux towards a carbon-neutral or even carbon-positive photorespiration. The implemented bypasses led to the rewiring of central metabolism, changes in plant physiology and a growth benefit in some of the transgenic lines. Analyzing the phenotypic, physiologic and metabolic changes caused by the implementation of the heterologous metabolic pathways enables a rational redesign of the pathways, iterating through the engineering cycle. Overall, we aim to contribute to the challenging goal of increasing photosynthetic efficiency, intertwining natural and synthetic metabolic networks, and shed light on the role of photorespiration in balancing plant metabolism.

S3 T5

**Discovery and characterization of a chloroplast ribose transporter in *Phaseolus vulgaris* and *Arabidopsis thaliana***

L. Voß<sup>1</sup>, I. Keller<sup>2</sup>, R. Schröder<sup>1</sup>, D. Mehner-Breitfeld<sup>3</sup>, M. Herde<sup>1</sup>, N. Medina-Escobar<sup>1</sup>, T. Brüser<sup>3</sup>, E. Neuhaus<sup>2</sup>, C. P. Witte<sup>1</sup>

<sup>1</sup>Leibniz University Hannover, Molecular Nutrition and Biochemistry of Plants, Hannover, Germany

<sup>2</sup>University Kaiserslautern, Plant Physiology, Kaiserslautern, Germany

<sup>3</sup>Leibniz University Hannover, Microbiology, Hannover, Germany

Chloroplasts are long known to exchange various monosaccharides with the cytosol but so far only glucose transporters have been identified while the ones for ribose and fructose are unknown. Ribose is released in the cytosol by nucleotide catabolism but phosphorylated in the plastids to ribose-5-phosphate by ribokinase. A cytosol to plastid transport is therefore required.

By cross-species comparative transcriptomics with nodule transcriptomes of different legumes, we have identified a plastid ribose transporter candidate. Legume species are ideally suited for such an approach. Ribose release and recycling are strongly induced in tropical legumes because they export ureides from nucleotide catabolism as nitrogen carriers of fixed nitrogen. This is not the case in legumes from temperate climate zones that export amides.

After identification of a plastid ribose transporter candidate in legumes, we first investigated the orthologous transporter in *Arabidopsis*. Ribose accumulates in corresponding mutants, which can be enhanced by the supply of nucleosides from outside. These are readily hydrolyzed to ribose and the nucleobase by the plant but ribose recycling is compromised in the mutants. In addition to ribose, constitutive fructose accumulation was also observed in the mutants, while glucose accumulated at the end of the night.

We were able to express the transporter in *Escherichia coli* mutants defective either in ribose or glucose transport and could directly demonstrate ribose and glucose transport in radioactive uptake experiments. Ribose transport is inhibited by fructose indicating that also this monosaccharide might be transported.

In transgenic nodulated hairy roots of *Phaseolus vulgaris* (common bean) we employed a transient CRISPR mutagenesis approach and could identify null mutants of the corresponding bean transporter. In the mutant nodules, ribose and fructose homeostasis were disturbed similar to what had been observed in *Arabidopsis*. We conclude that we have identified a universally conserved plastid ribose translocator of plants that also transports glucose and probably fructose *in vivo*.

S4 T1

Competition and soil fungi mediated priority effects in native and exotic European grassland plants

J. Dieskau<sup>1,2</sup>, I. Hensen<sup>1,2</sup>, N. Eisenhauer<sup>2,3</sup>, I. Gaborle<sup>1</sup>, W. Durka<sup>2,4</sup>, S. Lachmuth<sup>1,5</sup>, H. Auge<sup>2,4</sup>

<sup>1</sup>Biology, Geobotany and Botanical Garden, Halle a. d. Saale, Germany

<sup>2</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

<sup>3</sup>Biology, Leipzig University, Leipzig, Germany

<sup>4</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Community Ecology, Halle a. d. Saale, Germany

<sup>5</sup>University of Maryland Center for Environmental Science, Appalachian Laboratory, Frostburg, MD, United States

In natural plant communities, species often don't arrive simultaneously at a new site. The effect of an early-arriving species on the establishment, growth and reproduction of a late-arriving species is referred to as priority effect. Despite increasing evidence that priority effects play an important role in plant community assembly, the underlying mechanisms are not fully understood.

As a contribution to close this knowledge gap, we conducted a multi-species field experiment, with late-arriving species introduced two years after establishment of early-arriving species. To better understand how biogeographic history shapes priority effects, we used six native and six exotic species both as early- and late-arriving plants. To gain better insights into the roles of plant-plant and plant-fungi interactions, we furthermore implemented a fungicide treatment (for the removal of the soil fungi accumulated by early-arriving plants) and a herbicide treatment (for the removal of early-arriving plants and in consequence their direct competitive effects).

We tested the hypotheses that (1) competition-driven priority effects are negative, correlated to the biomass early arrivers, and stronger in exotic species, (2) the performance of late arrivers is hampered by soil fungi accumulated by early arrivers, but to a smaller extent in exotic species due enemy release, (3) soil fungi also affect early arrivers, in particular when they are native, potentially diminishing their competitive effect. In the oral presentation I will present the first results of the experiment.

S4 T2

Linking plant diversity–productivity relationships to plant functional traits and changes in soil properties

P. Dietrich<sup>1</sup>, N. Eisenhauer<sup>2</sup>, C. Roscher<sup>3</sup>

<sup>1</sup>Martin-Luther-Universität Halle, Geobotanik, Halle a. d. Saale, Germany

<sup>2</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Experimental Interaction Ecology, Leipzig, Germany

<sup>3</sup>Helmholtz Centre for Environmental Research – UFZ, Physiological Diversity, Leipzig, Germany

Positive relationships between plant diversity and productivity have been attributed to complementary resource use due to variations in plant functional traits. Additionally, it has been observed that soil properties related to nutrient availability change with plant diversity over time. However, the extent to which these soil changes contribute to long-term positive diversity–productivity relationships remains unclear. To explore this, we studied plant communities with varying species richness in a 15-year-old grassland biodiversity experiment. We assessed community biomass production and biodiversity effects (net biodiversity effects [NEs], complementarity effects [CEs], and selection effects [SEs]), along with community means of plant functional traits and soil properties.

We found that community biomass production, biodiversity effects, plant height, root length density (RLD), and all soil property variables changed with plant diversity and the presence of the dominant grass species, *Arrhenatherum elatius* (increasing except for soil pH, which decreased). Biomass production and biodiversity effects were positively related to increased plant height and RLD, and negatively related to soil pH. The presence of *A. elatius* was associated with increased soil organic carbon and decreased soil pH.

The results suggest that species richness and the presence of dominant species such as *A. elatius* alter soil organic carbon and soil pH, improving nutrient availability and favouring taller species with denser root systems. This leads to higher biomass production in species-rich communities. Our study reveals an additional mechanism, i.e. changes in soil properties, which in combination with functional trait diversity can explain the positive relationship between diversity and productivity. We recommend that future long-term experiments delve deeper into soil property changes and their interactions with plant characteristics to better understand the mechanisms shaping positive plant diversity–productivity relationships.

S4 T3

Relationship between endophytic fungi and leaf traits in a temperate biodiversity experiment

M. Köhler<sup>1</sup>

<sup>1</sup>Martin-Luther University, Institute of Geobotany, Halle a. d. Saale, Germany

Endophytic fungi living inside a leaf are a crucial part of the ecosystem. It was shown that the community composition of endophytic fungi on a large scale is mainly driven by host plant identity and geographic location. On a smaller scale, leaf traits have been shown to have a major impact on fungal endophyte communities. However, most of these studies focused on a limited number of host plant species and used plants grown under natural conditions. Using a tree biodiversity experiment, where all trees were the same age and grown under similar conditions, we were able to distinguish between effects brought by host identity, local host richness and host community assembly. We asked how leaf traits that characterize the habitat of endophytes influence the richness and community composition of endophytic fungi. Therefore, we used next-generation amplicon sequencing to derive the fungal endophyte community as well as near-infrared reflectance spectroscopy to calculate the mean values and variance of leaf traits at the individual tree level. We found that both mean trait values and within-individual variance had significant effects on fungal richness. Using mean trait values, we found that leaf dry matter content, leaf carbon and leaf carbon-nitrogen ratio had an overall negative effect on fungal endophyte richness, while leaf phosphorus content increased fungal endophyte richness, pointing towards a positive effect of leaf nutrient content on fungal richness. Additionally, we found an unexpected positive effect of within-tree trait variance to positively affect fungal endophyte richness. A probable reason for this effect of trait variance is the creation of more niche opportunities. Our study highlights the importance of investigating the understudied effect of leaf functional traits on plant-microbe interactions.

S4 T4

Role of plant functional traits in the naturalization and invasion success of Asteraceae

A. Kaur<sup>1</sup>, A. Sharma<sup>1</sup>, D. R. Batish<sup>1</sup>

<sup>1</sup>Panjab University, Botany, Chandigarh, India

Various attributes are hypothesized to facilitate the dominance of an invasive species in non-native regimes. To explore the characteristic invasive attributes of the family Asteraceae, a comparative study was conducted among nine species of this family co-occurring in the western Himalayan region. Based on their nativity and invasion status, the species were categorized as “*Invasive*”, “*Naturalized*”, and “*Native*”. Fifteen plant functional traits, strongly linked with invasion, were examined in the test species. The ANCOVA and Tukey’s analyses revealed a significant variation at  $p \leq 0.05$  between all the plant functional traits (except leaf carbon [Leaf C]) represented by “*Invasive*” and “*Native*” categories and most of the traits (except leaf area [LA], leaf nitrogen [Leaf N], Leaf C, and leaf carbon-nitrogen ratio [C:N]) represented by “*Naturalized*” and “*Native*” categories. Similarly, the “*Invasive*” and “*Naturalized*” categories also varied significantly at  $p \leq 0.05$  for most of the traits (except Leaf N, Leaf C, capitula/m<sup>2</sup> population [Cm<sup>2</sup>], seeds per capitula [Scapitula], and seed mass). Soil physicochemical properties showed an insignificant effect ( $p > 0.05$ ) on plant functional traits in different invasion categories except in the case of Leaf N, which was significantly affected by pH ( $p = 0.048$ ), N ( $p = 0.046$ ) and percent saturation ( $p = 0.019$ ). It was established that invasive species are characterized by high LA, specific leaf area [SLA], germination, and low C:N and leaf construction costs [LCC]. Most of the traits represented by native species justify their non-invasive behavior; whereas the naturalized species, despite having better size metrics (plant height), resource investment strategy (aboveground non-reproductive biomass [BNR], and aboveground reproductive biomass [BR]), and reproductive output (capitula per individual plant [Cplant], and seeds per individual plant [Splant]) failed to invade, which implies that the role of these functional aspects in imparting invasion potential to a species is not consistent in family Asteraceae. The results of PCA revealed that trait divergence plays a more imperative role in invasion success than naturalization in the species of family Asteraceae. The present study is intended to refine the pre-generalized invasion concepts associated with family Asteraceae to ensure more accurate identification of the potential invaders and better management of the existing ones.

S4 T5

Effects of Tree Species Richness on Within-Individual Leaf Trait Variation in the Tropical Rainforest

T. Proß<sup>1</sup>, H. Bruelheide<sup>1</sup>, C. Potvin<sup>2</sup>, M. Sporbert<sup>1</sup>, S. Trogisch<sup>1</sup>, S. Haider<sup>3</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg, Geobotany, Halle a. d. Saale, Germany

<sup>2</sup>McGill University, Montreal, Canada

<sup>3</sup>Leuphana University, Lüneburg, Germany

Understanding how tree species richness influences leaf traits is crucial for predicting the impact of biodiversity loss on tropical rainforest ecosystems. Traditional analyses have focused on between-species variation, known to affect ecosystem functioning. Recently, the importance of within-species variation has also been recognized. Extending this, our study examines within-individual trait variation, with the premise that each leaf uniquely adjusts to its micro-environment. Using forest plots varying in tree species richness (Sardinilla experiment, Panama), we analysed whether niche partitioning in mixed stands results in a decrease of within-species leaf trait variation and whether niche partitioning can be also observed at the level of individual trees. We focused on leaf traits that describe the growth strategy along the conservative-acquisitive spectrum of growth. We found a decrease in within-species variation of specific leaf area with increasing neighbourhood species richness. Both sampling height and local neighbourhood richness contributed to explaining within-species leaf trait variation, which however, varied in importance among different species and traits. Variation in leaf nitrogen decreased with increasing neighbourhood species richness, while the magnitude of within-individual variation of most traits was unaffected by neighbourhood species richness. Our results suggest an increased niche partitioning with increasing species richness both in a plant community and at the level of individual plants. Our findings highlight the importance of including within-individual trait variation to understand biodiversity-ecosystem functioning relationships.

S5 T1

High-resolution single-cell RNA sequencing dataset of primary root xylem reveals new developmental regulators

C. von der Mark<sup>1,2</sup>, A. Gokulendran Nair<sup>1,2</sup>, T. Depuydt<sup>1,2</sup>, T. Eekhout<sup>1,2</sup>, C. Grones<sup>1,2</sup>, M. Minne<sup>1,2</sup>, M. Saura-Sanchez<sup>1,2</sup>

J. Wendrich<sup>1,2</sup>, J. Nolf<sup>1,2</sup>, J. Staut<sup>1,2</sup>, K. Vandepoele<sup>1,2</sup>, B. De Rybel<sup>1,2</sup>

<sup>1</sup>Ghent University, VIB-UGent Center for Plant Systems Biology, Ghent, Belgium

<sup>2</sup>Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent, Belgium

Background and aim: Xylem serves an important dual role during plant growth and development as it is required for both physical support and water transport. Xylem cells produce distinct cell wall reinforcements to allow optimal water transport in the tracheary elements. To acquire these features, xylem cells follow a complex developmental trajectory during which meristematic precursors differentiate into elongated, fortified and hollow vessels. The complexity of these cellular and morphological changes is reflected in the dynamic transcriptional regulatory network during xylem formation. Numerous genes, phytohormones, signaling lipids and peptides are known to commonly contribute to xylem development. Finding new key developmental regulators and understanding their interactions thus requires a tool capable of capturing the dynamic spatiotemporal transcriptomic changes during the development accurately.

Findings: By combining single cell RNA-sequencing (scRNA-seq) with Fluorescence-Activated Cell Sorting (FACS) of developing xylem cells in Arabidopsis roots, we profiled the transcriptional changes during primary xylem development at high spatiotemporal resolution. The resulting scRNA-seq dataset allowed to discriminate between proto- and metaxylem cell types. Moreover, we observed divergent transcriptomic profiles between outer and inner metaxylem cells. As such, our dataset allowed to discriminate new sub-celltypes within the xylem lineage. We next constructed gene regulatory networks for these lineages and identified 14 putative transcriptional regulators. An elaborate functional characterization including the generation of gain- and loss-of-function lines was next initiated to validate the predicted role of these candidates during xylem development. Although the assessment of xylem phenotypes in these lines is still on-going, mutant lines for several regulators display defects in xylem development. This confirms the predictive power of our computational approach. We plan to expose these lines to drought to provide insights into the physiological relevance of the newly identified regulators and broaden our understanding regarding the function of different xylem subtypes.

Conclusion: The high-resolution xylem specific scRNA-seq dataset generated in this study provides a powerful tool to follow the developmental trajectory of xylem, explore novel developmental regulators controlling xylem formation and assess their physiological relevance during drought.

S5 T2

Reproducibly oriented cell divisions pattern the first flat body structures to set up dorsoventrality and de novo meristem formation in *Marchantia polymorpha*

E. S. Wallner<sup>1</sup>, L. Dolan<sup>1</sup>

<sup>1</sup>Gregor Mendel Institute, Vienna, Austria

Land plant bodies develop from stem cells located in meristems. However, we know little about how meristems are formed from non-meristematic cells. The haploid body of liverworts, such as *Marchantia polymorpha*, develops from unicellular spores in isolation from the parental plant, which allows all stages of development to be observed. Using timelapse imaging we discovered that the spore undergoes a spatio-temporally regulated series of oriented cell divisions that initiate development of a flat prothallus on which the first meristem is formed. The initial division of the spore is asymmetric: one daughter cell forms a rhizoid that defines the future basal pole and the other proliferates into an early cell mass (ECM) of isotropically growing cells. From this ECM one cell elongates and undergoes a formative division that produces the prothalloblast, which initiates formation of the first flat plant body - the prothallus. To form the prothallus, the prothalloblast divides transversely into a flat, four-celled plate. Oblique divisions in three of the four quadrants increase its size into a flat disc. Only one disc quadrant gives rise to a flat prothallus structure we designated as flabellum due to its fan-like appearance. A notch with apical stem cell and de novo meristem is formed on the flabellum as cell divisions become locally restricted to one precisely positioned domain of the flabellum margin. These first prothallus structures define the body axes, set up dorsoventrality of the flat *Marchantia* body and position the first meristem that produces all adult thallus tissues. The transcription factor Class III HD-Zip (MpC3HDZ) marks the developmental stages of the flat prothallus from disc to flabellum and polarises to the dorsal tissues of flabella and meristems. *Mpc3hdz* mutants are defective in setting up dorsoventrality and body flatness, which is a hallmark of the *Marchantia* body. In summary, we report how a regular set of cell divisions forms the prothallus – the first dorsoventral structure – and how cells on the margin of the prothallus develop a dorsoventralised meristem de novo. Identifying genes that regulate prothallus development and dorsoventrality will allow us to discover the molecular mechanisms for de novo meristem formation among a cell population derived from a single, isolated cell.

S5 T3

Why are some sugars toxic to plants? – Changes in root development

R. Tenhaken<sup>1</sup>, E. Ivanov Kakova<sup>1</sup>, M. Althammer<sup>1</sup>, M. Höftberger<sup>1</sup>, C. Regl<sup>2</sup>, K. Herburger<sup>3</sup>

<sup>1</sup>Universität Salzburg, Environment & Biodiversity, Salzburg, Austria

<sup>2</sup>Universität Salzburg, Biosciences, Bioanalytics, Salzburg, Austria

<sup>3</sup>University of Rostock, Rostock, Germany

Plant cells are surrounded by a carbohydrate-based cell wall, which is continuously remodelled, a process that release sugar monomers. Plants have established recycling pathways to reuse the activated sugars for future polymer biosynthesis. The first step is a phosphorylation of the sugars at the C1-position followed by a conversion to UDP-sugars by a UDP-sugar-pyrophosphorylase, which accepts many different sugar-1-phosphates. This recycling pathway must be tightly controlled as low concentrations (1-3 mM) of external arabinose or galactose cause severe alterations of the root development. Phenotypes include a programmed cell death, defects in cell plate formation after mitosis, or increased amounts of glycoproteins. In addition, we observe metabolic changes which likely explain some of the mentioned phenotypes.

References: Höftberger M et al. (2022) PLANTA 256 (26), Althammer M et al (2022) Plant Journal 109 (6):1416-1426



S5 T4

Splitting Hairs- Investigating the Role of Root Hairs in Perceiving Nutrient Availability and Local Plastic Physiological Responses

D. Jones<sup>1</sup>, C. Marcon<sup>2</sup>, H. Schneider<sup>1</sup>

<sup>1</sup>IPK Gatersleben, Genetics and Physiology of Root Development (GPW), Gatersleben, Germany

<sup>2</sup>University of Bonn, Bonn, Germany

Plant roots exhibit physiological plasticity in response to local nutrient availability. The primary site of nutrient uptake is at the root hairs, subcellular epidermal structures, which make up the majority of the root system surface area for nutrient capture and interaction with the soil environment. Homozygous mutants for genes controlling root hair length have been developed in maize, giving contrasting phenotypes of a severely reduced root hair length (*rth2*), or with a total absence of root hairs (*rth3*). Prior research suggests that root hairs are dispensable and that their presence or absence plays a less important role in root system dynamics than the soil environment including texture and water availability. However, it has also been observed that reduced root hair length results in a diminished degree of responsiveness to low nutrient availability. These mutants were used to investigate the role of the root hair in perception of local nutrient availability, and plasticity through a split-root system; leveraging the diverse spectrum of root hair lengths across the mutants and their respective background lines. We hypothesised that as root hair length decreases, so will the ability to perceive and thus respond physiologically to heterogeneous soil conditions. Single plants were grown in large (30 L) column mesocosms, with the root system split between two sub-compartments, and each compartment receiving either high, medium, or low nitrogen, to create either homogenous high or low nitrogen, or heterogenous nitrogen conditions experienced by the plant. The root architecture, anatomy of each root class, and respiration of each half root system, as well as shoot physiology, was analysed and the degree of plasticity in the root hair mutants (*rth2*, *rth3*) was determined. This split root system allows thorough investigation of local compared to systemic plasticity, providing greater insight into the role root hairs in these responses. In conducting this experiment, we have also developed high throughput and low-cost phenotyping solutions to compliment the formation of the new Genetics and Physiology of Root development group at the IPK Gatersleben.

S6 T1

Algal growth and morphogenesis-promoting factors released by cold-adapted bacteria contribute to the resilience and morphogenesis of the seaweed *Ulva* (Chlorophyta) in Antarctica (Potter Cove)

F. Ghaderiardakani<sup>1</sup>, J. F. Ulrich<sup>1</sup>, E. Barth<sup>2</sup>, M. L. Quartino<sup>3</sup>, T. Wichard<sup>1</sup>

<sup>1</sup>Friedrich Schiller University Jena, Institute for Inorganic and Analytical Chemistry, Jena, Germany

<sup>2</sup>Friedrich Schiller University Jena, Bioinformatics Core Facility, Jena, Germany

<sup>3</sup>Argentinean Antarctic Institute, Department of Coastal Biology, Buenos Aires, Argentina

Macroalgae are found in a variety of marine vegetation ecosystems around the world, contributing significantly to global net primary production. In particular, the sea lettuce, i.e., members of the genus *Ulva* (Chlorophyta), can be found in many ecological niches and are characterized by excellent adaptability to environmental changes but depend on essential associated bacteria, which release algal growth and morphogenesis-promoting-factors (AGMPFs). While it is evident that the warm-temperate Mediterranean macroalgae, such as *Ulva compressa* (cultivar *Ulva mutabilis*), may not develop under cold stress due to intrinsic physiological factors, our work investigated the hypothesis that bacteria need to be stress-adapted as well to be able to provide enough AGMPFs for growth and morphogenesis of *Ulva* throughout its life cycle, even under cold stress [1].

Our study thus aimed to understand which bacteria contribute to overcoming a variety of stressors for *Ulva* in polar regions. The green macroalga was collected from Potter Cove, King George Island (Isla 25 de Mayo), Antarctica, to study its associated microbiome and, subsequently, to identify AGMPFs releasing bacteria. Therefore, microbiome analysis was combined with morphogenetic bioassays and chemical analysis, identifying bacteria essential for algal growth under Antarctic conditions. Hereby, axenic cultures of *U. mutabilis*, previously developed as a model system for bacteria-induced algal growth and morphogenesis, were inoculated with freshly isolated and cultivable Antarctic bacteria to determine their morphogenetic activity.

The exploratory microbiome investigation recovered numerous cold-adapted AGMPF-producing bacteria. Unlike the reference bacterial strains isolated from the Mediterranean Sea, the cold-adapted isolates *Maribacter* sp. BPC-D8 and *Sulfitobacter* sp. BPC-C4, released sufficient AGMPFs, such as thalussin, necessary for algal morphogenesis even at 2°C. Our results illustrate the role of chemical mediators provided by bacteria in cross-kingdom interactions exposed to severe environmental conditions within aquatic systems. The newly isolated bacteria will enable further functional studies to understand the resilience of the holobiont *Ulva* and might applied in algal aquaculture to support growth even under adverse conditions.

[1] Ghaderiardakani, F. et al. (2020) Microbiome-dependent adaptation of seaweeds under environmental stresses: a perspective. Front. Mar. Sci. 7: 575228

S6 T2

**Plant-derived ROS licenses co-habitation with a potentially pathogenic leaf microbiota member**

F. Entila<sup>1</sup>, X. Han<sup>2</sup>, A. Mine<sup>3</sup>, P. Schulze-Lefert<sup>4,1</sup>, K. Tsuda<sup>2,1</sup>

<sup>1</sup>Max-Planck Institute for Plant Breeding Research, Köln, Germany

<sup>2</sup>Huazhong Agricultural University, Wuhan, China

<sup>3</sup>Kyoto University, Laboratory of Plant Pathology, Kyoto, Japan

<sup>4</sup>MPIZ, Plant-Microbe Interactions, Köln, Germany

Plants are perpetually exposed and inhabited by taxonomically distinct microbial assembly called microbiota. However some members of the microbiota can cause deleterious effects on plant hosts depending on its genetic predisposition or environmental conditions. The fundamental role of the plant innate immunity in controlling the microbiota residency and behavior to promote plant health remains scantily navigated. Through phenotyping of microbial colonization and immune read-outs using wild-type and immunocompromised plants, we found that commensal bacteria originally isolated from healthy *Arabidopsis thaliana* plants can induce a range of immune outputs in a strain-specific manner and does not coincide with their phylogeny. We have also revealed that the immune response RBOHD-mediated ROS generation restricted occupancy of a potentially pathogenic leaf strain *Xanthomonas* L148. The disease onset caused by *Xanthomonas* L148 in *rbohD* mutant plants is weakly mitigated by other leaf strains indicating strong host dependence of the disease phenotype. Through random genome-wide mutagenesis and subsequent genetic studies, we have shown that the *Xanthomonas* L148 *gspE* gene encoding a core type 2 secretion system (T2SS) component is crucial for the *Xanthomonas* L148 pathogenicity towards *rbohD* mutant plants. *In planta* bacterial transcriptomics also revealed that RBOHD-dependent ROS suppresses most carbohydrate-active enzymes (CAZymes) and T2SS gene expression including *gspE*. Moreso, *Xanthomonas* L148 colonization protected plants against a foliar pathogen and its protective function is independent of its pathogenic potential. Thus, plant-derived ROS turns a potentially detrimental leaf commensal into a beneficial bacterium for the host plant by suppressing T2SS allowing harmonious coexistence.

S6 T3

**Ectomycorrhiza-induced systemic defenses involve CERK-dependent and -independent pathways**

A. Polle<sup>1</sup>, S. Dreischhof<sup>1</sup>, M. Muhr<sup>2</sup>, F. Rezende<sup>1</sup>, D. Janz<sup>1</sup>, J. Schmidt<sup>1</sup>, M. Jakobi<sup>1</sup>, B. Kersten<sup>3</sup>, V. Lipka<sup>2</sup>, J. Schnitzler<sup>4</sup>

M. Fladung<sup>3</sup>, T. Teichmann<sup>2</sup>

<sup>1</sup>University of Göttingen, Forest Botany and Tree Physiology, Göttingen, Germany

<sup>2</sup>University of Göttingen, Department of Plant Cell Biology, Schwann-Schleiden Research Center for Molecular Cell Biology, Göttingen, Germany

<sup>3</sup>Thünen Institute, Großhansdorf, Germany

<sup>4</sup>Helmholtz Zentrum München (GmbH), Research Unit Environmental Simulation, Neuherberg, Germany

The interaction of mycorrhizal fungi with plant roots has beneficial effects for plant nutrition and defenses. For the exchange of nutrients, formation of a symbiotic structure is required, whereas other benefits may occur via interactions without a functional mycorrhiza. For example, volatile compounds released by mycorrhizal fungi without direct plant contact are involved in the recruitment of plant defenses. In non-host interactions, the ectomycorrhizal fungus *Laccaria bicolor* induced systemic defenses in *A. thaliana* in a CHITIN-ELICITOR-RECEPTOR Kinase (CERK)-dependent manner.

Here, we asked whether ectomycorrhizal systemic defense activation in its host species poplar (*Populus x canescens*) is dependent on CERK and involves volatile signaling.

We generated poplar CRISPR/Cas9 mutant lines, in which the chitin-inducible oxidative burst was abolished. The transgenic lines showed normal growth and mycorrhizal colonization. Wildtype poplars showed significant changes in leaf transcriptional profiles seven days after mycorrhizal colonization of roots in comparison with non-mycorrhizal poplars. The *cerk* mutants lacked an array of transcriptional responses related to immune signaling, while activation of flavonoid metabolism persisted in leaves of mycorrhizal wildtype and *cerk* lines. The flavonoid metabolism of leaves was also activated, when a direct contact between *L. bicolor* and poplar roots was prevented. The comparison of leaf transcriptomes of wildtype poplars with and without *L. bicolor* contact and of *cerk* mutants with and without mycorrhiza underpinned that the volatile-induced response was independent from CERK signaling.

Our study highlights the existence of CERK-dependent and –independent signaling pathways in a mycorrhizal host species and contributes to our understanding of plant-fungal signal transmission

S6 T4

The *Medicago truncatula* GRAS transcription factor SCL1 – a new regulator of cortical cell size and arbuscule development during mycorrhiza symbiosis

D. Schwarz<sup>1</sup>, S. Voß<sup>1</sup>, C. Seemann<sup>1</sup>, C. Heck<sup>1</sup>, N. Requena<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, JKIP - Molecular Phytopathology, Karlsruhe, Germany

Plant growth and crop productivity is greatly influenced by an interplay of the plant root and abiotic and biotic factors in the rhizosphere. The establishment and accommodation of the symbiotic arbuscular mycorrhizal (AM) fungi inside the root cortex is enabled by a major transcriptional reprogramming of the plant guiding a series of changes in root anatomy, especially of cortical cells that harbor fungal arbuscules. Many of the genes involved in the morphological reorganization of the root cortex belong to the large family of GRAS transcription factors. Several GRAS TF belonging to the MIG and SCL3 clade were shown to influence the size of arbuscule-containing cortical cells. They all can interact with DELLA proteins which are key regulators of arbuscule development and senescence through interactions with different types of mycorrhiza-induced transcription factors. Here we show that the *Medicago truncatula* GRAS transcription factor SCL1, an arbuscule-induced transcription factor, acts as an additional central regulator of cortical cell size interconnecting arbuscule developmental processes with morphological modulations of the root cortex. We could show that under non-symbiotic conditions, concomitant ectopic expression of MIG1 (a positive regulator of arbuscule-containing cell size) and DELLA1 were able to induce expression of SCL1. The downregulation of SCL1 causes a decrease in cortical cell size that subsequently leads to a severe impairment of arbuscule development. But most interestingly, ectopic expression of SCL1 also results in stunted cortical cells that accommodate fully developed but smaller arbuscules undergoing an accelerated senescence program. Taken together, these results suggest that SCL1 expression needs to be tightly controlled for the coordination of cortical cell expansion and arbuscule development. Understanding how mutualistic plant-microbe interactions can beneficially shape root architecture and impact on plant nutrient acquisition is pivotal for improving plant growth and crop productivity.

S6 T5

The fungal plant pathogen *Verticillium dahliae* engages in stable interactions with terrestrial microalgae

H. Rovenich<sup>1</sup>, E. Schnell<sup>1</sup>, A. Kraege<sup>1</sup>, H. Haeweker<sup>1</sup>, O. Nielsen<sup>1</sup>, E. Chavarro-Carrero<sup>1</sup>, B. Thomma<sup>1</sup>

<sup>1</sup>University of Cologne, Institute of Plant Sciences, Köln, Germany

*Verticillium dahliae* is a soil-borne fungal pathogen of many plant species. Despite its pathogenic lifestyle on plants, *V. dahliae* spends part of its life cycle in the soil where it encounters numerous other organisms, including unicellular green algae. Like vascular plants, these microalgae belong to the green plant lineage and are known to interact with fungi. While only few parasitic interactions have been reported, arguably the most ubiquitous and extensively characterized associations are beneficial and are represented by lichens. However, whether their capacity to interact with fungi extends to other species that are not associated with lichens remains unknown. Co-culture experiments showed that *V. dahliae* engages in stable interactions with phylogenetically diverse terrestrial microalgae, ranging from beneficial to detrimental. These interactions were characterized by close physical associations, often resulting in the formation of interspecies biofilms. In the fungus, these differential interactions involved the activation of fundamentally different transcriptional programs. The identity of fungal candidate genes suggest that molecular mechanisms involved in fungal-algal interactions resemble those governing the interactions between fungi and vascular plants.

S7 T1

**Two weeks to survive: molecular mechanisms harmonizing seed conditioning, germination, and haustoriogenesis in parasitic weeds of the Orobanchaceae family**

G. Brun<sup>1</sup>, F. Schindler<sup>2</sup>, A. Bouyrakhen<sup>1</sup>, O. Dayou<sup>1</sup>, W. Weckwerth<sup>2</sup>, S. Wicke<sup>1</sup>

<sup>1</sup>Humboldt-Universität zu Berlin, Evolution und Biodiversität der Pflanzen, Berlin, Germany

<sup>2</sup>University of Vienna, Department of Functional and Evolutionary Biology, Vienna, Austria

Specialist plants have turned into fully parasitic weeds that withdraw water, nutrients, and macromolecules from crops via a specialized feeding structure, the haustorium, thereby causing tremendous yield losses. Parasitism brought about numerous molecular and functional adaptations for seed and seedling development in obligate parasitic species of the Orobanchaceae family. Seeds must undergo a conditioning period, after which germination requires host-derived compounds like strigolactones. Then, seedlings develop a haustorium upon perception of host-derived haustorium-inducing factors (HIFs), guiding the parasite to a host within a few days before it exhausts its resources. Outstanding spatiotemporal precision is required for this developmental sequence, yet its molecular foundations remain elusive.

Here, integrating seed transcriptome data for 13 Orobanchaceae species of varying host-dependency for germination using a stationary Ornstein-Uhlenbeck model highlighted gene sets with low expression conservation that discriminate species according to their host requirements. Additionally, we combined time-series transcriptomics with targeted gene and hormone quantitation of parasite seeds during host-induced germination. We identified that the limited transcriptional variations in the early stages of germination stimulant response modulate abscisic acid (ABA) and gibberellins synthesis and signaling. We found that an early decrease in ABA levels controls downstream signaling, with a predominant role for histone deacetylation and a significant reduction in bioactive cytokinins. Finally, we demonstrated that contrary to the current paradigm, haustorium development is naturally host-independent in parasitic weeds, as seedlings use their own HIFs in a dose-dependent manner. HPLC-MS time-series analysis highlighted increases in bioactive cytokinins as the seedlings develop. We also demonstrated that new classes of HIFs, including phytosterols, quinones, and flavonoids act synergistically at hormonal concentrations. These findings coupled with transcriptomics suggests that lignin neosynthesis during conditioning is a template for producing bioactive HIFs through post-germinative oxidative stress. Our research elucidates the key molecular processes occurring during the critical first two weeks from conditioning to haustorium development, prompting for a re-evaluation of our understanding of allelopathic communication between parasitic Orobanchaceae and their hosts.

S7 T2

**Zauderer1 and Zauderer2 Encode Two F-box Proteins Involved in Embryo/seed Development and Timing of Flowering**

D. Tysiachnyi<sup>1,2</sup>, J. E. Leuendorf<sup>3</sup>, T. Schmülling<sup>3</sup>, W. Brenner<sup>1</sup>

<sup>1</sup>Universität Leipzig, General and applied botany, Leipzig, Germany

<sup>2</sup>Taras Shevchenko National University of Kyiv, Department of Biochemistry, Kyiv, Ukraine

<sup>3</sup>Freie Universität Berlin, Applied Genetics, Berlin, Germany

During their life cycle, plants pass through distinct developmental phases: embryo development, dormant seed, germination, juvenile phase, adult vegetative and reproductive phase, and finally senescence. The transitions between subsequent stages are developmentally timed and also dependent on environmental cues or triggers.

In plants, numerous processes are regulated by protein turnover, as targeted protein degradation is part of signal transduction. About 700 F-box proteins are encoded by the Arabidopsis genome but their function is not known for most of them. They are the variable substrate adapters of SCF E3 ubiquitin ligases, binding to the core complex by means of the F-box domain. They possess further domains to bind target proteins, which are thereby recruited to the ubiquitin ligase complex. The resulting poly-ubiquitination marks the target proteins for degradation by the proteasome.

We present an F-box protein mutant that is impaired at two points in its development. Firstly, the start of seed and embryo development after fertilization is apparently delayed to a variable extent. Secondly, flowering is delayed by about 5 days and the plants have produced 5 to 6 more rosette leaves before the onset of flowering. This phenotype is coupled to mutations in two as of yet uncharacterized, highly similar F-box protein-encoding genes named *ZAUDERER1* and *ZAUDERER2* (Zauderer being the German word for procrastinator). Remarkably, plants carrying homozygous mutant alleles in both genes never emerged from crossing experiments; instead, undeveloped ovules were found in the siliques, meaning that *ZAUDERER* activity is indispensable for seed development. Single mutants of either gene were indistinguishable from the wild type, pointing towards redundancy between these genes. Both aspects of the phenotype were linked and occurred only in plants carrying mutant alleles homozygous for one of the genes and heterozygous for the other one.

We hypothesize that the mode of action of the *ZAUDERER* proteins involves the degradation of one or more target proteins, which is necessary to start embryo and seed development and to start flowering. Since F-box proteins are not yet known to play a role in developmental phase transitions, this would be a novel role for F-box proteins. Therefore, we are in the process of identifying *ZAUDERER*-interacting proteins by proximity labeling in order to learn about the signaling pathway in which they are implicated.

S7 T3

Exploring the role of peptide hormones in plant growth and fruit development by altering their maturation processes

C. Francesse<sup>1</sup>, A. Pavanello<sup>1</sup>, M. Boschin<sup>1</sup>, M. Armellini<sup>1</sup>, U. Salvagnin<sup>1,2</sup>, L. Trainotti<sup>1,3</sup>

<sup>1</sup>University of Padova, Biology, Padova, Italy

<sup>2</sup>Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

<sup>3</sup>University of Padova, Botanical Garden, Padova, Italy

Plant growth mechanisms are complex genetically and environmentally determined processes along with fruit development and ripening. The former are fundamental to vegetative growth but also enable the occurrence of the latter. The latter, in turn, are essential for the successful plant reproduction and for economically valuable food sources production.

Different plant species share common regulatory networks relying on hormones, TFs, and various signaling molecules. Among these, peptide hormones (PHs), involved in both long and short distance signaling and fulfilling numerous functions<sup>1</sup>, are good candidates for regulating different developmental processes.

Recent studies have demonstrated their involvement in different plant processes, but some of their functions, especially in fruit development, are still overlooked. To shed light on this, we took advantage of the evidence that many PH families share common steps in their biosynthetic pathways<sup>2</sup>. Therefore, we decided to deregulate the expression of multiple PHs by disrupting the post-translational modifications responsible for their biological activity. Specifically, we focused on two types of post-translational modifying enzymes: proteases of the subtilase family and the Tyrosyl Protein Sulfotransferase (TPST)<sup>3</sup>. For the first targets, we employed two different approaches: one based on the overexpression of microbial protease inhibitors<sup>4</sup>, and another based on silencing through amiRNAs, as for TPST silencing. With the use of different promoters, we impaired PHs synthesis constitutively in tobacco, and in a fruit-specific manner in tomato.

By this approach, we were able to demonstrate the relevance of secreted PHs in many plant processes related to the development of different organs and to prove their important role in the regulatory dynamics occurring in fruit.

References

1. Tavormina, P., De Coninck, B., Nikonorova, N., De Smet, I. & Cammue, B. P. A. The Plant Peptidome: An Expanding Repertoire of Structural Features and Biological Functions. *Plant Cell* 27, 2095–2118 (2015).
2. Olsson, V. *et al.* Look Closely, the Beautiful May Be Small: Precursor-Derived Peptides in Plants. *Annu. Rev. Plant Biol.* 70, 153–186 (2019).
3. Stührwohldt, N. & Schaller, A. Regulation of plant peptide hormones and growth factors by post-translational modification. *Plant Biol.* 21, 49–63 (2019).
4. Stührwohldt, N., Schardon, K., Stintzi, A. & Schaller, A. A Toolbox for the Analysis of Peptide Signal Biogenesis. *Mol. Plant* 10, 1023–1025 (2017).

S7 T4

Deciphering the 3D cellular basis of morphogenesis: how the ovule bends into its final shape

T. A. Mody<sup>1</sup>, R. Ray<sup>1</sup>, G. Mosca<sup>2</sup>, K. Schneitz<sup>1</sup>

<sup>1</sup>Technical University of Munich, TUM School of Life Sciences, Plant Developmental Biology, Freising, Germany

<sup>2</sup>University of Tübingen, ZMBP, Biomechanical Modeling of Morphogenesis, Tübingen, Germany

Tissue morphogenesis remains poorly understood. In plants, a central problem is the interplay between cellular behavior of mechanically connected cells and tissue-level processes that lead to emergent properties. We use ovule curvature as a model to address this aspect, taking a comparative approach that exploits the diversity of ovule curvature across angiosperms. We apply advanced imaging and machine learning based cell segmentation to generate 3D digital ovules with single cell resolution. This allows us to investigate the hidden functional complexity of the 3D cellular architecture underlying ovule curvature. We then combine quantitative comparative morphometry and topological analysis to explore similarities and differences in the 3D cellular architectures of ovules from a variety of angiosperm species. The cellular parameters obtained are used in finite element modeling (FEM) to develop plausible models that explain the differences in ovule curvature, which in turn are functionally tested by genetics where possible. Here we present the results of our work on two species with differently shaped ovules: *Arabidopsis thaliana* and *Cardamine hirsuta*. We first generated 3D digital atlases of ovule development at single cell resolution for both species. Analysis of these 3D digital ovules combined with FEM suggests that subtle differences in cellular growth patterns in the region flanked by the integuments result in the striking differences in ovule curvature between the two species. Our work demonstrates the power of comparative 3D cellular morphometry and the importance of internal tissues and their developing cellular architecture in inducing the emergent properties characteristic of tissue morphogenesis.

S7 T5

Uncovering Gene-Phenotype Associations in Leaf Development within a Vast Wild  
*Arabidopsis thaliana* Population by Landscape Transcriptomics

E. Y. Mjema<sup>1</sup>, S. Laubinger<sup>1</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg, General Genetics, Halle a. d. Saale, Germany

Understanding the complex association between genes and phenotypic traits is crucial to unravelling molecular mechanisms driving plant development, adaptation and survival. Studying the transcriptome profiles of wild plants in their natural habitat offers significant advantages for association modelling, as it provides an accurate representation of changes due to environmental conditions and selection pressures, thereby making the observed responses ecologically relevant. Here, we explore the transcriptomic variation within wild *Arabidopsis thaliana* across diverse locations in Germany to identify genes associated with leaf development by leveraging machine learning and statistical approaches. 2,500 wild-grown plants were phenotyped for 15 distinct traits. In addition, we obtained the transcriptome of 490 individual plants. We employed LASSO regression and RFE algorithms for selecting genes linked to leaf length (LL), leaf width (LW), leaf area (LA), leaf aspect ratio (LAR, length/width) and petiole length ratio (PLR, petiole/length). Comparative assessments using Random Forest and XGBoost models enabled a robust evaluation of methodological efficacy and highlighted genes crucial for leaf development. Future work will focus on functional validations to confirm the roles of these in phenotypic trait variability, thereby advancing our understanding of plant genetic adaptability. Furthermore, these findings can serve as a valuable resource for studies aimed at improving agronomic traits in other plant species.

References

1. Cheng, C.Y., Li, Y., Varala, K., Bubert, J., Huang, J., Kim, G.J., Halim, J., Arp, J., Shih, H.J.S., Levinson, G. & Park, S.H. Evolutionarily informed machine learning enhances the power of predictive gene-to-phenotype relationships. *Nature communications*, 12, 5627 (2021)
2. Danilevicz MF, Gill M, Anderson R, Batley J, Bennamoun M, Bayer PE and Edwards D (2022) Plant Genotype to Phenotype Prediction Using Machine Learning. *Front. Genet.* 13:822173. doi: 10.3389/fgene.2022.822173
3. Breiman, L. Random forest. *Machine learning* 45, 5-32, <https://doi.org/10.1023/A:1010933404324> (2001)

S8 T1

Natural variation in a node-expressed MTP-type Zn transporter gene modulates shoot development and Zn allocation to grains in barley

J. Guo<sup>1</sup>, Z. Jia<sup>1,2</sup>, J. Kumlehn<sup>3</sup>, M. Mascher<sup>4</sup>, E. Peiter<sup>5</sup>, N. von Wirén<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research, Molecular Plant Nutrition, Physiology and Cell Biology, Gatersleben, Germany

<sup>2</sup>China Agricultural University, State Key Laboratory of Nutrient Use and Management, College of Resources and Environmental Sciences, National Academy of Agriculture Green Development, Beijing, China

<sup>3</sup>Leibniz Institute of Plant Genetics and Crop Plant Research, Plant Reproductive Biology, Physiology and Cell Biology, Gatersleben, Germany

<sup>4</sup>Leibniz Institute of Plant Genetics and Crop Plant, Genebank, Gatersleben, Germany

<sup>5</sup>Martin Luther University Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, Halle a. d. Saale, Germany

Zinc (Zn) is an essential micronutrient for plant development and human nutrition but also toxic when exceeding an optimal range. Employing molecular mechanisms governing Zn accumulation in cereal grains provides opportunities to generate naturally Zn-biofortified or Zn deficiency-tolerant crops. Barley (*Hordeum vulgare* L.) is one of the most important staple food crops and shares many similar gene functions with wheat, but the genetic and molecular mechanisms underlying Zn allocation in barley are still elusive. By exploiting natural variation in barley accessions, we identified *HvMTP1.2* which is member of the Metal Tolerance Protein 1 family as the likely causal gene in a quantitative trait locus for grain Zn concentration in barley. The corresponding *HvMTP1.2* protein localizes to the tonoplast, and in yeast it appears to mediate vacuolar sequestration of Zn<sup>2+</sup>, but not of iron, manganese, copper or cadmium. Strong promoter activity of *HvMTP1.2* is observed in the developing nodes, especially in the parenchyma cell bridge, inflorescence meristem and spikelet, which is suppressed by Zn deficiency but induced by excess Zn. Disruption of *HvMTP1.2* by Cas9-mediated genome editing reduces shoot development and yield production, probably due to Zn toxicity in nodes, but promotes Zn allocation from the uppermost node to grains. Additionally, haplotype analysis in 110 re-sequenced barley accessions revealed a threonine to serine substitution at position 224 (T224S) in *HvMTP1.2*, which abolishes Zn transport ability in yeast but increases Zn concentrations in barley grains. We further show that the S224 variant is less common in domesticated than wild barley lines and is associated with soil Zn levels at a global scale. Collectively, our results indicate that *HvMTP1.2* represents a valuable breeding target to improve grain quality and yield in barley.

S8 T2

Involvement of auxin in nutritropism of rice

T. Fujiwara<sup>1</sup>, K. Yamazaki<sup>1</sup>

<sup>1</sup>Univ. Tokyo, Graduate School of Agricultural and Life Sciences, Tokyo, Japan

Nutritropism is a phenomenon of roots changing its direction of elongation towards sources of mineral nutrients. We found this phenomenon in rice roots and demonstrated that rice roots respond to gradients of ammonium and grow towards regions of high ammonium concentrations (Yamazaki et al 2020). Nutritropism is likely to play a key role in acquisition of nutrients in soils with uneven distribution of mineral nutrients and understating its mechanism is important for physiological understanding of plants and also for efficient nutrient acquisition from soil for improved nutrient uptake from soils. We also revealed that rice nutritropism is affected by other nutrients such as phosphate (Yamazaki et al 2022). We also conducted expression profile analysis of root tissues between near and far sides from the nutrient source and found a number of genes regulated between inside and outside (Yamazaki et al 2024). To better understand mechanism of nutritropism, we have conducted mutant analysis of rice that have defects in its nutritropic response and from such mutants we identified involvement of an auxin transporter in nutritropism. Exogenous application of auxin analogs restored nutritropism of the mutant, suggesting the importance of auxin in nutritropism. The updated results will be presented at the meeting.

S8 T3

Navigating Iron Deficiency: The Dynamic Role of MTP10 in Arabidopsis

B. Meier<sup>1</sup>, S. Höller<sup>2</sup>, M. Friesch<sup>3</sup>, D. Brueckner<sup>4</sup>, G. Falkenberg<sup>4</sup>, E. Peiter<sup>1</sup>

<sup>1</sup>Martin Luther University, Plant Nutrition, Halle a. d. Saale, Germany

<sup>2</sup>University Düsseldorf, Department of Biology, Düsseldorf, Germany

<sup>3</sup>Leibniz Institute of Plant Biochemistry (IPB), Cell and Metabolic Biology, Halle a. d. Saale, Germany

<sup>4</sup>Deutsches Elektronen-Synchrotron (DESY), Photon Science, Hamburg, Germany

In plants, iron (Fe) is an essential micronutrient playing critical roles in photosynthesis and as cofactor of various enzymes. Both, deficiency and excess of Fe can result in impaired plant growth and symptoms of toxicity, respectively. A member of the Cation Diffusion Facilitator (CDF) family, the Metal Tolerance Protein (MTP) 8, is involved in metal, including Fe, homeostasis in Arabidopsis. During seed development, MTP8 mediates Mn localization to the abaxial side of the cotyledons and to cortical cells of the hypocotyl but also mediates the reallocation of Fe during germination<sup>[1]</sup>. Under Fe deficiency, MTP8 sequesters excess Mn into the vacuoles of cortex cells to secure Fe acquisition<sup>[2]</sup>. Our research identified another CDF family member, MTP10, also involved in Fe homeostasis. Yeast drop assays demonstrated that MTP10, like MTP8, complements Mn- and Fe-sensitive yeast strains, indicating its role in transporting both metals. Histochemical GUS studies revealed that *MTP10* is merely expressed in the vasculature of roots and shoots, suggesting its involvement in elemental translocation within the plant. Intriguingly, knockout mutants of MTP10 displayed a significant impairment in Fe translocation from older to younger leaves under Fe deficiency, which enhanced photosynthetic activity in older leaves but adversely affected younger leaves. This finding was supported by Perl's/DAB-staining and  $\mu$ XRF measurements which showed an overaccumulation of Fe in the vasculature of these mutants leading to altered transcriptional changes of Fe deficiency marker genes and thus indicating a disrupted regulatory response in Fe signalling. Further analyses showed that MTP10's subcellular localization changes depending on the Fe status of the plant. Under sufficient Fe supply, MTP10 localizes to the endoplasmic reticulum (ER) and Golgi vesicles, but it relocates to the plasma membrane upon Fe deficiency. In conclusion, our findings reveal MTP10 to play a prominent role in the plant's response to varying Fe availability, ensuring proper metal homeostasis and overall health.

<sup>[1]</sup>Eroglu S. et al. (2017). Metal Tolerance Protein 8 Mediates Manganese Homeostasis and Iron Reallocation during Seed Development and Germination. *Plant Physiology* 174: 1633-1647

<sup>[2]</sup>Eroglu S. et al. (2016). The vacuolar manganese transporter MTP8 determines tolerance to iron deficiency-induced chlorosis in Arabidopsis. *Plant Physiology* 170: 1030-1045

S8 T4

## Deciphering novel mechanisms of sulfur homeostasis

D. Ristova<sup>1,2</sup>, S. Basu<sup>1</sup>, S. Kopriva<sup>1,2</sup><sup>1</sup>University of Cologne, Köln, Germany<sup>2</sup>Cluster of Excellence on Plant Sciences (CEPLAS), Köln, Germany

As sessile organisms, plants are constantly challenged by fluctuations of environmental factors, including mineral nutrients. However, understanding how these nutrients are integrated at transcriptional, developmental and metabolic level is frequently limited. Sulfur (S) is an essential nutrient for all organisms. Nevertheless, research on S signaling and homeostasis is lagging, compared to other macronutrients, mostly because S deficiency in modern agriculture was not an issue until recently. However, S deficiency is becoming a threat to modern agriculture practice, especially when combined with other deficiencies. Apart from the importance of S for primary metabolism, S plays important role in the synthesis of secondary metabolites that are crucial for biotic interactions.

Here, we identified seven differentially expressed genes (DEGs) common in four species, including two monocots (*Os. Kitaake* and *S. viridis*) and two dicot species (*A. thaliana* and *S. lycopersicum*). Only one of the seven genes has established role in S signaling and homeostasis. We characterize four of the remaining six genes, and show that all have altered S homeostasis, play role in glucosinolate (GSLs) synthesis, and are link between S metabolism and other processes, such as TCA cycle and photorespiration.

S8 T5

## Role of novel plant inositol pyrophosphate synthases and phosphohydrolases in phosphate signaling

G. Schaaf<sup>1</sup>, E. Lange<sup>1</sup>, R. Schneider<sup>1</sup>, K. Lami<sup>1</sup>, V. Gaugler<sup>1</sup><sup>1</sup>University of Bonn, Institute of Crop Science and Resource Conservation, Department of Plant Nutrition, Bonn, Germany

Phosphorus (P) is an essential element and often limits crop growth as only a minor fraction of soil P is plant available. Given that worldwide P deposits that can be exploited to produce P fertilizer are limited, and considering that P is a strong global pollutant of open water bodies, there is a high interest in increasing crop P-efficiency. Moreover, a large portion of P is present as phytic acid in plant seeds, which acts as an antinutrient for humans and non-ruminant animals by rendering essential micronutrients such as iron and zinc unavailable. In plants, phosphate (Pi) homeostasis is regulated by the crosstalk between Pi starvation response transcription factors (PHRs) and stand-alone SPX proteins, which act as sensors for inositol pyrophosphates (PP-InsPs), highlighting the importance of these molecules in Pi signaling. Using a PP-InsP pulldown approach and a genome-wide screen, we recently identified novel enzymes involved in the synthesis and turnover of these enigmatic messengers. We characterized these enzymes both *in vitro* and *in planta*, and will provide insights into how these new players complement our understanding of P signaling and how they could be used to improve P use efficiency in crops.

S9 T1

## Phytochrome C and Photoperiod Response 1 Interact to Control Floral Development in Barley Under High Temperatures

K. E. Colpan Karisan<sup>1</sup>, M. Von Korff Schmising<sup>1</sup><sup>1</sup>CEPLAS / Heinrich-Heine-University Düsseldorf, Institute of Plant Genetics, Düsseldorf, Germany

The increase in the average ambient temperatures due to climate change threatens crop production globally. Barley (*Hordeum vulgare* L.) is an important cereal crop and model plant to elucidate the genetic control of high temperature adaptation in cereals. In *Arabidopsis*, phytochromes and the circadian clock genes control the growth and development in response to high ambient temperatures. In barley, *PHOTOPERIOD RESPONSE 1* (*PPD-H1*) and *EARLY FLOWERING 3* (*ELF3*) have been implicated in the control of development under high ambient temperatures. However, the genetic control of flowering time and reproductive development in response to high ambient temperatures in barley remains unclear. Natural variation at *PYHTOCHROME C* (*PhyC*) has been shown to interact with *PPD-H1* to accelerate flowering in barley under different photoperiods. Nevertheless, little is known about their interaction with high ambient temperatures. Therefore, we tested the effects of *PhyC* and its interactions with *PPD-H1* on development under high ambient night and day temperatures. For this purpose, we analysed the genetic, hormone and metabolite networks in the leaves and shoot apical meristem (SAM), which control flowering time, spike development and floret fertility downstream of *PhyC* and *PPD-H1* and in response to high ambient temperatures.



S9 T2

Effects of light fluctuations on kernel number and compensation of yield potential  
via thousand kernel weight are cultivar- and phase-specific in winter wheat

K. Sabir<sup>1</sup>, H. Stützel<sup>1</sup>, T. W. Chen<sup>2</sup>

<sup>1</sup>Leibniz Universität Hannover, Institute of Horticultural Production Systems, Hannover, Germany

<sup>2</sup>Humboldt Universität zu Berlin, Berlin, Germany

Short-term environmental fluctuations (EF) during critical phenological sub-phases of yield formation may have significant impact on grain yield in winter wheat. In addition, the magnitude of sensitivity to EF can be also genotype-specific. A better understanding of the three-way interactions between genotype, phenology and EF can help to reduce the gap between potential and actual yields under enhanced climatic variability. Here we analyzed multiple environment field trials (3 seasons, 6 sites and 3 different cropping intensities) by a novel statistical approach to estimate the sensitivities of three yield components to short-term EF in light intensity (LI), temperature and precipitation in 220 cultivars across 81 time-windows ranging from double ridge to seed desiccation. The most sensitive yield component was kernel number per spike (KpS), which was affected by short-term fluctuations of LI and temperature, especially during the sub-phase between yellow anther and tipping and at pre-grain filling. To confirm the outcome of this statistical approach, we further tested the effects of LI on yield components of 16 cultivars during identified specific sub-phases using a growth chamber experiment under five light treatments. In the experiments, sensitivity of KpS to LI during the yellow anther and tipping phases was explained by effects on floret development and increases kernel abortion, especially on the basal and middle spikelets. Interestingly, the ability to compensate the reduction of KpS in response to LI by an increase of thousand kernel weight (TKW) showed four-times variation between genotypes. To test if the combined effects of multiple sub-phase aggravate the development of KpS, another growth chamber experiment with seven light treatments and four cultivars was conducted. KpS was reduced by 17% if fluctuation occurred in only one sub-phase but by 40% in two consecutive sub-phases (yellow anther and heading), showing significant additive effects. Our results offer deep insights into complex genotype x environment interactions and highlight the significance of cultivar- and phase-specific sensitivity to environmental fluctuations. These findings provide a new avenue to search for physiological mechanisms that can be used to mitigate effects of climate variability and to maximize yield potential.

S9 T3

Homeologue-aware binding analysis of *Brassica napus* seed development transcription factors

A. Meierhenrich<sup>1</sup>, B. Verwaaijen<sup>1</sup>, D. Knoch<sup>2</sup>, T. Altmann<sup>2</sup>, A. Bräutigam<sup>1</sup>

<sup>1</sup>University Bielefeld, CeBiTec, Faculty of Biology, Computational biology, Bielefeld, Germany

<sup>2</sup>IPK Gatersleben, Molecular Genetics, Seeland / OT Gatersleben, Germany

*Brassica napus* is one of the most important oil crops, holding significant socioeconomic importance due to the high nutritional value of its oil and its use for biofuel production. The seed filling stage is particularly important for oil content, seed vigor and germination rate. Some regulators of seed development have already been discovered, such as the LEC1, ABI3, FUS3 and LEC2 genes, which form the LAFL network. However, there are many additional transcription factors (TFs) that are expressed in seeds that are not well understood. Due to the recent allotetraploidization between *B. oleracea* and *B. rapa*, *B. napus* harbors two highly similar subgenomes (A and C). As a result, and due to the whole genome triplication in the Brassicaceae family, each TF with known function can exist with multiple gene copies, including homeologues in the two subgenomes. To identify additional seed process relevant transcription factors and to test homeologue specificity, we computed a gene regulatory network (GRN) based on the associations between TFs and target genes to study the TFs. The network was constructed with GENIE3 from 2,990 publicly accessible wildtype RNA-seq datasets for *B. napus* from the NCBI Sequence Read Archive (SRA). We also test TF binding experimentally with DNA affinity purification sequencing (DAP-seq).

Using a walk-trap algorithm, the network was partitioned into communities and tested for GO term enrichment. DAP-seq and independent, tissue-specific expression analyses were carried out with >50 TFs with either known or unknown roles in seed filling from the “seed development” and “lipid localisation” communities. Many but not all TFs show shared expression patterns in the independent data. DAP-seq revealed that the majority of the candidates share a conserved binding motif with their orthologous *A. thaliana* genes. Homeologous TFs indeed share bound genes and enriched GO terms. Many TFs bind to genes that are significantly enriched in seed-relevant GO terms. Some of the TFs also appear to have regulatory properties on genes involved in photosynthesis.

S9 T4

The pan-epigenome of barley reveals epigenetic consequences of structural variations  
Z. Zhu<sup>1</sup>, S. Padmarasu<sup>1</sup>, T. Lux<sup>2</sup>, M. Spannagel<sup>2</sup>, A. Himmelbach<sup>1</sup>, M. Mascher<sup>1</sup>, N. Stein<sup>1</sup>  
<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany  
<sup>2</sup>Helmholtz Zentrum München, Neuherberg, Germany

The pan-genome of barley (*Hordeum vulgare* L.) has revealed a complex landscape of variations in presence/absence and structural variants, which are crucial for genetic and breeding studies. Understanding the functional consequences of these genetic variations, however, requires insights into regulatory sequences and epigenetic regulation. Our project aims to address this by constructing a comprehensive barley pan-epigenome, integrating data on DNA methylation, histone modifications, chromatin accessibility, and chromatin interactions. Through whole genome bisulfite sequencing, we generated the pan-methylome for the first twenty accessions of the barley pan-genome. We observed that average DNA methylation levels were context-dependent, highest in CpG context within transposable elements and substantially lower in genic regions, including coding sequences. Based on orthologous relationships, we identified differentially methylated orthogroups to explore the diversity in DNA methylation patterns across these accessions. Integrating ATAC-seq data revealed that regions of accessible chromatin, typically devoid of DNA methylation, are predominantly associated with annotated genes located on chromosome arms and marked by acetylation on histone H3 lysine 9 (H3K9ac), indicating promoter regions and potential enhancers. Conversely, trimethylation on histone H3 lysine 27 (H3K27me3) suggested gene repression. These epigenomic marks enabled us to analyze the haplotype-specific impacts of structural variations. For instance, a 141 Mb inversion on chromosome 7H in the cultivar RGT Planet led to the inversion of gene positions, along with corresponding changes in chromatin status, including accessibility and methylation profiles. While the inversion's effect on individual genes near the breakpoint needs detailed investigation, our findings suggest that large chromosomal inversions do not generally disrupt overall chromatin status.

S9 T5

Genome-wide analysis of 24-nt siRNA-mediated *de novo* DNA methylation and its role in regulating plant growth-defence trade-offs in wheat (*Triticum aestivum*)  
L. Han<sup>1</sup>, Z. Zhou<sup>1</sup>, M. Schemmel<sup>1</sup>, H. Kage<sup>2</sup>, D. Cai<sup>1</sup>

<sup>1</sup>Christian-Albrechts-University of Kiel, Institute of Phytopathology, Department of Molecular Phytopathology and Biotechnology, Kiel, Germany

<sup>2</sup>Christian-Albrechts-University of Kiel, Institute of Crop Science and Plant Breeding, Department of Agronomy and Crop Science, Kiel, Germany

The demand for wheat continues to increase to meet the world's growing population. "Wheat yield decline", a phenomenon where the yield falls drastically after successive cultivation, is still a detrimental factor for wheat production, and the underlying mechanism is not well understood. The observed changes in expression profiles of non-coding small RNAs under successive wheat cultivation prompted us to focus on 24-nt-siRNA-guided *de novo* DNA methylation and explore its role in wheat yield decline.

Our data showed that successive wheat cultivation resulted in drastic alternations of the abundance of wheat 24-nt siRNAs, which tended to cluster as hotspots across the wheat genome. WGBS (Whole Genome Bisulfite Sequencing) analyses revealed that these hotspots correlated significantly with differentially methylated regions (DMRs) in the genome. Most DMRs were located in the promoter region of genes, thus suggesting their role in regulating the expression of genes at the epigenetic level. By functional annotation of the targeted genes, we found that the majority of hyper-methylated genes were involved in physiological processes such as photosynthesis and nutrient uptake, while most hypo-methylated genes were involved in plant responses to *a/biotic* stresses. Strikingly, these results perfectly matched transcriptomic data obtained by RNAseq analyses, which showed that a large number of genes and signalling pathways related to photosynthesis in the leaf and nutrient uptake in the roots were severely suppressed, and several genes involved in plant response to various *a/biotic* stresses were highly activated, especially in roots.

These data demonstrate for the first time that *de novo* DNA methylation guided by 24-nt siRNAs plays a role in the adaptation of plants to cropping systems by regulating plant growth-defence tradeoffs. This, under successive wheat cultivation, allows the plant to prioritize its limited resources and energy primarily to adapt to changing growing conditions (e.g. defence) but at the detriment of plant growth, development and yield formation. This finding opens a new aspect for developing a sustainable wheat cropping system and for genetic improvement e.g. through genome editing. Understanding the mode of action of 24-nt-siRNA-directed *de novo* DNA methylation in regulating plant growth-defence tradeoffs and identifying the corresponding "epi-alleles" are in progress.

S10 T1

**Developmental pathways underlying sexual differentiation in a U/V sex chromosome system**

D. Liesner<sup>1</sup>, G. Cossard<sup>1</sup>, M. Zheng<sup>1</sup>, O. Godfroy<sup>2</sup>, J. Barrera-Redondo<sup>1</sup>, F. B. Haas<sup>1</sup>, S. M. Coelho<sup>1</sup>

<sup>1</sup>Max Planck Institute for Biology Tübingen, Algal Development and Evolution, Tübingen, Germany

<sup>2</sup>Station Biologique de Roscoff CNRS, Sorbonne Université,

UMR8227 Laboratory of Integrative Biology of Marine Models, Roscoff, France

In many multicellular organisms, male and female developmental fates are not determined by the classic XX/XY or ZW/ZZ systems but rather by a third type of sex chromosomes, the U/V sex chromosomes. In U/V systems, sex is expressed in the haploid phase, with U chromosomes confined to females and V chromosomes to males. Here, we explore several male, female and partially sex-reversed male lines of giant kelp (*Macrocystis pyrifera*), a brown alga (Phaeophyceae) with a U/V system, to decipher the role of U/V sex chromosomes and autosomes in the initiation of male versus female developmental programs. Using comparative transcriptomics and experimental approaches, we identify a small set of genes located on the V- and U sex chromosomes that play a role in triggering the male versus female developmental programs, and we uncover a subset of autosomal effector genes. We describe the transcriptomic pathways underlying sexual differentiation and show that male, but not female, developmental fate involves large-scale transcriptome reorganization with pervasive enrichment in regulatory genes affecting the expression of more than half of the giant kelp genome. Furthermore, male-biased genes are more species-specific and exhibit faster evolutionary rates than unbiased genes, whereas genes underlying female developmental fate are more evolutionary conserved. Our observations imply that a female-like phenotype is the “ground state” of giant kelp morphology, which is complemented by the presence of a U-chromosome, but overridden by a dominant male developmental program in the presence of a V-chromosome.

S10 T2

**The effect of flavonoids on cyanobacterial motility and their role in symbiotic relationship with land plants**

D. Büyüktas<sup>1</sup>, W. D. M. Mesquita<sup>1</sup>, A. Dadras<sup>1</sup>, C. G. Köhne<sup>1</sup>, A. Pöhlein<sup>2</sup>, C. Herfurth<sup>3,4</sup>, I. Feussner<sup>3,4</sup>, R. Daniel<sup>2</sup>

T. Darienko<sup>1</sup>, M. Lorenz<sup>5</sup>, S. de Vries<sup>1</sup>

<sup>1</sup>University of Goettingen, Department of Applied Bioinformatics, Göttingen, Germany

<sup>2</sup>University of Goettingen, Department of Genomic and Applied Microbiology, Göttingen, Germany

<sup>3</sup>Goettingen Center for Molecular Biosciences (GZMB), Service Unit for Metabolomics and Lipidomics, Göttingen, Germany

<sup>4</sup>University of Goettingen, Department of Plant Biochemistry, Göttingen, Germany

<sup>5</sup>University of Goettingen, Department of Experimental Phycology and SAG Culture Collection of Algae, Göttingen, Germany

Cyanobacteria have symbiotic relationships with diverse land plants, from bryophytes to ferns, gymnosperms, and angiosperms. One specific example is the symbiosis between *Azolla filiculoides* and its cyanobiont *Nostoc azollae*: the cyanobiont is vertically inherited, and both partners co-evolve, which results in full co-dependency. Essential for all cyanobacterial symbioses is the ability to differentiate into motile filaments, hormogonia, upon plant cues. In *Azolla*, hormogonia is required to colonize new tissue and inheritance; however, the molecular cues underlying the symbiotic transfer are only poorly understood. Salicylic acid (SA) is produced by *A. filiculoides* and in land plants as a defense response to different pathogenic microbes in the environment. When SA is exogenously applied to *A. filiculoides*, its cyanobiont's abundance and gene expression are altered. Our preliminary transcriptomic data suggest that SA may control flavonoids, metabolites that in the past have been suggested to influence cyanobacterial motility. We want to test whether SA and flavonoids play a role in cyanobacterial symbiotic relationships. To do that, we investigated the effect of SA and flavonoids produced by *A. filiculoides* on cyanobacterial symbionts. We induced motility in cyanobacteria using far-red light and tested the ability of the phenolic compounds to modulate the transition into hormogonia stages. The results of the treatments were documented using light microscopy and RNA was extracted for further analyses and then sequenced. We set up a machine-learning-based image analysis pipeline and analysed light microscopy images to quantify differentiation into hormogonia and the amount of vegetative cells. We found that SA and the majority of flavonoids tested enhanced hormogonia transition in a symbiotic cyanobacterium. Now, we are analysing the RNA-Seq data obtained from this experiment to connect differential gene expression to the motility phenotype, hereby focusing on genes known to modulate motility, nitrogen-fixation, and those suggested to be related to the communication between cyanobacteria and their hosts.

S10 T3

**Starch reorganizes and becomes more accessible in maturing cells of the green alga *Zygnema* sp.**Q. Wang<sup>1</sup>, C. Uwizeye<sup>2</sup>, P. H. Jouneau<sup>3</sup>, D. Falconet<sup>2</sup>, E. Marechal<sup>2</sup>, A. Holzinger<sup>4</sup>, K. Herburger<sup>1</sup><sup>1</sup>University of Rostock, Institute of Biological Sciences, Rostock, Germany<sup>2</sup>INRAE, University Grenoble Alpes, Laboratoire de Physiologie Cellulaire et Végétale, CEA, CNRS, Grenoble, France<sup>3</sup>University Grenoble Alpes, Laboratoire Modélisation et Exploration des Matériaux, IRIG, CEA, Grenoble, France<sup>4</sup>University of Innsbruck, Department of Botany, Innsbruck, Austria

Starch is the fundamental storage polysaccharide of plants and deposited in the form of granules in pyrenoids of Zygnematophyceae, the green algal group most closely related to all land plants. These granules are mainly composed of two  $\alpha$ -1-4-linked glucose homopolymers: linear amylose and branched amylopectin. Interestingly, our FIB-SEM, SEM and particle analyzing data showed that in maturing cells of filamentous *Zygnema* sp., the granules become smaller and fragmented, suggesting a reorganization of the starch pool with cell age.

To explore the purpose of this reorganization, we thoroughly investigated the mostly unknown multiscale structure of Zygnematophyceae starch in young (~1 month) and old (~12 months) *Zygnema* sp. cultures. XRD analysis revealed a significant decrease in starch crystallinity in old cells, which can be explained by increased chain lengths and branching degrees of the amylopectin fraction and a decreased amylose content (SEC, <sup>1</sup>H NMR and HPAEC-PAD data). Enzymatic digestion models suggested a smaller fraction of resistant starch in old cells, aligning with the lower amylose content.

When exposing axenic *Zygnema* sp. to starch preparations as a carbon source, we found higher growth rates on “old” starch than on “young” starch. This was not true for de-branched starch preparations. Moreover, axenic algae grew best on their own starch, while different land plant starches (maize, wheat, rice, etc.) produced much lower growth rates, suggesting that algae customize their own starch to some extent, even though the composition resembles that of land plants.

In summary, structural analyses show that young and old algal starch differ significantly in their multiscale structure and that old starch can be broken down more efficiently. We suggest that this equips algae with a rapidly usable carbon pool that enables high growth rates, for instance, when mature resistant cells (akinetes) with low photosynthesis rates are able to rapidly germinate into new filaments.

S10 T4

Unraveling adaptive responses of the green alga *Chlamydomonas reinhardtii* and its bacterial interactions in a nature-like environment

T. Vuong<sup>1</sup>, E. Kurtoglu<sup>1</sup>, C. Schultz<sup>2</sup>, L. Schrader<sup>1</sup>, P. Then<sup>3</sup>, J. Petersen<sup>1</sup>, M. Westermann<sup>4</sup>, A. Rredhi<sup>1</sup>, S. Chowdhury<sup>5</sup>  
R. Mukherji<sup>5</sup>, M. Schmitt<sup>6</sup>, J. Popp<sup>2,6,7</sup>, P. Stallforth<sup>5,7</sup>, M. Mittag<sup>1,7</sup>

<sup>1</sup>Matthias Schleiden Institute of Genetics, Bioinformatics and Molecular Botany, Friedrich Schiller University Jena, Jena, Germany

<sup>2</sup>Leibniz Institute of Photonic Technology (IPHT), Member of Leibniz Health Technologies,  
Member of the Leibniz Center for Photonics in Infection Research (LPI), Jena, Germany

<sup>3</sup>Microverse Imaging Center, Balance of the Microverse Cluster of Excellence, Friedrich Schiller University Jena, Jena, Germany

<sup>4</sup>Electron Microscopy Center, Jena University Hospital, Jena, Germany

<sup>5</sup>Department of Paleobiotechnology, Leibniz Institute for Natural Product Research and Infection Biology,  
Hans Knöll Institute (HKI), Jena, Germany

<sup>6</sup>Institute of Physical Chemistry (IPC) and Abbe Center of Photonics (ACP),

Member of the Leibniz Center for Photonics in Infection Research (LPI), Friedrich Schiller University Jena, Jena, Germany

<sup>7</sup>Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Jena, Germany

Photosynthetic microorganisms including microalgae and cyanobacteria are responsible for approximately 50% of global CO<sub>2</sub> fixation. The unicellular green alga *Chlamydomonas reinhardtii* serves as a model organism to study the influence of biotic interactions and abiotic factors. *C. reinhardtii* was originally isolated from a potato field and is also found in rice paddy fields. It was previously shown that the antagonistic bacterium *Pseudomonas protegens* exhibits algicidal activity against *C. reinhardtii* by secreting toxins (Aiyar *et al.*, 2017; Hotter *et al.*, 2021; Rose *et al.*, 2021). A third bacterium, *Mycetocola lacteus*, can rescue the alga by inactivating one of the bacterial toxins. All three genera *Pseudomonas*, *Mycetocola* and *Chlamydomonas* are found together in the microbial metagenome of rice wash water (Carrasco Flores *et al.*, 2024). Here, we investigated the adaptive responses of *C. reinhardtii* in a rice soil-like environment by using non-shaken cultures, a medium with acetate as found in rice soil and spatially structured 3-dimensional components (S3-D) as solid support. We found that the growth of *C. reinhardtii* is enhanced in S3-D compared to pure liquid culture under these conditions where the algae grow mixotrophically. Under these conditions, the algal cells undergo major alterations in their morphology and physiology in S3-D compared to pure liquid culture. Changes in the algal cell size, ciliary length, cell wall, eyespot area, starch and lipid accumulation as well as pigment biosynthesis were observed. Moreover, we found changes in algal-bacterial interactions under these conditions. Our data reveal how a nature-like environment influences a green soil alga as well as its bacterial interactions.

References

Aiyar *et al.*, 2017, *Nat Comm* 8: 1–13.

Hotter *et al.*, 2021, *PNAS* 118: e2107695118.

Rose *et al.*, 2021, *Environ Microbiol* 23 9: 5525-5540.

Carrasco Flores *et al.* 2024, *PNAS* 96 121: e2401632121.

S10 T5

Surviving in an extreme environment: Cyanobacteria and microalgae of biological soil crusts in Spitsbergen (High Arctic)

B. Becker<sup>1</sup>, E. Pushkareva<sup>1</sup>, E. Hejdukova<sup>2</sup>, A. Kologmiets<sup>2</sup>, O. Bren<sup>2</sup>, P. Pribyl<sup>2</sup>, J. Elster<sup>2</sup>

<sup>1</sup>University of Cologne, Institute for Plant Sciences, Köln, Germany

<sup>2</sup>University of South Bohemia, Centre for Polar Ecology, Ceske Budejovice, Czech Republic

**Introduction:** The High Arctic deserts are extreme polar habitats characterised by low temperatures, extreme light conditions (approximately 90 days of polar night and midnight sun each year) and temporary shortages of liquid water and nutrients. However, cyanobacteria, algae, lichens, microfungi and bryophytes in various combinations form a cover of biological soil crusts that are important primary producers, fix nitrogen and stabilise the soil. However, the microbial communities within Arctic biocrusts and their strategies for surviving harsh conditions are poorly understood.

**Methods:** Field and laboratory experiments were carried out during 2022-2024. For the field experiment, three sites at different altitudes (47, 409 and 519 m a.s.l.) were established near Longyearbyen (Central Spitsbergen) in August 2022, and various environmental factors such as temperature and irradiance were monitored during the study. Several algal and cyanobacterial strains were isolated and some algal strains were selected for further cryo- and desiccation experiments, cell vitality assessment and transcriptomic studies. The field manipulation experiment included additional water or nutrient supply and winter thaw experiments. Photosynthetic activity was measured throughout the year using a fluorophotometer.

**Results:** Metagenomic and metatranscriptomic analyses revealed significant differences in microbial community composition between sites at different altitudes. The fungal community was dominated by Ascomycota and Basidiomycota, with lichenisation and saprotrophic functional traits. Cyanobacteria were the dominant primary producers and consisted mainly of heterocystous cyanobacteria. Our study identified molecular mechanisms underlying cold adaptation, including the expression of heat shock proteins and cold-inducible RNA helicases in cyanobacteria and fungi. Photosynthetic activity, measured throughout the year in the field, showed that biocrusts are active at near-zero or even mild sub-zero temperatures and recover very quickly when artificially thawed in the laboratory.

**Conclusion:** Overall, the microbial communities appear to be permanently well adapted to the extreme environment.

S11 T1

Phosphoinositides modulate auxin-dependent transcription by controlling the histone acetyltransferase GCN5 in Arabidopsis

F. Daamen<sup>1</sup>, M. Heilmann<sup>2</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg, Plant Nutrition, Halle a. d. Saale, Germany

<sup>2</sup>Martin Luther University Halle-Wittenberg, Plant Biochemistry, Halle a. d. Saale, Germany

Phosphoinositides (PIs) are signaling lipids and their biosynthesis in the cellular membranes is regulated by families of lipid kinases. In addition to their functions in the cytoplasm and at the plasma membrane, PIs and phosphatidylinositol 4-phosphate 5-kinases (PIP5Ks) are also found in the nucleus of plants, so far with unclear function<sup>[1,2]</sup>. Here, we show that in adult Arabidopsis plants, overexpression or underexpression of PIP5K1 or PIP5K2 affects the degree of nuclear histone acetylation. Systematic interaction tests between PIP5K2 and histone-modifying enzymes revealed an interaction with the histone acetyltransferase GENERAL CONTROL NON REPRESSIBLE 5 (GCN5). GCN5 regulates the expression of several developmentally and stress-regulated genes through acetylation of histone H3, and effects of GCN5 on the transcription of the auxin-dependent expressed gene *GRETCHEN HAGEN 3.3* (*GH3.3*) is well studied<sup>[3]</sup>. Effects of PIs on the GCN5/auxin-mediated activation of *GH3.3* transcription were tested by overexpressing PIP5K2 in Arabidopsis mesophyll protoplasts. The expression with PIP5K2 attenuated auxin-triggered *GH3.3* induction, whereas overexpression of an inactive PIP5K2 K470A variant or the functionally divergent PIP5K6 isoform had no effect. The histone acetylation activity of recombinant GCN5 was inhibited *in vitro* by PIs, indicating a direct effect of PIs on GCN5 function. In addition, GCN5 can bind to PIs *in vitro* via three basic amino acid residues. This PI-binding ability affects the nuclear localization of GCN5, and GCN5 substitution variants incapable of binding PIs showed a relaxed and significantly more cytosolic localization than wild type GCN5, which was almost exclusively observed in the nucleus. Overall, the data suggest that GCN5, and possibly other components of the histone acetylation complex, can be recruited by PIs to active transcription sites in the nucleus, as has previously been proposed<sup>[4]</sup>. Modification of PI levels by nuclear PI4P 5-kinases results in altered PI composition, thereby modulating GCN5 activity and localization, thus contributing to the regulation of the transcriptional activity of specific genes.

[1] Gerth et al., (2017) Plant J; [2] Ischebeck et al., (2013) Plant Cell; [3] Weiste and Dröge-Laser, (2014) Nat Commun; [4] Soliman and Riabowol, (2007) Trends Biochem Sci

S11 T2

Role of the ER-bound transcription factor ANAC013 and its cleavage by rhomboid-like protease RBL2  
in mitochondrial retrograde signalling under hypoxia stress

T. Renziehausen<sup>1</sup>, E. Eysholdt-Derzso<sup>2</sup>, S. Frings<sup>1</sup>, S. Frohn<sup>3</sup>, M. Sauter<sup>2</sup>, I. De Clercq<sup>4</sup>, J. van Dongen<sup>5</sup>, J. Schippers<sup>3</sup>  
R. Schmidt-Schippers<sup>1</sup>

<sup>1</sup>Bielefeld University, Plant Biotechnology, Bielefeld, Germany

<sup>2</sup>University of Kiel, Plant Developmental Biology and Plant Physiology, Kiel, Germany

<sup>3</sup>IPK Gatersleben, Department of Molecular Genetics, Seeland, Germany

<sup>4</sup>Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent, Belgium

<sup>5</sup>RWTH Aachen University, Institute of Biology I, Aachen, Germany

During flooding, which increasingly occurs due to climate change, impaired oxygen availability interferes strongly with plant growth, development and survival. For growth and metabolic adjustment to stress, plants must quickly sense oxygen deficiency and activate transcriptional responses for adaptation. We identified an early-bird transcription factor of the ANAC-family in Arabidopsis, which is involved in mitochondrial retrograde signalling and quickly translocates from the ER to the nucleus under hypoxia. Importantly, this ANAC factor activates crucial hypoxia core genes such as *ADH1*, *PDC1* and *PGB1* under stress by physical binding to specific *cis*-elements in the target promoters, as shown by transactivation assays, electrophoretic mobility shift assays (EMSA) and CHIP-SEQ analysis. Certain residues in the ANAC transmembrane domain were identified to be crucial for transcription factor release from the ER and we provide evidence that a specific RHOMBOID-LIKE (RBL) protease is responsible for ANAC cleavage during stress. In line with this, knock-out lines for *ANAC* and *RBL* genes display reduced anoxia tolerance, underlining their importance in hypoxia signalling. In summary, a novel ANAC-RBL module is introduced, which acts during the onset of hypoxia enabling fast mitochondrion-dependent transcriptional reprogramming for stress adaptation and plant survival.

S11 T3

*In vivo* detection of dynamic light-induced H<sub>2</sub>O<sub>2</sub> release from chloroplasts for putative retrograde communication

J. M. Ugalde<sup>1</sup>, A. Meyer<sup>1</sup>

<sup>1</sup>University of Bonn, Bonn, Germany

Chloroplasts as endosymbiotic organelles rely on the nuclear genome for most of their proteins. To ensure appropriate gene expression and translation on demand, multiple retrograde control mechanisms have evolved by which chloroplasts communicate their current state to the nucleus. Among these, redox-dependent signals take a prime role since they are directly linked to photosynthetic activity. Upon illumination, the photosynthetic machinery inevitably generates reactive oxygen species (ROS), such as superoxide and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), that are potentially damaging and thus need to be detoxified. For retrograde communication, H<sub>2</sub>O<sub>2</sub> as the most stable ROS form may diffuse to the cytosol and other compartments, where it can ultimately affect thiol-based redox switches on proteins including transcription factors. Due to H<sub>2</sub>O<sub>2</sub> detoxification along the ascorbate-glutathione pathway, H<sub>2</sub>O<sub>2</sub> may also cause transient oxidation of the glutathione pool leading to a less negative glutathione redox potential ( $E_{\text{GSH}}$ ). Hydrogen peroxide has long been considered a retrograde signal but direct evidence for light-dependent H<sub>2</sub>O<sub>2</sub> release from chloroplasts and its dynamics is missing. Genetically encoded sensors, such as HyPer7 for H<sub>2</sub>O<sub>2</sub> and Grx1-roGFP2 for  $E_{\text{GSH}}$  offer opportunities for studying these parameters in live cells. Yet, time-resolved measurements of light-dependent processes with fluorescent reporters are challenging as illumination can only be done between measurements. Here, we developed a novel setup with automatized internal illumination of plants inside a fluorescence plate reader that allows to minimize the delay between illumination and the measurements. With this setup, we measured the dynamic changes in  $E_{\text{GSH}}$  and H<sub>2</sub>O<sub>2</sub> during dark-light transitions in chloroplasts and the cytosol of Arabidopsis using the different sensors targeted to both compartments. Upon illumination with different qualities and intensities of light, there is a fast and transient H<sub>2</sub>O<sub>2</sub> increase in chloroplasts, and a decrease of  $E_{\text{GSH}}$ , followed by a slower increase of H<sub>2</sub>O<sub>2</sub> and limited glutathione oxidation in cytosol. After transition to darkness, these parameters rapidly recover in both compartments. The ability to record the amplitude and dynamics of light-induced H<sub>2</sub>O<sub>2</sub> release from chloroplasts enabled us to investigate the effects of pharmacologically imposed photo-oxidative stress and to genetically dissect the generation of H<sub>2</sub>O<sub>2</sub> signals and the putative decoding machinery in the cytosol.

S11 T4

**Defining a novel peroxisome-mediated strategy of plant viruses to combat RNA interference**

S. Wirling<sup>1</sup>, L. M. Halscheid<sup>1</sup>, C. Jeffries<sup>2</sup>, U. Krämer<sup>3</sup>, S. Reumann<sup>1</sup>

<sup>1</sup>Universität Hamburg, Biology, Hamburg, Germany

<sup>2</sup>EMBL Hamburg, Hamburg, Germany

<sup>3</sup>Ruhr-Universität Bochum, Faculty of Biology and Biotechnology, Bochum, Germany

RNA interference (RNAi) is the primary defense mechanism of plants against viral pathogens. In the evolutionary arms race, viruses developed several ways to evade this mechanism. The peanut clump virus (PCV) encodes a 15 kDa protein (P15) that functions as a viral suppressor of RNAi by binding small interfering (si) RNAs in the cytosol and co-exporting them by the peroxisome targeting signal type 1 (PTS1) of P15 into the lumen of peroxisomes (Incarbone et al., 2017). This mechanism of piggy-back transport of nucleic acids into peroxisomes is so far unique for plant-virus interactions. We first investigated whether this siRNA elimination mechanism evolved also in other plant viruses. Even though P15 is highly variable in primary sequence, we were able to identify 18 orthologs in six different viral genera, with nine of them containing a predicted PTS1. Peroxisome targeting of these putative orthologs was experimentally validated *in vivo* in *Arabidopsis thaliana* seedlings and *Nicotiana benthamiana* leaves. The shared cellular localization suggests a broader distribution of the mechanism among viruses in the *Virgaviridae* family. PCV P15 consists of a cysteine-rich N-terminal  $\alpha/b$ -fold and a C-terminal coiled-coil domain. To elucidate the structure and siRNA binding mechanism of P15, we recombinantly produced it in *E. coli*, and we optimized the challenging purification process. The affinity of P15 to different siRNAs of varying lengths was investigated using microscale thermophoresis and revealed a size-selective binding mechanism for double-stranded siRNA of 21-24 nt as well as a KD in the lower micromolar range. We identified a zinc-binding site in the N-terminal domain, and experimentally determined Zn<sup>2+</sup> binding to P15 in a 1:1 molar stoichiometry. The formation of P15 dimers was shown by size-exclusion chromatography and mass photometry. With the introduction of over 20 different mutations into P15, the functions of domains and single amino acid residues in oligomerization and siRNA binding were determined. The coiled-coil domain was found to mediate P15 dimerization. Currently, we are using small-angle X-ray scattering in addition to crystallization to resolve the 3D structures of the apo and siRNA-bound forms of P15.

References

Incarbone M, Zimmermann A, Hammann P, Erhardt M, Michel F, Dunoyer P. Neutralization of mobile antiviral small RNA through peroxisomal import. *Nat Plants*. 2017 Jun 19;3:17094. doi: 10.1038/nplants.2017.94.

S11 T5

**Plastidial amino acid transporter proteins in *A. thaliana***

F. Kuhnert<sup>1</sup>, K. Vogel<sup>1</sup>, P. Westhoff<sup>2</sup>, P. Lundquist<sup>1</sup>, C. Rosar<sup>1</sup>, T. Goss<sup>1</sup>, V. Valencia<sup>1</sup>, A. Weber<sup>1</sup>

<sup>1</sup>Heinrich Heine University Düsseldorf, Plant Biochemistry, Düsseldorf, Germany

<sup>2</sup>Heinrich Heine University Düsseldorf, Metabolomics and Metabolism Laboratory, Düsseldorf, Germany

Amino acids are essential building blocks in all living organisms. They are involved in protein biosynthesis, nitrogen storage and transport, plant growth and development, and signaling, amongst others. Plants can synthesize all proteinogenic amino acids *de novo* in leaf tissue. In addition, plants can take up amino acids from soil by the roots and transport them throughout the plant by the xylem and phloem.

*De novo* amino acid biosynthesis is highly compartmentalized and most amino acids are synthesized inside plastids. Amino acid transporters are required inside the plastid envelope to export the amino acids and supply the remaining cell compartments. We identified a protein family involved in amino acid metabolism containing a domain of unknown function (DUF) 3411. The designation RETICULATA (RE) stems from the reticulate leaf phenotype displayed by some knock-out mutants of this family due to altered mesophyll development. *Arabidopsis thaliana* RE1 is localized in the inner chloroplast membrane. RE1 mutants show the reticulate phenotype, growth deficiencies on media with externally supplied basic amino acids and altered basic amino acid content. Functional redundancy is implied for RE1 and RER1, its closest homolog, due to a lethal phenotype in double knockout mutants. These and further results obtained through yeast complementation analyses and uptake assays with reconstituted proteins in liposomes provide evidence for novel basic amino acid carriers in plastids of *Arabidopsis*.



S12 T1

Reducing the sinapine levels of *Camelina sativa* seeds through targeted genome editing of REF1

K. Feussner<sup>1</sup>, A. Kelly<sup>1</sup>, M. Fulda<sup>1</sup>, M. Aden<sup>1</sup>, I. Abreu<sup>1</sup>, I. Feussner<sup>1</sup>

<sup>1</sup>University of Goettingen, Plant Biochemistry, Göttingen, Germany

Sinapine (sinapoylcholine) is the major phenolic metabolite typically found in the oil-rich seeds of Brassicaceae such as *Camelina sativa* or *Brassica napus*. Here it imparts a bitter taste to the seeds as a defense mechanism against herbivores, but it also renders them less palatable to livestock. To allow more *Camelina* flour to be used for human consumption or as animal feed, we aimed at a reduction of the sinapine content through CRISPR/Cas9 - based genome editing of *REF1*, which encodes the NADP<sup>+</sup> dependent coniferaldehyde/sinapaldehyde dehydrogenase (CALDH/SALDH or REDUCED EPIDERMAL FLUORESCENCE (REF1)), and which has been identified as a key enzyme for sinapine biosynthesis in *Arabidopsis thaliana* and oilseed rape. Inactivation of all 3 homeoalleles found in *C. sativa* lowered the sinapine content in seeds by ~ 60 % in the two cultivars IPK139 and Ligena, but without impacting the total lipid or protein content of the seeds, seed weight or lipid breakdown during germination. It was however accompanied by changes within the phenylpropanoid metabolism in both seeds and leaves, as revealed by UPLC-ESI-QTOFMS - based metabolite fingerprinting. In summary, the lines produced here provide a valuable trait, which can be combined with other traits through gene stacking to obtain crops with a significantly improved product quality.

S12 T2

Exploring the Micromechanical and Self-Healing Properties of Apple Fruit Cuticles  
as Influential Factors Preserving Fruit Quality

D. Heinemann<sup>1,2</sup>, T. Landes<sup>1,2</sup>, B. P. Khanal<sup>1,2</sup>, H. Bethge<sup>1,2</sup>, M. Zabic<sup>1,2</sup>

<sup>1</sup>Leibniz University Hannover, Institute of Horticultural Production Systems, Hannover, Germany

<sup>2</sup>Leibniz University Hannover, Hannover Centre for Optical Technologies, Hannover, Germany

The cuticular membrane (CM) is a non-living biological polymeric membrane covering most aerial organs of plants, including fruits. It is an important protective layer against water movements, environmental conditions, and pathogens, and therefore represents a major factor influencing fruit quality and food safety. In apple fruit, the CM encounters a strong strain because of the large increase of surface area during growth seasons, leading to mechanical stress. Despite the importance of maintaining the CM's barrier function, understanding of the micromechanical and self-healing properties of the CM remains limited. Here, we investigated the micromechanical composition of mature apple fruit CM using Brillouin spectroscopy. Firstly, results of Brillouin spectroscopy were validated using macroscopic tensile testing. Secondly, mechanical micro-maps of isolated apple fruit CM were recorded. Significant differences in Brillouin frequency shift (BFS) between anticlinal and periclinal regions of the CM were detected, with higher BFS in the periclinal region, indicating a higher stiffness. Further analysis involved dewaxing and HCl treatment, showing that waxes mainly contribute to stiffness in periclinal regions, while polysaccharides sustain stiffness in anticlinal regions post-dewaxing. Additionally, artificial microcracks were induced using ultra-short laser processing to study passive self-healing properties of the non-living CM. Observations of temperature-dependent wax crystal formation over time within the artificial microcracks and their close vicinity support the existence of a self-healing mechanism within the CM previously postulated by Curry et al. 2009 as "shear, stitch, and stretch" mechanism.

These findings enhance our understanding of the CM's protective functions and its capacity for self-repair, which are crucial for improving fruit quality and food safety.

S12 T3

Molecular analysis of *Salicornia* spp. to better exploit its potential as new crop plantA. Fussy<sup>1</sup>, J. Papenbrock<sup>1</sup><sup>1</sup>Institut für Botanik, Leibniz Universität Hannover, Hannover, Germany

Freshwater is becoming an increasingly scarce resource [1]. Hence, it is crucial to explore alternative resources like saline water and soils. Therefore, a profound molecular understanding of processes related to salt stress in potential crop plants is increasingly important. This investigation focusses on the halophyte *Salicornia europaea* and analyses its gene expression, yield and total phenolic compounds under hydroponic cultivation at five salinity levels (0, 7.5, 15, 22.5, 30 g/L NaCl) over five harvests recording plant development at 15-day intervals. The aim is to clarify whether the roots and shoots of *S. europaea* display different salt management strategies and further elucidate variations in plants reactions to deficient, optimal and excessive salinity during their development. Normalization strategies for gene expression levels had to be found to draw conclusions about regulatory processes as an equilibrium response to the prolonged saline treatments, which allows insights beyond physiological and biochemical analyses [2].

The main results included the selection of potential reference genes and the classification of specific gene expression patterns, along with variations in fresh mass production and phenolic compound content that depend on developmental stage and salinity levels. Highest fresh mass of *S. europaea* was observed four months after germination in 15 g/L NaCl. Notably, at 0 g/L NaCl, a unique set of genes in shoots showed increased expression levels, highlighting a NaCl-deficient stress response. These genes are coding for a tonoplast Na<sup>+</sup>/H<sup>+</sup>-antiporter (*SeNHX1*), a vacuolar H<sup>+</sup>-ATPase (*SeVHA-A*), two H<sup>+</sup>-PPases (*SeVP1*, *SeVP2*), a hkt1-like transporter (*SeHKT*), a vinorine synthase (*SeVinS*), a peroxidase (*SePerox*), and a plasma membrane Na<sup>+</sup>/H<sup>+</sup>-antiporter (*SeSOS1*). Other genes coding for an amino acid permease (*SeAAP*) and a proline transporter (*SeProT*) demonstrated marginal or dispersing salinity influence, though implying their nuanced regulation in plant's development. Interestingly, fundamentally differentiating regulatory processes in *S. europaea* involved in dealing with deficient compared to excessive salinity were observed.

[1] Gleick, P. H., & Cooley, H. (2021). Freshwater scarcity. *Annu. Rev. Environ. Resour.*, 46, 319-348.

[2] Rozentsvet, O. A., Nesterov, V. N., Kosobryukhov, A. A., Bogdanova, E. S., & Rozenberg, G. S. (2021). Physiological and biochemical determinants of halophyte adaptive strategies. *Russ. J. Ecol.*, 52, 27-35.

S12 T4

Impact of changing air temperatures on the growth and phytochemical contents in spearmint (*Mentha spicata*)D. Chatterjee<sup>1</sup>, A. Mitra<sup>1</sup><sup>1</sup>Indian Institute of Technology Kharagpur, Agricultural & Food Engineering Department, Kharagpur, India

*Mentha spicata* (spearmint) is a commercially available mint species from the Lamiaceae family, having huge economic importance in the field of herbal medicines, food flavourings and condiments industry because of their bioactive properties, unique aroma and flavour. Several studies have been conducted to understand the impact of different temperatures and photoperiods on the volatile profile of mint essential oils but no proper study has been undertaken on the internal pool of leaf volatiles. In this study, impact of five different temperatures on the vegetative growth, photosynthetic pigment profile, surface ultrastructure and histochemistry of glandular trichomes, antioxidant capacity as well as internal pool of leaf volatiles of spearmint were investigated under controlled environment inside a plant growth chamber with 16 h photoperiod. The results indicated that the vegetative growth of the aerial parts in terms of number of leaves, nodes and internodes was best at the warmer temperatures (highest at 28°C). The photosynthetic pigments such as total chlorophyll and carotenoid contents were highest at 24°C with reduced contents at all other conditions. The peltate glandular trichomes responsible for the biosynthesis and accumulation of major terpenoids exhibited highest density at 28°C. Additionally, the trichomes were also found to be intensely stained by terpene-specific Nadi stain at 28°C. The enzymatic and non-enzymatic antioxidant activities were elevated at the lower temperatures, 16°C showing the highest values, indicating increased stress. In case of the internal pool of leaf volatiles, nearly 40 compounds were identified by gas chromatography-mass spectrometry, with unilateral dominance of the marker compound (-)-carvone, a monoterpene ketone. Other compound classes were identified as monoterpenes, their alcohols and esters along with sesquiterpenes and sesquiterpenoids. Highest contents of (-)-carvone and its precursor D-limonene were found at 28°C, correlating with the highest density and intense staining of trichomes at the same temperature. In conclusion, 28°C was found to be the best suited temperature for good vegetative growth along with enhanced accumulation of terpenoids having high economic value. Thus, this study paves a new perception for year-round indoor cultivation of spearmint leading to enhanced accumulation of terpene compounds of economic importance.

S12 T5

Agroforest systems with grapevines and trees, advances and disadvantages

C. Zörb<sup>1</sup>, P. P. Lehr<sup>1</sup>

<sup>1</sup>Universität Hohenheim, Kulturpflanzenwissenschaften, Stuttgart, Germany

To introduce agroforestry systems is a new trend in agriculture to reduce monocultures and to increase biodiversity and enable greater ecosystem service. Here we show one of the few well established agroforest systems with grapevine and trees *i.e.* Riesling and Sauvignon blanc in combination with oak and poplar in a more than 10 years old experimental field trial. Grapevine was planted on tree stems in all combination *e.g.* Riesling with oak and Riesling with poplar and in a randomized block design with n=5 at a steep vineyard at Saar river. Effects of shading of trees on grapevine and water availability were evaluated. It was also tested with tracer nitrogen compounds if there are possible effects of nitrogen shift from tree roots to grapevine. Moreover the effect on plant biodiversity was started to be evaluated. The microbiome of rhizosphere soil and the metabolites in this environment were also analysed. Positive and negative effects were discussed. Among the positive side effects is the option to achieve high prices for such wines because of the exclusivity of the trial.

S13 T1

The influence of the circadian clock and environmental conditions on calcium-dependent responses to pathogens

M. Knight<sup>1</sup>, B. Jacobs<sup>1</sup>

<sup>1</sup>Durham University, Durham, United Kingdom

Abiotic and biotic environmental stimuli are sensed and transduced by signalling networks in plants leading to an appropriate pattern of protective gene expression. We are interested in how calcium, involved in response to so many different primary signals, can encode specific information to elicit the correct downstream responses. Different external stimuli elicit unique spatiotemporal patterns of elevations in cellular calcium concentration known as calcium signatures and these encode stimulus-specific information that is decoded by plant cells. Through a combination of experimental and mathematical approaches, we are examining the molecular mechanisms for decoding calcium signatures by specific transcription factors to lead to appropriate specific gene expression responses. This presentation will focus on our work on calcium-dependent signalling in response to pathogens. We have found that the circadian clock modifies calcium signatures to PAMPs and consequently downstream defence responses. We have determined the mechanism by which the clock regulates calcium-regulated PTI signalling. Similarly, we found that ambient growth temperature has a profound effect upon calcium-dependent PTI signalling, and have investigated the mechanism using both pharmacological and genetic approaches. Together our work reveals different points in calcium-dependent defence signalling that are regulated by both endogenous and exogenous information to achieve the appropriate response.

S13 T2

**Inositol pyrophosphates are master regulators of arbuscular mycorrhiza**

K. Raj<sup>1</sup>, V. Gaugler<sup>2</sup>, G. Schaaf<sup>2</sup>, M. K. Ried-Lasi<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry, Molecular Signal Processing, Halle a. d. Saale, Germany

<sup>2</sup>University of Bonn, INRES, Bonn, Germany

Tight regulation of nutrient homeostasis is vital for every cell. Plants have evolved elaborate systems to sense and signal extracellular and intracellular e.g. phosphate levels and to regulate cellular nutrient concentrations. Most land plants establish Arbuscular Mycorrhiza (AM) with phosphate-acquiring fungi, and selected members of the Fabales, Fagales, Cucurbitales and Rosales engage in root nodule symbiosis with diazotrophic bacteria<sup>[1,2]</sup>. While many genes involved in symbiont perception and subsequent genetic reprogramming have been well characterized, the signalling events that take place between the plasma membrane and the nucleus and the signalling hubs connecting symbiosis with nutrient homeostasis remain largely obscure. Plant phosphate homeostasis is regulated by inositol pyrophosphates (PP-InsPs). PP-InsPs are low abundant high energy messengers that bind to SPX domains - selective high-affinity PP-InsP receptors – and mediate the interaction of SPX and PHR-like transcription factors, thereby regulating the expression of phosphate starvation-induced genes<sup>[3]</sup>. There is accumulating evidence that phosphate homeostasis and AM signalling are interconnected *via* SPX and PHR<sup>[4-7]</sup>. Moreover, there is a direct link between phosphate and nitrogen homeostasis employing SPX. When SPX is degraded, PHR and NIN-like proteins migrate to the nucleus, where they activate phosphate starvation- and nitrate-induced genes, respectively, and coordinate in concert phosphate and nitrate utilisation<sup>[8]</sup>. It is our goal to scrutinize the role of PP-InsP ligands and putative precursors during symbioses and nutrient homeostasis in *Lotus japonicus* and thus to illuminate the interplay of these different plant strategies to overcome nutrient limitations.

References

1. M. Parniske (2008). *Nat. Rev.*, **6**, 763-775.
2. J. J. Doyle (2011). *MPMI*, **24**, 1289-1295.
3. J.-Y. Jung, M. K. Ried, M. Hothorn, Y. Poirier (2017). *Curr. Opin. Biotechnol.*, **49**, 156-162
4. P. Wang, R. Snijders, W. Kohlen, J. Liu, T. Bisseling, E. Limpens (2021). *Plant Cell*, **33**, 3470-3486
5. J. Shi, B. Zhao, S. Zheng, X. Zhang, X. Wang, W. Dong, Q. Xie, G. Wang, Y. Xiao, F. Chen, *et al.* (2021). *Cell*, **184**, 5527–5540.
6. D. Das, M. Paries, K. Hobecker, M. Gigl, C. Dawid, H. M. Lam, J. Zhang, M. Chen, C. Gutjahr (2022). *Nat. Commun.*, **12**, 477
7. D. Liao, C. Sun, H. Liang, Y. Wang, X. Bian, C. Dong, X. Niu, M. Yang, G. Xu, A. Chen, S. Wu (2022). *Plant Cell*, **34**, 4045-4065.
8. B. Hu, C. Chu (2019). *New Phytol.*, **225**, 1455-1460.

S13 T3

**Ethylene-mediated reduction of root colonization by arbuscular mycorrhiza fungi requires the repressor of the karrikin signalling pathway, *SMAX1***

K. Varshney<sup>1,2</sup>, D. Das<sup>3,4</sup>, S. Ogawa<sup>5,6</sup>, S. Torabi<sup>1,2</sup>, R. Hüttl<sup>2</sup>, D. Nelson<sup>5</sup>, C. Gutjahr<sup>1,2,3</sup>

<sup>1</sup>Max Planck Institute of Molecular Plant Physiology, Root Biology and Symbiosis, Potsdam-Golm, Germany

<sup>2</sup>Technical University of Munich (TUM), Plant Genetics, Freising-Weihenstephan, Germany

<sup>3</sup>University of Munich (LMU), Plant Genetics, Planegg-Martinsried, Germany

<sup>4</sup>Redox Bio-Nutrients, Burley, ID, United States

<sup>5</sup>University of California, Department of Botany and Plant Sciences, Riverside, CA, United States

<sup>6</sup>RIKEN Center for Sustainable Resource Science, Yokohama, Japan

The interaction of plants with arbuscular mycorrhiza (AM) fungi is tightly regulated at the cellular, genetic, and molecular level. Phytohormones play a key role in the establishment of AM symbiosis. The gaseous hormone ethylene is often involved in stress responses and has also been shown to negatively regulate the interactions of plants with AM fungi, but the underlying molecular mechanism is yet unclear. Another small molecule signaling pathway, the karrikin signaling pathway plays a key role in promoting root colonization by AM fungi. Rice and *Lotus japonicus* mutants of the karrikin signaling repressor gene, *SMAX1*, show increased colonization levels. Interestingly, in *L. japonicus*, the *smax1* mutant displays increased ethylene biosynthesis, while still being colonized to levels higher than the wild type. Here we show that in *L. japonicus*, the negative effect of ethylene on colonization by AM fungi requires a functional *SMAX1*. It further involves reduced expression of strigolactone biosynthesis genes and of common SYM-pathway genes, such as *CCaMK*, which is likely caused by increased *SMAX1* accumulation through ethylene action.

S13 T4

The interplay between *aba*, *ja* and *sa* during drought stress response in *arabidopsis* and potato

U. Voithknecht<sup>1</sup>, S. Bhattacharyya<sup>1</sup>, S. Mahmud<sup>2</sup>, E. Rodriguez<sup>1</sup>, B. Wurzing<sup>3</sup>, M. Teige<sup>3</sup>, C. Bleker<sup>4</sup>, K. Gruden<sup>4</sup>, F. Chigri<sup>1</sup>

<sup>1</sup>IZMB, University of Bonn, Plant Cell Biology, Bonn, Germany

<sup>2</sup>University of Missouri, Dept. Biochemistry & Interdisciplinary Plant Group, Columbia, SC, United States

<sup>3</sup>University of Vienna, Dept. Functional & Evolutionary Ecology, Vienna, Austria

<sup>4</sup>National Institute of Biology, Dept. Biotechnology and Systems Biology, Ljubljana, Slovenia

Question: How does the interplay of hormone-dependent signaling pathways balance the often-contrary needs of growth, development and stress protection.

Methods: During a recent study we performed RNA-seq analysis of *Arabidopsis* plants kept under well-watered or progressive drought conditions for 14 days. At this point, drought-treated wild type plants still retained >85% of their leaf water content and showed no outward signs of wilting. On a molecular level, we could observe significant changes in the expression of over 3400 genes. From these, we extracted those genes that showed the strongest upregulation, in an attempt to identify novel and important candidates of the drought response system.

Results: We identified approximately 150 genes with a logFC change over 5.0 (FDR <0.01). Under control conditions these genes had different expression levels ranging from no expression (TPM =0) to medium expression levels (TPM = 11 to 1000). Not unexpected, within this group of highly drought induced genes (HDIGs) we found many LEAs/putative LEAs, putative ABA-responsive transcriptional repressors and ABA-responsive proteins. About 80 of the genes encode proteins of unknown or putative function. For other genes, a putative or experimentally proven function is assigned but no prior relationship to drought has been reported. For two of these genes, HDIG15 and HDIG88, we performed a more thorough analysis of expression in relation to drought by qRT-PCR analysis. The data were compared to the expression of genes such as RD29A, a well described ABA-responsive drought marker. We also investigated the phenotype of *hdig15* and *hdig88* single mutants as well as a *hdig15/88* double mutant under control and drought stress conditions. In a case study, we furthermore analysed the differential role of ABA, JA and SA on the expression of drought induced genes with a special focus on RD29A and its ortholog from potato.

Conclusion: Together, our results further the understanding of the intricate cross-talk between various hormones in drought response.

S13 T5

A new connection with the matrix: Structural role of peptides to sustain pollen tube expansion

S. Moussu<sup>1</sup>, H. K. Lee<sup>1</sup>, K. Haas<sup>2</sup>, C. Broyart<sup>1</sup>, -. Ursina<sup>3</sup>, D. de Bellis<sup>4</sup>, T. Levasseur<sup>5</sup>, E. Bonnin<sup>5</sup>, N. Geldner<sup>3</sup>, B. Cathala<sup>5</sup>

H. Höfte<sup>2</sup>, J. Santiago<sup>1</sup>

<sup>1</sup>University of Lausanne, The Plant Signaling Mechanisms Laboratory, Department of Plant Molecular Biology, Lausanne, Switzerland

<sup>2</sup>Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (JJPB), Versailles, France

<sup>3</sup>University of Lausanne, Department of Plant Molecular Biology, Lausanne, Switzerland

<sup>4</sup>University of Lausanne, Electron Microscopy Facility, Lausanne, Switzerland

<sup>5</sup>Biopolymères, Interactions et Assemblages, INRAE, Nantes, France

Plant cells coordinate with the surrounding cell wall during expansion for optimal growth and morphogenesis. This is key in pollen tubes that requires the support of the cell wall to counterbalance the internal turgor pressure while maintaining optimal elongation rate. The cell wall surveillance receptor complex, LRX8-RALF4, has been characterized to maintain cell wall integrity in the pollen tube. Yet, the exact molecular mechanism of how the LRX8-RALF4 complex contributes to the pollen tube cell wall integrity remains elusive. Here, we identified that the LRX8-RALF4 complex physically interacts with pectin in a charge dependent manner via a newly polycationic surface formed upon RALF4 binding to LRX proteins. Disruption of this basic surface on the peptide impairs RALF4- pectin binding capability, disrupting male fertility and optimal growth dynamics of the pollen tube. Furthermore, super-resolution and electron microscopy techniques reveal that the LRX8-RALF4-pectin complex display a reticulated pattern in the cell wall supporting the overall cell wall integrity of the pollen tube. Our work demonstrates how the mechanical support created from the network of the cell wall receptor complex with pectin is vital for pollen tube elongation.

S14 T1

**An interdisciplinary study to investigate the plant-soil-microbiome continuum under drought**

R. Hartwig<sup>1</sup>, M. Santangeli<sup>2</sup>, H. Würsig<sup>3</sup>, M. Martín Roldán<sup>3</sup>, B. Yim<sup>4</sup>, E. Lippold<sup>3</sup>, A. Tasca<sup>5</sup>, E. Oburger<sup>2</sup>, M. T. Tarkka<sup>3</sup>  
D. Vetterlein<sup>3</sup>, P. Bienert<sup>5</sup>, E. Blagodatskaya<sup>3</sup>, K. Smalla<sup>4</sup>, S. Wienkoop<sup>6</sup>, M. Wimmer<sup>1</sup>

<sup>1</sup>Universität Hohenheim, Fg. Qualität pflanzlicher Erzeugnisse (340e), Stuttgart, Germany

<sup>2</sup>University of Natural Resources and Life Sciences, Vienna, Austria

<sup>3</sup>Helmholtz Centre for Environmental Research, Halle a. d. Saale, Germany

<sup>4</sup>Julius Kühn-Institut, Braunschweig, Germany

<sup>5</sup>Technical University of Munich, München, Germany

<sup>6</sup>University of Vienna, Vienna, Austria

Drought stress is one of the most detrimental abiotic stresses in crop production. While knowledge is abundant about the drought responses of plants or soil microbes individually, we have a very limited understanding of the complex interactions occurring in the plant-soil-microbiome continuum under water limitation. Drought-induced changes in microbial composition may be beneficial to plants and thus alter their stress response, while at the same time drought-induced changes in root exudates may attract specific microbial taxa and in turn alter the response of the whole rhizosphere microbiome. As sites of root exudation and microbial hotspots, root hairs appear to play a role in these processes, especially under limiting conditions like drought. How and to which extent root hairs contribute to the formation of the rhizosphere is so far largely unclear. In addition, the plant shoot as carbon source for root exudates and root growth should also be considered. To link changes and adaptations of maize shoot, root and rhizosphere under water limitation, we conducted an interdisciplinary study with a root hairless maize mutant (*rth3*) and its corresponding wildtype B73 (WT), grown for 22 days in loamy soil under controlled conditions. Roots and shoots were analyzed for physiological stress responses, total gene expression, proteome and metabolome, root exudates as well as enzyme activities and microbial composition in the rhizosphere and bulk soil. After 3-4 days of water limitation, transpiration rates started to decline, and reached values near zero after 7 days without watering in both genotypes. At this time, we observed genotypic differences in soil and plant water relations, shoot nutrient content, root gene expression (including aquaporins), root exudation rates, rhizosphere enzyme activity and microbial diversity. Metabolomic and proteomic data of shoots and roots are currently being analyzed and will be included to shed light especially on the role of root hairs under water limitation. Our results indicate that the rhizosphere microbiome can be significantly altered after 7 days of water limitation, and that this response correlates with changes in the amount and composition of root exudates. We have first indications that root hairs may be involved in the stress response.

S14 T2

**Mechanisms of root branching under heterogenous water availability**

P. Mehra<sup>1</sup>

<sup>1</sup>University of Nottingham, School of Biosciences, Nottingham, United Kingdom

Water scarcity is a threat to agriculture, given the impact of climate change. Root branching is an agronomically important trait that determines foraging capacity of plants. To adapt to heterogenous soil water availability, roots exhibit remarkable plasticity in their branching patterns. Discovering how plant root sense and continuously adapt to fluctuating water availability at cellular and organ scale is vital for futureproofing crops. Xerobranching serves as a valuable model for studying root adaptive mechanisms in response to fluctuating soil water availability. A xerobranching response is triggered when a growing root tip loses contact with soil moisture (e.g. in an air-gap) leading to suppression of branching until the root tip re-enters moist soil (Mehra et al., 2022). Our recent research has uncovered that xerobranching utilizes Reactive Oxygen Species (ROS) to inhibit root branching. Lack of moisture triggers nuclear ROS accumulation within the basal meristem and elongation zone of growing root tips. ROS induces multimerization of auxin repressor protein IAA3/SHY2 (Roy et al., 2024 bioRxiv). Mutations in specific cysteine residues in IAA3/SHY2 disrupt its redox-mediated multimerization and interaction with co-repressor TPL, but not with auxin response partner ARF7 and auxin receptor TIR1. ROS-mediated oligomerization of IAA3/SHY2 is required for efficient ARF-mediated target gene repression during xerobranching and lateral root emergence. Our findings demonstrate that AUX/IAA proteins vary in their redox mediated multimerization, revealing a new auxin response regulatory mechanism that directly connects ROS sensing to auxin signalling. Our study reveals how ROS, auxin and water stress intersect to shape adaptive responses in plant roots and maintain their phenotypic plasticity

References:

Mehra, P., Pandey, B. K., Melebari, D., Banda, J., Leftley, N., Couvreur, V., ... & Bennett, M. J. (2022). Hydraulic flux-responsive hormone redistribution determines root branching. *Science*, 378(6621), 762-768.

Roy, D., Mehra, P., Mukkavar, V., Clark, L., Bellande, K., Vermeer, J. E., ... & Sadanandom, A. (2024). ROS responsive Aux/IAA multimerization modulates auxin responses. *bioRxiv*, 2024-02.

S14 T3

Old but gold: exploiting the underutilized oilseed *Camelina sativa* to uncover and promote tolerance to abiotic stress for improving climate resilience in crops

D. Großkinsky<sup>1</sup>, S. Silvestre<sup>2</sup>, A. Forgatti-Hell<sup>2</sup>, R. Haslam<sup>2</sup>, A. Da Costa<sup>3</sup>, J. D. Faure<sup>3</sup>, F. Zanetti<sup>4</sup>, A. Monti<sup>4</sup>, J. Prieto Ruiz<sup>5</sup>, P. León<sup>5</sup>, B. Usadel<sup>6</sup>, M. Le Boulch<sup>7</sup>, C. Cassan<sup>7</sup>, P. Pétriacq<sup>7</sup>, S. Prigent<sup>7</sup>, Y. Gibon<sup>7</sup>, C. Jonak<sup>1</sup>

<sup>1</sup>AIT Austrian Institute of Technology, Tulln an der Donau, Austria

<sup>2</sup>Rothamsted Research, Harpenden, United Kingdom

<sup>3</sup>INRAE, Versailles, France

<sup>4</sup>Università di Bologna, Bologna, Italy

<sup>5</sup>Camelina Company, Madrid, Spain

<sup>6</sup>Forschungsinstitut Jülich, Jülich, Germany

<sup>7</sup>Inrae, Bordeaux, France

*Camelina sativa* (camelina, gold-of-pleasure) is an old, re-emerging European oilseed crop without intense breeding history. Camelina is characterized as a low-input crop for bioenergy as well as for food and feed that can be grown on poor soils and marginal land, and it shows inherent tolerances towards adverse environmental conditions such as heat and drought. Thus, camelina is a promising source of functional diversity and has a great potential as a climate-smart crop needed to cope with climate change-driven challenges in agriculture. To uncover camelina's stress adaptation strategies, the EU-Horizon 2020 project UNTWIST<sup>1</sup> (GA 862524) is using an interdisciplinary systems approach to dissect its multi-layered responses to heat and drought. Screening the performance of 54 genetically diverse lines in field trials as well as under controlled heat and drought conditions combined with genomic and metabolomic analyses allowed the selection of four contrasting focus lines. These lines are further characterized in additional field trials under different cropping systems and complementary large-scale experiments under controlled heat and drought conditions for in-depth analyses. Results of the four focus lines from a controlled drought stress experiment in an automated high-throughput phenotyping setup will be presented in detail. This approach allowed to discriminate differential behaviour of the focus lines under well-watered and drought conditions at multiple morphological and physiological levels through RGB, hyperspectral reflectance, chlorophyll fluorescence and thermal imaging techniques. These results will be integrated with (epi)genomic, physiological, metabolic, proteomic, and transcriptomic data to feed into mechanistic and predictive models and to derive markers for crop improvement. Moreover, implications for the understanding of genetic interplay and plasticity in plant adaptation, which can be exploited for increasing crop yield stability in adverse and changing environmental conditions, will be discussed.<sup>2</sup>

References

<sup>1</sup> <https://www.untwist.eu>

<sup>2</sup> Großkinsky DK, Faure J-D, Gibon Y, Haslam RP, Usadel B, Zanetti F, Jonak C (2023) The potential of integrative phenomics to harness underutilized crops for improving stress resilience. *Front Plant Sci* 14:1216337.

S14 T4

A constitutive drought-stressed status is provoked by the complete loss of plasma membrane aquaporins in *Arabidopsis thaliana*

T. Zhu<sup>1</sup>, P. K. Hsu<sup>2</sup>, Z. Yang<sup>3</sup>, K. Jhala<sup>1</sup>, B. Geist<sup>1</sup>, E. Grill<sup>3</sup>, J. I. Schroeder<sup>2</sup>, A. Schäffner<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München, Molecular Plant Physiology, Institute of Biochemical Plant Pathology, Neuherberg, Germany

<sup>2</sup>University of California San Diego, Cell and Developmental Biology Department, School of Biological Sciences, La Jolla, CA, United States

<sup>3</sup>Technical University of Munich, Institute of Botany, School of Life Sciences Weihenstephan, Freising, Germany

Plasma membrane-localized aquaporins (PIPs, plasma membrane intrinsic proteins) are abundant proteins facilitating water and neutral solute permeation. All higher plants harbor many isoforms split into highly homologous PIP1 and PIP2 isoforms. *Arabidopsis thaliana* harbors five PIP1 and eight PIP2 members. To address their principal function, we aimed to generate a mutant eliminating all PIP2 isoforms. An octuple *pip2<sup>Δ1to8</sup>* mutant could be generated by combining insertion lines and CRISPR/Cas9 approaches. Notably, *pip2<sup>Δ1to8</sup>* exhibited a concomitant loss of PIP1 proteins; thus, it constitutes a complete loss-of-PIP function mutant. Although viable and fertile, *pip2<sup>Δ1to8</sup>* resulted in substantial growth retardation when grown on soil in a regular growth chamber. *pip2<sup>Δ1to8</sup>* experienced constitutive drought stress symptoms under medium-to-high vapor pressure deficit (VPD) growth conditions as indicated by (i) an altered, drought-related paraheliotropic leaf movement rhythmicity and by (ii) a reduced stomatal conductance. The *pip* loss-of-function mutant also was more drought resistant when cultivated under a progressively severe drought regime. Interestingly, the retarded growth phenotype was at least partially rescued when *pip2<sup>Δ1to8</sup>* was cultivated at low VPD. This restoration by high air humidity could not be achieved by growth in hydroponic culture facilitating water availability at the roots nor by sucrose supplementation or enhanced ambient CO<sub>2</sub>. The constitutively lower stomatal conductance of *pip2<sup>Δ1to8</sup>* was not linked to an altered stomatal index. Elevated ambient CO<sub>2</sub> led to a further closure of stomata, whereas low CO<sub>2</sub> induced a rapid opening like wild type. However, in the latter case, the low-CO<sub>2</sub>-induced opening of *pip2<sup>Δ1to8</sup>* was transient and followed by a rapid closing. Thus, the complete loss of plasma membrane aquaporins underscores the impact of PIPs on plant water relations as their principal role and air humidity as a major factor that enables almost wild type-like growth of this severe mutant.

S14 T5

Reinforcement Learning-Supported Metabolic Modeling Enables Dynamic Simulation of Plant Seed-to-Seed Growth Cycle in a Changing Environment

D. Koch<sup>1</sup>, S. Camborda La Cruz<sup>2</sup>, J. N. Weder<sup>2</sup>, D. Psaroudakis<sup>1</sup>, N. Töpfer<sup>2,3</sup>, J. Szymanski<sup>1,4,3</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research, Molecular Genetics, Seeland, Germany

<sup>2</sup>University of Cologne, Köln, Germany

<sup>3</sup>Cluster of Excellence on Plant Sciences (CEPLAS), Düsseldorf, Germany

<sup>4</sup>Forschungszentrum Jülich GmbH, IBG-4 Bioinformatics, Jülich, Germany

Plants have developed a variety of strategies that enable them to complete the seed-to-seed growth cycle in challenging environments. Many of these strategies are tied to the timing and prioritization of specific metabolic objectives. This study integrates metabolic modeling and machine learning to explore and simulate these survival strategies by combining a whole-plant metabolic growth model with the adaptive learning capabilities of AI agents, within the interactive format of a strategy game.

To achieve this, a whole-plant, genome-scale metabolic model was developed to simulate interactions between major plant organs and the environment using a Dynamic Flux Balance Analysis framework. The model was coupled with environmental parameters derived from historical weather data representing 24-hour cycles across different seasons and climates. To manage dynamic growth regulation, we employed two approaches: firstly, using reinforcement learning to fine-tune plant growth strategies in response to environmental fluctuations, plant biomass, and developmental timing; secondly, implementing the model as an interactive educational game tested on human agents through a citizen science approach. The game loop integrates with the model at multiple levels, providing real-time visual feedback and immersing players in the plant life cycle.

Results demonstrated that both AI and human players adopted strategies that reflected the growth programs and physiological cycles observed in plants. This included the sequencing of organ growth, differential growth based on nutrient availability, and carbon storage management in day-night cycles. The findings suggest that a relatively simple plant metabolic model, controlled by a set of regulatory inputs, can simulate complex growth patterns in a changing environment.

This represents the first step towards a digital twin of a growing plant, offering significant potential for applications in agriculture and metabolic engineering.



S15 T1

*Agrobacterium*-based protein transfer system for transgene-free genome engineering

J. Macholl<sup>1</sup>, T. Jores<sup>1,2</sup>

<sup>1</sup>University of Düsseldorf, Institute of Synthetic Biology, Düsseldorf, Germany

<sup>2</sup>CEPLAS -, Cluster of Excellence on Plant Sciences, Düsseldorf, Germany

Improved crop resilience and yield are required to ensure food security, which is threatened by population growth and climate change. Genetic engineering holds promise to create more resilient and high-yielding crops. However, current plant engineering methods can lead to the integration of foreign DNA into the plant genome, which raises concerns about transgenic organisms and potential off-target effects. Although transgene-free genome engineering approaches exist, they are technically challenging and labour-intensive as the resulting plants have to be regenerated from transformed protoplasts or embryos. To overcome these issues, we are developing a transgene-free method for plant transformation that uses *Agrobacteria* to transfer proteins into plant cells, without a concomitant transfer of T-DNA. While such a protein transfer system has been previously described, it suffered from low efficiency, likely due to low protein expression. We created a Golden Gate-compatible vector toolkit that maintains plasmids with high copy number in *Agrobacteria*. Additionally, we identified inducible promoters for high protein expression in *Agrobacteria*, and we are optimizing the efficiency of protein transfer. Our enhanced protein transfer system opens up new avenues for improving crop traits by genome engineering, without the drawbacks associated with stable integration of foreign DNA into the plant genome.

S15 T2

Engineered CRISPR endonucleases favoring homology-directed repair in plants

T. Schreiber<sup>1</sup>, A. Prange<sup>1</sup>, P. Schäfer<sup>1</sup>, T. Iwen<sup>2</sup>, R. Grützner<sup>1</sup>, S. Marillonnet<sup>1</sup>, A. Lepage<sup>3</sup>, M. Javelle<sup>3</sup>, W. Paul<sup>3</sup>, A. Tissier<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry, SZB, Halle a. d. Saale, Germany

<sup>2</sup>Philipps University Marburg, AG Bange, Marburg, Germany

<sup>3</sup>Limagrain, Chappes, France

The targeted modification of plant genetic material offers a wide range of potential applications. Modifications of larger sections of DNA, such as insertions or replacements, are difficult to achieve using existing techniques. These modifications are usually triggered by DNA double-strand breaks (DSBs) in the area to be modified and occur via homology-directed repair (HDR). Two main pathways are involved in the repair of DNA DSBs: non-homologous end-joining (NHEJ) and HDR. A key and specific step for the processing of DSBs by HDR is the production of single-stranded 3'-overhangs. However, programmable endonucleases induce blunt ends or short 5' sticky ends, which result in substrates that are primarily repaired by NHEJ. To enhance the efficiency of homology-mediated repair, we equipped CRISPR endonucleases Cas9 and Cas12a with a 5' exonuclease. Among the tested candidates, we identified 5' exonucleases that led to a significant increase in HDR frequency. In *Arabidopsis*, these endonucleases led to a 10-fold increase in HDR efficiency, while in wheat they resulted in an editing rate of 1-2%. The tools described herein facilitate the routine gene targeting in plants.

S15 T3

### Taking the Next Step in Plant Genome Editing: Precision and Efficiency with Advanced Base Editors

L. Lang<sup>1</sup>, V. Albinus<sup>1</sup>, M. Rumpf<sup>1</sup>, D. Becker<sup>2</sup>, H. Schmidt<sup>1</sup>

<sup>1</sup>DNA Cloning Service e.K., Hamburg, Germany

<sup>2</sup>University of Hamburg, Institute of Plant Sciences and Microbiology, Hamburg, Germany

The increasing challenges posed by climate change and continuous population growth necessitate rapid advancements in plant genome editing. Traditional CRISPR/Cas9 methods often lack the specificity and efficiency required for precise gene edits, leading to largely unpredictable outcomes and the requirement to screen a large number of offspring. Our innovative base editor system leverages deaminases that are able to modify singular adenines (adenosine base editors; ABEs) or cytosines (cytosine base editors; CBEs) within a customizable target sequence without the need for generating double strand breaks to achieve accurate and swift genome editing in plants. Use cases for the base editor system include not only gene knockout but also the fine-tuning of gene expression through targeted modifications of regulatory elements as well as semi-random mutagenesis by introducing single nucleotide mutations in genes of interest. Furthermore, by applying a multiplex approach, base editors can edit several targets at once.

Our system was tested on the model plant *Arabidopsis thaliana*, where we could achieve a near 100% editing efficiency in the T<sub>1</sub> generation on different targets using both Tad8e (ABE) and APOBEC3A (CBE). Making use of an FY-APOBEC3A mutant enzyme, we were able to alter the editing window of the base editor. Further, we could improve the achieved editing efficiency by optimising our choice of promoter. Our results so far indicate that combining PAM-modified Cas9 variants, such as the NG-Cas9 or the near PAM-less SpRY-Cas9, with an advanced base modification toolbox will allow us to freely customise any base of a given genome in the near future. To be able to introduce any desired base in place of another, we have further implemented uracil N-glycosylase (UNG) and N-methylpurine DNA glycosylase (MPG) into our system, which both allow for more complex base conversions. Our approach is currently in the process of being transferred to and tested in a variety of crops. Ultimately, our goal is to equip researchers with the means to save a whole plant generation cycle to retrieve a pre-determined mutant germplasm, enabling the faster development of plant material for both basic research and industry applications. By addressing the limitations of current CRISPR/Cas9 technologies in this way, we provide a robust tool for both academic and agricultural development, and we enable breeders to generate new varieties with an unprecedented speed.

S15 T4

### SpaceEx – Developing a new platform for spatial transcriptomics in plants and beyond

P. Rizzo<sup>1</sup>, E. Schaudy<sup>2</sup>, M. Giridhar<sup>3</sup>, I. Grosse<sup>4</sup>, M. Müller-Hannemann<sup>4</sup>, A. Schmidt<sup>4</sup>, B. Chavez<sup>1</sup>, I. Mora-Ramirez<sup>1</sup>

N. Shanmugaraj<sup>1</sup>, T. Schnurbusch<sup>1</sup>, A. Himmelbach<sup>1</sup>, L. Altschmied<sup>1</sup>, M. Somoza<sup>3</sup>

<sup>1</sup>IPK Gatersleben, Seeland, Germany

<sup>2</sup>University of Vienna, Vienna, Austria

<sup>3</sup>Leibniz Institute for Food Systems Biology, Freising, Germany

<sup>4</sup>Martin Luther University Halle-Wittenberg, Institut für Informatik, Halle a. d. Saale, Germany

Cellular decisions, crucial in shaping an organism's growth and development, are intricately tied to spatiotemporal gene expression patterns on the cellular level. Hence, obtaining information about spatiotemporal expression patterns is extremely valuable for understanding developmental trajectories that lead to a specific cell fate.

Spatial transcriptomics and, more generally, spatial biology are revolutionizing many research fields, providing novel technologies and enabling scientists to address unprecedented biological questions.

We are a team of developmental biologists, chemical engineers, and bioinformaticians from IPK-Gatersleben, Martin Luther University Halle-Wittenberg, Vienna University, and LSB Freising who joined forces to develop a new technology for studying transcriptomes of plants, animals, and other multicellular organisms in a spatially resolved way. Here, we present SpaceEx, a novel platform for performing spatial transcriptomics at cellular resolution and reasonable cost using a combination of (i) DNA arrays for capturing polyadenylated RNA and (ii) position-specific barcodes that provide spatial coordinates for the captured transcripts.

S15 T5

Creating an artificial CMS system by knocking out a functional gene in tobacco mitochondria

J. Forner<sup>1</sup>, D. Kleinschmidt<sup>1</sup>, E. H. Meyer<sup>2</sup>, J. Gremmels<sup>1</sup>, R. Morbitzer<sup>3</sup>, T. Lahaye<sup>3</sup>, M. A. Schöttler<sup>1</sup>, R. Bock<sup>1</sup>

<sup>1</sup>Max-Planck-Institut für Molekulare Pflanzenphysiologie, Potsdam, Germany

<sup>2</sup>Martin-Luther-Universität, Halle a. d. Saale, Germany

<sup>3</sup>Eberhard Karls Universität, Tübingen, Germany

Targeted modification of the plant mitochondrial genome has been a long-standing desired goal of the research community and only in recent years it has become feasible. Key to this are transcription activator-like effectors (TALEs), or more precisely base editors and nucleases (TALENs) that build on these. TALENs are protein-only, freely programmable site-specific DNA endonucleases. TALEN-encoding transgenes can be delivered to the nucleus via classical transformation techniques, while the resulting proteins can be easily targeted to the mitochondria by adding an N-terminal mitochondrial presequence.

Using TALENs, we had previously created a series of point mutations in the *nad9* gene of tobacco exploiting the gene drive-like properties of TALEN activity in plant mitochondria (Nature Plants 2022, PMID: 35301443).

Now, we have managed to completely remove the *nad9* gene from the mitochondrial genome of *Nicotiana tabacum*, creating a full knock-out. While the removal of *nad9* was coupled to genome rearrangements via recombinations in most of the mutant lines, we also succeeded in isolating a few lines with a clean deletion, without any additional alterations in the mitochondrial genome.

The mutant plants lack the entire complex I in their respiratory chain, but are viable. They display a distinct phenotype, most notably delayed germination and growth as well as altered leaf and flower morphology. They are also male sterile. By allotopic expression of *nad9* in the nucleus, we could fully rescue the *nad9* knock-out phenotype, including reversion to male fertility. We thus have created an artificial cytoplasmic male sterility (CMS) system (Nature Plants 2023, PMID: 37814021), paving the way to more efficient plant hybrid breeding.

S16 T1

How do viscoelastic properties differ in Arabidopsis root tissues and zones?

L. Alonso Baez<sup>1</sup>, A. Bjørkøy<sup>1</sup>, B. Stokke<sup>1</sup>, T. Hamann<sup>1</sup>

<sup>1</sup>Norwegian University of Science and Technology, Department of Biology, Trondheim, Norway

During the lifespan of a plant cell, the balance between turgor pressure and tensile stresses acting on its cell wall drives cell expansion and provides mechanical strength. The composition and structure of cell walls, together with the experienced mechanical forces, determine the viscoelastic properties of plant cells, which are crucial for proper growth, development and adaptation to stresses. However, little is known about how different plant tissues, which possess unique characteristics, respond to external signals, and regulate viscoelasticity as well as growth.

Traditional methods for studying viscoelastic properties, such as assessing stiffness and elastic modulus, often involve invasive techniques that necessitate surface contact, potentially altering or damaging the material properties and limiting what can be accessed and characterized. Moreover, conventional approaches typically require fixed samples, limiting investigation of molecular processes *in vivo*.

Here we used Brillouin microscopy, a non-invasive, contactless, and label-free technique, to probe viscoelastic properties of cell walls and cytoplasm in different tissues and zones in living Arabidopsis roots with subcellular resolution. We studied different developmental stages and used chemicals to disrupt cell wall organization and/or change the osmotic conditions. Positional information from a fluorescence marker is correlated with viscoelastic maps to characterize mechanical properties of plant cell walls exposed to modified turgor pressure and affected cell wall composition in different root tissues. This approach will enable us to understand how mechanical stimuli influence plant development and adaptation to environmental stresses.

S16 T2

**Phytochrome B photobody live imaging - how do phytochrome B photobodies form?**

F. Stamm<sup>1</sup>, S. Haque<sup>1</sup>, M. Sahu<sup>1</sup>, O. Heliel<sup>1</sup>, J. Schmidt<sup>1</sup>, I. Zubcic<sup>1</sup>, K. van Gelderen<sup>1</sup>

<sup>1</sup>Heidelberg University, Centre for Organismal Studies, Heidelberg, Germany

Light perception and responses are essential for plant life and how light can be converted into a biochemical signal is a fundamental question in biology. Plants use red and far-red light to probe their environment in order to adapt their growth. Phytochromes are the main red-light sensors in plants and phytochromes form small (~400nm) liquid-liquid phase separated subnuclear bodies, photobodies, which also contain supporting cofactors and downstream transcription factors. phyB photobodies have been extensively studied, however, it is not clear how this process is regulated dynamically and which factors are the main drivers of phyB photobody formation. We study phyB photobodies as dynamical compartments. Therefore, we have set up live confocal imaging of phyB photobodies and manipulate light conditions at the microscope with flexible LEDs, that can irradiate the sample, without interfering with imaging. We have been able to form and reform photobodies using red and far-red light on the minute timescale, showing the light-switch nature of phyB photobodies. Surprisingly, we have identified mutants that form photobodies in far-red, rather than light, challenging the model that active phyB forms photobodies. To study the dynamic response of photobodies to ambient temperature changes, and manipulate these conditions at the microscope, we are using a new microelectronic circuit called VAHEAT. With VAHEAT we are able to control the temperature accurately and quickly, in order to see the minute-by-minute changes in photobody dynamics. Experiments show that a temperature increase from 21 to 30 degrees already results in visible effects after several minutes. Together, these observations show that phase separation and light together shape phyB photobodies and further our understanding of light perception and phase separation in the nucleus.

S16 T3

**Characterizing the function of the VIPP1/2 auxiliary VPL proteins in the chloroplast unfolded protein response in *Chlamydomonas reinhardtii***

K. König<sup>1</sup>, A. Probst<sup>1</sup>, S. Zeifelder<sup>1</sup>, L. Weber<sup>1</sup>, M. Kubik<sup>1</sup>, R. Hüttmann<sup>1</sup>, K. Hieronimus<sup>1</sup>, F. Sommer<sup>1</sup>, M. Schroda<sup>1</sup>

<sup>1</sup>RPTU Kaiserslautern-Landau, Molecular Biotechnology & Systems Biology, Kaiserslautern, Germany

In *Chlamydomonas reinhardtii*, VIPP1 and VIPP2 play roles in the sensing, signaling and coping with membrane stress - the chloroplast unfolded protein response (cpUPR), and in the biogenesis of thylakoid membranes (1-3). TurboID-mediated proximity-labeling with VIPP1/2 as baits under ambient and H<sub>2</sub>O<sub>2</sub> stress conditions confirmed known interactions of VIPP1 with VIPP2, HSP70B and CDJ2. Proteins in the proximiomes of VIPP1/2 can be grouped into proteins involved in the biogenesis of thylakoid membrane complexes and the regulation of photosynthetic electron transport. A third group comprises the VIPP PROXIMITY LABELING (VPL1-11) proteins of unknown function whose genes are upregulated under chloroplast stress conditions (4). Our aim is to elucidate how VPL1-11 guide VIPP1/2 effector and signaling functions in the cpUPR to allow for acclimation. We already confirmed VIPP1 in the proximiome of VPL2 in a reciprocal TurboID approach (4). Using mNeonGreen and mScarlet fusions, VPL1 was found to co-localize with VIPP1 in few distinct spots inside the chloroplast, whereas VPL5 localized in many spots, just like VIPP1. Currently, we are analyzing *vpl* mutant phenotypes under various stress conditions. Moreover, we investigate whether VPLs have an impact on the various oligomeric structures formed by VIPP1 *in vitro* and *in vivo*.

References

1. Perlaza et al. (2019) The Mars1 kinase confers photoprotection through signaling in the chloroplast unfolded protein response. *ELife*, 8, e49577
2. Nordhues et al. (2012) Evidence for a role of VIPP1 in the structural organization of the photosynthetic apparatus in *Chlamydomonas*. *The Plant Cell*, 24(2), 637-659
3. Theis et al. (2020) VIPP2 interacts with VIPP1 and HSP22E/F at chloroplast membranes and modulates a retrograde signal for HSP22E/F gene expression. *Plant, Cell and Environment*, 43(5), 1212-1229
4. Kreis et al. (2023) TurboID reveals the proximiomes of *Chlamydomonas* proteins involved in thylakoid biogenesis and stress response. *Plant Physiology*, 193(3), 1772-1796

S16 T4

Being in the right place – Peripheral membrane protein(s) during polar growth of tobacco pollen tubes

C. Fritz<sup>1</sup>, B. Kost<sup>1</sup>

<sup>1</sup>Friedrich-Alexander University Erlangen-Nürnberg, Cell biology, Erlangen, Germany

Tobacco pollen tube tip growth is required for fertilization and serves as an excellent model system to investigate polar cell expansion, a process essential for plant morphogenesis. At the pollen tube tip, regulatory proteins and lipids with key functions in the control of tip growth are specifically associated with clearly distinct plasma membrane (PM) domains. These domains stably retain their specific positions despite constant remodeling of the highly dynamic PM at the pollen tube tip by massive apical secretion and lateral endocytic recycling.

A quantitative map has been developed of the relative positions of PM domains at the pollen tube tip, which 1) are enriched in key signaling proteins (e.g. RAC/ROP signaling) or lipids (e.g. phosphoinositide), 2) display high membrane order, or 3) are in contact with cytoplasmic structures (e.g. actin, vesicles, TGN) playing important roles in apical membrane traffic. This map establishes striking PM and cytoplasmic compartmentalization, which appears to underlie rapid and efficient pollen tube tip growth. Furthermore, molecular mechanisms responsible for the association of the phospholipase C NtPLC3 specifically with the lateral pollen tube PM were investigated. Truncation analysis identified N-terminal and C-terminal regions, which together are necessary and sufficient for correct NtPLC3 PM targeting. This process was further demonstrated to require 1) intramolecular interactions at NtPLC3 domain interfaces (i.e. a salt bridge and a hydrophobic cluster), 2) reversible S-acylation of two cysteine residues at the NtPLC3 N-terminus, which depends on an adjacent polybasic region (PBR), and 3) a C-terminal C2 domain containing several Ca<sup>2+</sup> binding sites along with another PBR. Interestingly, NtPLC3 binds to the phospholipids PI4P, PI4,5P<sub>2</sub> (its substrate) and PA *in vitro*, and remarkably colocalizes with PA *in vivo*.

S16 T5

Calmodulin, IQDs, and Microtubules: Integrating Calcium Signaling with Cytoskeletal Regulation during Cell Division

J. Buhl<sup>1</sup>, P. Dahiya<sup>1</sup>, G. Stamm<sup>1</sup>, K. Bürstenbinder<sup>1</sup>

<sup>1</sup>Philipps Universität Marburg, Plant Cell Biology, Marburg, Germany

The interior of a cell is densely packed with organelles, proteins, nucleic acids, and other (macro)molecules. Within this crowded environment, numerous cellular processes operate simultaneously, the spatial and temporal regulation of which is crucial for cell survival. To manage this complexity cells have evolved sophisticated strategies, including compartmentalization, colocalization, the cytoskeleton, and scaffold proteins. These strategies help transform the cell from an unorganized, crowded space into an organized, highly regulated unit.

In plants, the identity, function, and regulation of scaffold proteins are still poorly understood. The plant-specific IQ67-DOMAIN (IQD) protein family is proposed to function as intrinsically disordered scaffolds at membranes and microtubules, coordinating macromolecular complex assemblies. A characteristic feature of IQD proteins is the presence of the eponymous IQ67 domain, which serves as a complex interaction platform for the Ca<sup>2+</sup>-sensor Calmodulin (CaM). However, the role and biological function of this interaction as well as the stoichiometry, Ca<sup>2+</sup> dependency, the functionality of individual CaM-interacting motifs, and their relation to cellular Ca<sup>2+</sup> signaling are not well understood. Our previous work identified Arabidopsis IQD8 as key determinant in cell plate positioning during cell division suggesting that Ca<sup>2+</sup> and CaM functions are linked to cell division control (1). To elucidate Ca<sup>2+</sup>-CaM-dependent mechanisms during cell division and to investigate the mechanisms and consequences of IQD8-CaM interaction for its cellular function, we use a combination of mutagenesis approaches, protein biochemistry and cell biology. Ultimately, our work focusing on the IQD8-CaM interaction will contribute to a better understanding of signal integration during cell division and the role of scaffold proteins in plants.

References

(1) Kumari, P., Dahiya, P., Livanos, P. et al. IQ67 DOMAIN proteins facilitate preprophase band formation and division-plane orientation. *Nat. Plants* 7, 739–747 (2021)

S17 T1

**Plant enhancers: how they work and where to find them**

T. Jores<sup>1,2,3</sup>, J. Tonnies<sup>3</sup>, N. A. Mueth<sup>3</sup>, S. Gorjifard<sup>3</sup>, J. T. Cuperus<sup>3</sup>, S. Fields<sup>3,4</sup>, C. Queitsch<sup>3,5</sup>

<sup>1</sup>Heinrich Heine University Düsseldorf, Institute of Synthetic Biology, Düsseldorf, Germany

<sup>2</sup>Heinrich Heine University Düsseldorf, Cluster of Excellence on Plant Science (CEPLAS), Düsseldorf, Germany

<sup>3</sup>University of Washington, Department of Genome Sciences, Seattle, WA, United States

<sup>4</sup>University of Washington, Department of Medicine, Seattle, WA, United States

<sup>5</sup>University of Washington, Brotman Baty Institute for Precision Medicine, Seattle, Germany

Enhancers are *cis*-regulatory elements that shape gene expression in response to numerous developmental and environmental cues. In animals, genome-wide maps of enhancer activity exist, and several models have been proposed to explain how enhancers integrate the activity of multiple transcription factors. However, in plants, we know only a few enhancers and it remains unclear how plant enhancers integrate transcription factor activity.

To learn how plant enhancers function, we characterized 3 light-responsive plant enhancers—*AB80*, *Cab-1*, and *rbcS-E9*—derived from genes associated with photosynthesis. Saturation mutagenesis revealed mutations, many of which clustered in short regions, that strongly reduced enhancer activity in the light, in the dark, or in both conditions. When tested in the light, these mutation-sensitive regions did not function on their own; rather, cooperative interactions with other such regions were required for full activity. Epistatic interactions occurred between mutations in adjacent mutation-sensitive regions, and the spacing and order of mutation-sensitive regions in synthetic enhancers affected enhancer activity. In contrast, when tested in the dark, mutation-sensitive regions acted independently and additively in conferring enhancer activity. Taken together, this work demonstrates that plant enhancers show evidence for both cooperative and additive interactions among their functional elements. In a parallel approach, to map enhancers genome-wide, we measured the enhancer activity of over 350,000 sequences derived from accessible chromatin regions of the *Arabidopsis*, tomato, sorghum, and maize genomes. By testing these sequences in the dicot tobacco and the monocot maize, and under different light or temperature regimes, we identified constitutive, species-specific, and condition-specific enhancers and determined the features that are responsible for their activity. Using this data, we trained computational models that can accurately predict the enhancer activity of novel sequences and help design condition-specific enhancers.

Taken together, our work contributes to our understanding of plant gene regulation and will enable the design of strong, condition-specific enhancers.

S17 T2

**Positioning of pyrimidine motifs around cassette exons defines their PTB-dependent splicing in *Arabidopsis***

R. Burgardt<sup>1</sup>, D. Lambert<sup>2</sup>, C. Heuwieser<sup>2</sup>, M. Sack<sup>3</sup>, G. Wagner<sup>2</sup>, Z. Weinberg<sup>3</sup>, A. Wachter<sup>1,2</sup>

<sup>1</sup>Johannes Gutenberg University, Mainz, Germany

<sup>2</sup>University of Tübingen, Tübingen, Germany

<sup>3</sup>Leipzig University, Leipzig, Germany

Alternative splicing (AS) is a complex and versatile process that generates multiple transcript variants from a single pre-mRNA and is involved in numerous biological functions. Many RNA-binding proteins are known to regulate AS; however, little is known about the underlying mechanisms, especially outside the mammalian clade. Here, we show that polypyrimidine tract binding proteins (PTBs) from *Arabidopsis thaliana* regulate AS of cassette exons via pyrimidine (Py)-rich motifs close to the alternative splice sites. Mutational studies on three PTB-dependent cassette exon events revealed that only some of the Py motifs in this region are critical for the AS response. Moreover, *in vitro* binding of PTBs did not reflect a motif's impact on AS *in vivo*. Our analysis further suggested that the position of PTB binding relative to the cassette exon defines whether its inclusion or skipping is induced. Bioinformatic investigation of all known PTB-regulated cassette exons from *A. thaliana* and human supported these characteristic patterns of Py motif distribution for PTB-dependent AS events. Exon skipping is thereby associated with a higher frequency of Py stretches within the cassette exon, and in human also upstream of it, whereas exon inclusion is characterized by increased Py motif occurrence downstream of said exon. Enrichment of Py motifs downstream of PTB-activated 5' splice sites is also seen for PTB-dependent intron removal and alternative 5' splice site events from *A. thaliana*, suggesting this is a common step of exon definition. In conclusion, the position-dependent AS-regulatory mechanism by PTB homologs has been conserved during the separate evolution of plants and mammals, while other critical features, in particular intron length, have considerably changed.

S17 T3

Control of Arabidopsis immune and developmental gene expression through phosphorylation of VQ-motif containing transcriptional co-regulators

J. Marx<sup>1</sup>, P. Pecher<sup>1</sup>, M. Weyhe<sup>1</sup>, J. Lee<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry, Biochemistry of Plant Interactions, Halle a. d. Saale, Germany

(Current address: PP: Dept. for Bioorganic Chemistry, IPB. MW: Landeskriminalamt Niedersachsen).

Mitogen-activated protein kinases (MAPKs) are highly conserved in all eukaryotes and control cellular responses through phosphorylation of target proteins, which are often transcriptional regulators. Our interest in MAPKs relevant for plant immunity led us to analyse a subclass of VQ-motif containing proteins (VQPs) that we termed MVQs (for MAPK-targeted VQPs). MVQs bind specific WRKY transcription factors to modulate their functions. Here, selected examples from our studies on MAPK-MVQ-WRKY network during immunity and plant development will be shown. Since the motif for WRKY interaction overlaps with WRKY-DNA binding domains, it has been speculated that DNA association might be affected. Here, we will present results that support our working model that MVQs act as transcriptional co-regulators while bound to *cis* elements of target genes. Signalling specificities and repertoire can thus be determined by the combinatorial WRKY-VQP variations. Besides phosphorylation-mediated effects, we will also highlight examples where gene regulation may be controlled by formation of biomolecular condensates in the nucleus.

S17 T4

Single-cell sequencing of C<sub>3</sub>-C<sub>4</sub> intermediate Brassicaceae species

S. Triesch<sup>1</sup>, U. Schlüter<sup>1</sup>, A. Weber<sup>1</sup>

<sup>1</sup>Heinrich-Heine-Universität Düsseldorf, Institute for Plant Biochemistry, Düsseldorf, Germany

Plants exhibiting C<sub>3</sub>-C<sub>4</sub> intermediate photosynthesis occur in over 20 Angiosperm lineages and their phenotypes can be seen as interim steps on the evolutionary trajectory towards C<sub>4</sub> photosynthesis. As C<sub>4</sub> photosynthesis is highly complex, C<sub>3</sub>-C<sub>4</sub> intermediate plants offer valuable insights into the early stages of the evolutionary path of biochemical, anatomical and developmental adjustments *en route* to C<sub>4</sub>. We are specifically interested in C<sub>3</sub>-C<sub>4</sub> intermediate species from the Brassicaceae, a family containing multiple important crop and well-characterized model species. We are conducting pan-genomic association mapping across 30 sequenced Brassicaceae species with several independent origins of the C<sub>3</sub>-C<sub>4</sub> intermediate photosynthesis trait.

One frequently observed principle underlying C<sub>3</sub>-C<sub>4</sub> photosynthesis is differential cell autonomy between mesophyll and bundle-sheath cells that form a characteristic anatomy around the leaf veins. To shed light on the underlying genetic mechanisms for the specialized leaf anatomy and biochemistry in the leaf of C<sub>3</sub>-C<sub>4</sub> intermediate Brassicaceae, we performed single-cell RNA sequencing for selected species from this family. Initial results show a remarkable functionalization of the bundle-sheath tissue, e.g. by a large shift of components of the photorespiratory pathway to this cell type.

Focusing on target genes underlying differential spatial expression, we analyzed variation in *cis*-regulatory patterns and their putative association to cell-specific expression in C<sub>3</sub>-C<sub>4</sub> intermediate plants. To this end, we integrated experimental data from DNA methylation sequencing, STARR-seq and single-cell sequencing with predictions from our deep learning pipelines *Helixer* and *Predmoter*, predicting gene models and chromatin state of selected C<sub>3</sub>-C<sub>4</sub> intermediate Brassicaceae genomes.

In doing so, we found in four independent C<sub>3</sub>-C<sub>4</sub> intermediate lineages that the insertion of different transposable elements in the same promoter region of a component of the glycine decarboxylase complex correlates with the differential cell-specific expression of the gene. Notably, this transposon insertion occurs in species forming a polyphyletic group, which hints at a major convergent event in the evolution of C<sub>4</sub> photosynthesis.

S17 T5

## panomiX: a panomics data integration and interpretation tool

A. Sahu<sup>1</sup>, D. Psaroudakis<sup>1</sup>, J. Szymanski<sup>1,2</sup><sup>1</sup>Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Molecular Genetics, Gatersleben, Germany<sup>2</sup>Forschungszentrum Jülich, Institute of Bio- and Geosciences, Jülich, Germany

Recent advancements in high-throughput technologies have catalyzed the generation of vast amounts of multi-omics data, revolutionizing our understanding of plant systems biology. In this study, we present a novel integrative approach, accompanied by the development of a comprehensive toolbox named panomiX, aimed at unraveling the intricate molecular relationships underlying plant mechanisms by seamlessly integrating diverse omics datasets. panomiX, designed with a user-friendly graphical interface, offers a wide array of functionalities, including data preprocessing, uni- and multivariate statistical analysis, multi-block omics integration, geno-pheno analysis, and visualization. Its intuitive interface empowers users to effortlessly navigate through each step of the analytical process. Leveraging state-of-the-art machine learning and regression techniques, panomiX empowers users to navigate through complex biological datasets with ease. Additionally, it employs advanced optimization algorithms, ensuring robust and efficient analysis of complex biological datasets. By applying panomiX to real-world biological datasets encompassing transcriptomics, genomics, phenomics, and Fourier-transform infrared spectroscopy data, we validate its efficacy in advancing omics research. Our study underscores the synergy between integrated multi-omics approaches and computational tools, demonstrating their pivotal role in unraveling the complexity of plant systems at multiple biological levels. The insights gained from this research hold promise for accelerating discoveries and driving innovation in various fields of plant biology. By enabling a comprehensive understanding of gene expression, metabolic interactions, and phenotypic traits, panomiX significantly contributes to the field, advancing plant breeding, stress response analysis, and overall crop improvement.

S18 T1

Dissecting gene-metabolite relationships in the *Medicago truncatula* terpenome after *Aphanomyces euteiches* infectionE. Harding<sup>1</sup>, H. Yadav<sup>1</sup>, S. Marillonnet<sup>1</sup>, S. Rosahl<sup>1</sup>, A. Tissier<sup>1</sup>, B. Hause<sup>1</sup>, P. Ochoa<sup>1</sup><sup>1</sup>Leibniz Institute of Plant Biochemistry, Cell and Metabolic Biology, Halle a. d. Saale, Germany

Plants biosynthesize a broad range of secondary metabolites through specialized and species-specific metabolic pathways. Among these, terpenoids form the largest group of specialized metabolites, with pivotal roles in development, adaptation to external cues, and invaluable antimicrobial properties. Combining transcriptomics and metabolomics approaches, we are reporting on the discovery and functional analysis of two terpene synthases (TPSs), *MtTPS10* and *MtTPSX*, upregulated after infection with *Aphanomyces euteiches* in the roots of two different *Medicago truncatula* ecotypes, A17 and B21, respectively. The TPSs are implicated as being responsible for releasing a complex blend of sesquiterpenoid phytoalexins mediating plant defense. Heterologous expression in yeast revealed that *MtTPS10* catalyzes the formation of himachalol from farnesyl diphosphate (FPP) as its primary sesquiterpene product (Yadav et al., 2019). In contrast, *MtTPSX* catalyzes the formation of copaene from FPP as the most abundant sesquiterpenoid. The volatile terpenes of these two TPSs showed antimicrobial activity against *A. euteiches*. Accordingly, *M. truncatula* plants expressing either *MtTPS10* or *MtTPSX* were found to be resistant to *A. euteiches* infection, whereas the respective mutants exhibited an enhanced susceptibility to the pathogen (Dreher et al., 2017, Yadav et al., 2019). Our studies support the roles of species-specific sesquiterpenoids in root-microbe interactions. This discovery sheds light on legume resistance to root rot caused by *A. euteiches* and offers a potential target for breeding durable, disease-resistant legume cultivars.

## References

Dreher et al. (2017) PeerJ 5, e3713

Yadav et al. (2019) Plant Physiol. 180, 1598-1613



S18 T2

The biosynthesis of thymol, carvacrol, and thymohydroquinone in Lamiaceae proceeds via cytochrome P450s and a short-chain dehydrogenase

S. T. Krause<sup>1</sup>, P. Liao<sup>2</sup>, C. Crocoll<sup>3</sup>, B. Boachon<sup>4</sup>, J. Gershenzon<sup>5</sup>, N. Dudareva<sup>2</sup>, J. Degenhardt<sup>1</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg, Pharmaceutical Biotechnology, Halle a. d. Saale, Germany

<sup>2</sup>Purdue University, Department of Biochemistry, West Lafayette, IN, United States

<sup>3</sup>University of Copenhagen, Department of Plant and Environmental Science, Copenhagen, Denmark

<sup>4</sup>UJM Saint-Etienne, Saint-Etienne, France

<sup>5</sup>Max Planck Institute for Chemical Ecology, Jena, Germany

Thymol and carvacrol are phenolic monoterpenes found in thyme, oregano, and several other species of the Lamiaceae. Long valued for their smell and taste, these substances also have antibacterial and anti-spasmodic properties. They are also suggested to be precursors of thymohydroquinone and thymoquinone, monoterpenes with anti-inflammatory, antioxidant, and antitumor activities. Thymol and carvacrol biosynthesis has been proposed to proceed by the cyclization of geranyl diphosphate to  $\gamma$ -terpinene, followed by a series of oxidations via *p*-cymene. Here, we show that  $\gamma$ -terpinene is oxidized by cytochrome P450 monooxygenases (P450s) of the CYP71D subfamily to produce unstable cyclohexadienol intermediates, which are then dehydrogenated by a short-chain dehydrogenase/reductase (SDR) to the corresponding ketones. The subsequent formation of the aromatic compounds occurs via keto–enol tautomerisms. Combining these enzymes with  $\gamma$ -terpinene in *in vitro* assays or *in vivo* in *Nicotiana benthamiana* yielded thymol and carvacrol as products. We also identified and characterized two P450s of the CYP76S and CYP736A subfamilies that catalyze the hydroxylation of thymol and carvacrol to thymohydroquinone when heterologously expressed in yeast and *N. benthamiana*. Our findings alter previous views of thymol and carvacrol formation and provide targets for metabolic engineering of high-value terpenes in plants.

S18 T3

Neighbourhood matters: Consequences of individual *versus* neighbourhood plant chemodiversity on visiting insects

C. Müller<sup>1</sup>, D. Ziaja<sup>1</sup>, R. Sasidharan<sup>1</sup>, E. Eilers<sup>1</sup>, R. Jakobs<sup>1</sup>

<sup>1</sup>Bielefeld University, Chemical Ecology, Bielefeld, Germany

Some plant species exhibit a fascinatingly high intraspecific chemodiversity which may affect interacting organisms. The Asteraceae *Tanacetum vulgare* is characterised by various terpenoids, which differ in composition among individuals, forming distinct chemotypes. We thus investigated how visiting insects, such as herbivorous aphids and flower feeders but also pollinators, are affected by the chemodiversity of individual plants versus the chemodiversity of intraspecific neighbours. Therefore, we set up a common garden with patches consisting either of five *T. vulgare* plants of one chemotype (homogenous plots) or of five different chemotypes (heterogenous plots) and observed visiting insects across two seasons. Different aphid specialists of *T. vulgare* showed distinct patterns. *Macrosiphoniella tanacetii* was positively influenced by the individual plant chemodiversity, while *Uroleucon tanacetii* was negatively affected by plot chemodiversity. Flower feeding beetles of the genus *Olibrus* showed clear preferences for certain chemotypes but plot type had no effect, while pollinators were overall more abundant on heterogenous than on homogenous plots. Our data highlight that not only the individual plant chemodiversity matters but that neighbourhood is likewise highly important for driving antagonists and mutualists, with a high species-specificity in insect responses. Intraspecific diversity in plant specialised metabolism has thus numerous ecological implications.

S18 T4

**Selecting for Quantity and Quality of Acylsugars in Tomato Whitefly Resistance Breeding**

J. W. de Kraker<sup>1</sup>, J. Bleeker<sup>1</sup>, D. Cano<sup>2</sup>, I. Degeling<sup>1</sup>, T. Montoro<sup>2</sup>, J. Romers<sup>1</sup>, I. Roobeek<sup>1</sup>, N. Solo<sup>1</sup>, F. Villanueva<sup>2</sup>, M. Ykema<sup>1</sup>

<sup>1</sup>ENZA Zaden R&D, Enkhuizen, Netherlands

<sup>2</sup>Enza Zaden R&D, Almería, Spain

Economically, whitefly (*B. tabaci*) is one of the most important pests in tomato (*S. lycopersicum*). It causes direct damage through feeding on plant phloem sap, but moreover indirect damage through plant virus transmission. For several years it is known that presence of acylsugars can effectively reduce the risk of whitefly infestation, but in domesticated tomato varieties these compounds are almost absent<sup>1</sup>. In tomato wild species these secondary metabolites are present in glandular trichomes and consist of a sucrose moiety decorated with 2-5 acylchains of amino acid origin and/or fatty acid origin, creating a wide structural diversity<sup>2</sup>. In recent years several research groups were very successful in identifying the structural genes involved in biosynthesis of acylsugars<sup>3,4</sup>. We focussed however on (re)introducing acylsugars in cultivated tomato by a combination of QTL-mapping and efficient chemotyping of vast numbers of plants. This resulted in the identification of an *AP2e* gene encoding an APETALA2 ethylene-responsive transcription factor that is introduced from the wild tomato source and upregulates the acylsugar production for which most structural (biosynthetic) genes still seem to be present in domesticated tomato lines. Selection for *AP2e* would increase total acylsugar content (quantity) and resulted in elevated levels of resistance against whitefly. Yet, in the continuation of the breeding process, it became clear that besides quantity, the type of acylsugar (quality) matters as well for a good resistance. Hence, in addition to *AP2e*, we need to retain the correct structural genes to assure tomato plants have predominantly acylsugars that really contribute to whitefly resistance.

References

<sup>1</sup>Leckie BM, *et al.* (2012) *Mol Breeding* 30: 1621-163

<sup>2</sup>Ghosh B, *et al.* (2014) *Metabolomics* 10: 497-507

<sup>3</sup>Fan P, *et al.* (2015) *PNAS* 113: E239-248

<sup>4</sup>Fan P, *et al.* (2020) *eLife* 9: e56717

S18 T5

**Synthetica Botanica: Engineering the Future of Plant Metabolism**

J. D'Auria<sup>1</sup>

<sup>1</sup>Leibniz IPK, Molecular Genetics / Metabolic Diversity, Seeland, Germany

Question - Can gene discovery and enzyme characterization for structural genes involved in specialized metabolism be enhanced or expedited using synthetic biology?

Recent advancements in plant biochemistry have shed light on the intricate biosynthetic pathways of two significant alkaloids: tropane alkaloids (TAs) in Erythroxylaceae and Solanaceae, and gramine in grasses. Our comprehensive research program reveals the polyphyletic nature of TA biosynthesis, characterized by the independent evolution of unique enzymatic mechanisms across different plant orders. Utilizing yeast as a versatile screening platform, we identified the missing links in the TA biosynthetic pathway of *Erythroxylum coca*. This includes the discovery of bifunctional spermidine synthase/N-methyltransferases and flavin- and copper-dependent amine oxidases responsible for the initial tropane ring closure. Further, a SABATH family methyltransferase was found to be crucial for adding the 2-carbomethoxy moiety, a hallmark of Erythroxylaceae TAs. Coexpression with methylecgonone reductase facilitated the production of a novel hybrid TA, integrating structural elements from both Erythroxylaceae and Solanaceae lineages. Additionally, clustering analysis of *Erythroxylum* transcriptome datasets led to the identification of a CYP81A family cytochrome P450 enzyme, orchestrating the second tropane ring closure, culminating in the de novo biosynthesis of methylecgonine in yeast.

In parallel, our research into the defensive alkaloid gramine, which serves as a deterrent to insects yet reduces palatability in ruminants, has pinpointed the elusive gene responsible for its synthesis. The gene, encoding the cytochrome P450 monooxygenase CYP76M57—dubbed AMI synthase (AMIS)—enables gramine production in various species, including *Nicotiana benthamiana*, *Arabidopsis thaliana*, and *Saccharomyces cerevisiae*. By introducing AMIS into the gramine-free barley variety Golden Promise and removing it from the cultivar Tafeno via Cas-mediated gene editing, we have demonstrated the potential for precise manipulation of gramine content. This breakthrough is underpinned by the discovery of a cryptic oxidative rearrangement process, converting an amino acid into a biogenic amine.

S19 T1

Epidermal bladder cells of *Chenopodium quinoa* as a herbivore defense mechanism

M. W. Moog<sup>1</sup>

<sup>1</sup>University of Copenhagen, Department of Plant and Environmental Sciences, Frederiksberg C, Denmark

The aerial surfaces of quinoa (*Chenopodium quinoa*) and common ice plant (*Mesembryanthemum crystallinum*) are covered with a layer of epidermal bladder cells (EBCs), which are modified non-glandular trichomes previously considered to be key to the extreme salt and drought tolerance of these plants. Screening mutant plants with substantially reduced numbers of EBCs revealed that these cells only play minor roles, if any, in abiotic stress tolerance and in fact are detrimental under conditions of water deficit. EBCs instead function as deterrents to a broad range of generalist arthropod herbivores, through their combined function of forming both a chemical barrier, by accumulating the toxin oxalic acid, as well as a physical barrier, due to their morphology. Furthermore, EBCs also serve a protective function against a phytopathogen. This work overturns current models that link EBCs to salt and drought tolerance and assigns new functions to these structures that might provide novel possibilities for protecting crops from arthropod pests.

S19 T2

A metabolic arms race between barley (*Hordeum vulgare* L.) and two fungal pathogens

D. Esposito<sup>1</sup>, Y. Liu<sup>1</sup>, L. Mahdi<sup>2</sup>, A. Porzel<sup>3</sup>, P. Stark<sup>3</sup>, H. Hidayat<sup>3</sup>, A. Scherr-Henning<sup>1</sup>, U. Bathe<sup>1</sup>, I. Acosta<sup>4</sup>, A. Zuccaro<sup>2</sup>  
G. Balcke<sup>1</sup>, A. Tissier<sup>1</sup>

<sup>1</sup>Leibniz Institute for Plant Biochemistry (IPB), Cell and Metabolic Biology (SZB), Halle a. d. Saale, Germany

<sup>2</sup>Institute for Plant Sciences, Cluster of Excellence on Plant Sciences (CEPLAS), Köln, Germany

<sup>3</sup>Leibniz Institute for Plant Biochemistry (IPB), Bioorganic Chemistry (NWC), Halle a. d. Saale, Germany

<sup>4</sup>Max-Planck Institute for Molecular Plant Physiology, Golm, Germany

In this work [1], we show a metabolic interaction between barley (*H. vulgare* L.) and the fungal pathogens *Bipolaris sorokiniana* [Bs] and *Fusarium graminearum* [Fg], involving hordedanes, a previously undescribed set of labdane-related diterpenoids, with antimicrobial properties. Both fungi are the pathogenic agents of several diseases in wheat and barley and represent a major threat for these crops. Using previously established systems, we characterized a gene cluster induced upon infection with Bs and reconstructed major parts of a network of diterpenoids and characterized several of the intermediates and products by NMR. Among the newly discovered genes we found homologs of copalyl diphosphate synthase (HvCPS2), kaurene synthase-like (HvKSL4), and several cytochrome P450 oxygenase (CYPs). By analysing the barley root exudates via high resolution HPLC-MS, we also discovered that hordedanes are mostly secreted into the rhizosphere, suggesting a role in the interaction between plant and microorganisms. While *Hvcps2* mutants are unable to produce hordedanes, *Hvksl4* mutants retained the ability to generate copalol-derivatives. When confronted with Fg, *Hvcps2* mutants show an increase in root colonization, confirming the importance of hordedanes in the defence against this pathogen. *Hvksl4* mutants were not more colonized than WT by Fg, suggesting the copalol-derivatives are still toxic to Fg. Unexpectedly though, both mutants showed reduced colonization by Bs. Moreover, incubation of Bs with one of the most abundant hordedanes in the exudates (compound 21) enhances the fungal growth in a concentration-dependent manner. Thus, Bs seems to take advantage of this compound. This is also supported by the results of a RNAseq experiment carried out on Bs in the presence of compound 21. Over the course of three days, Bs undergoes transcriptional remodelling that shows the induction of detoxification enzymes as well as changes in genes involved in pathogenicity. Furthermore, confocal microscopy of barley roots infected by Bs suggest an earlier switch to the necrotrophic phase in *Hvcps2* mutants. These results constitute an illustration of the metabolic arms race between plants and pathogens and highlight the importance of studying metabolic interactions between them.

References

[1] Liu et al., 2023 (cite BioRxiv paper)

S19 T3

Exploring the Functional Diversity of NLR Immune Receptors in Divergent Land Plant Lineages

K. S. Chia<sup>1</sup>, P. Carella<sup>1</sup>

<sup>1</sup>John Innes Centre, CDB, Norwich, United Kingdom

Nucleotide-binding domain and leucine-rich repeat (NLR) immune receptors are crucial for plant immunity against pathogens. To date, most studies of NLR proteins are centered on the evolutionarily young angiosperm (flowering plant) lineage. By comparison, we know much less about the conservation and functional diversification of NLRs across the full diversity of land plant lineages. To begin to explore plant NLR diversity, we first focused on broadly distributed NLR N-terminal domains (CCs and TIRs) as they are directly involved in executing immune responses in flowering plants. Through heterologous expression in the model angiosperm *Nicotiana benthamiana*, we demonstrated functional conservation of CC and TIR families across all major plant lineages, from algae to angiosperms. Moreover, we identified comparable CC-induced immune-like responses in the liverwort *Marchantia polymorpha*, which hints to the existence of an ancestral CC-mediated response in plants. In addition, we are also exploring the function of novel lineage-specific N-terminal domains (protein kinases and alpha/beta hydrolases) of bryophytes. This represents the first functional validation of a non-canonical immune receptor domains in plants. Moving forward, we are examining the extent to which full-length NLRs are transferable across distantly related plants like *Marchantia* and *Nicotiana*. Our initial data supports the deep functional conservation of NLR proteins and challenges the established notion that immune receptors have limited transferability across the plant kingdom.

References

Khong-Sam Chia, Jiorgos Kourelis, Albin Teulet, Martin Vickers, Toshiyuki Sakai, Joseph F Walker, Sebastian Schornack, Sophien Kamoun, Philip Carella, The N-terminal domains of NLR immune receptors exhibit structural and functional similarities across divergent plant lineages, *The Plant Cell*, 2024;, koae113, <https://doi.org/10.1093/plcell/koae113>

S19 T4

Carbohydrate availability and homeostasis modulate pathogen susceptibility of *Arabidopsis thaliana*

K. S. Munzert<sup>1</sup>, L. Zanon<sup>1</sup>, H. Leicher<sup>2</sup>, C. Rautengarten<sup>3</sup>, B. Ebert<sup>3</sup>, M. Stegmann<sup>2,4</sup>, L. Voll<sup>1</sup>, T. Engelsdorf<sup>1</sup>

<sup>1</sup>Philipps-Universität Marburg, Biology / Molecular Plant Physiology, Marburg, Germany

<sup>2</sup>TUM School of Life Sciences, Biology / Phytopathology, Freising, Germany

<sup>3</sup>Ruhr University Bochum, Biology / Molecular Evolution of Plants, Bochum, Germany

<sup>4</sup>Ulm University, Molecular Botany, Ulm, Germany

Plant carbohydrate availability has profound effects on susceptibility to fungal pathogens. Starch-free *Arabidopsis thaliana* plastidic phosphoglucosyltransferase (*pgm*) mutants suffer from nocturnal carbohydrate shortage and impaired defence against the hemibiotrophic pathogen *Colletotrichum higginsianum*. Previous studies revealed that preformed defence, like the composition of the plant cell wall as a penetration barrier, as well as induced defence, like the induction of salicylic acid-dependent responses, are impaired in *pgm* mutants, leading to hypersusceptibility to *C. higginsianum* (Engelsdorf et al., 2013, 2017).

To identify genes involved in the regulation of carbohydrate-dependent pathogen susceptibility, we performed a forward genetic screening and identified suppressors of *pgm* hypersusceptibility. One of the suppressor candidates was mapped to *OUTER ENVELOPE PROTEIN 40* (*OEP40*), coding for a  $\beta$ -barrel solute channel in the chloroplast outer envelope membrane. *OEP40* is *in vitro* permeable for glucose and glucose phosphates and the channel properties are regulated by trehalose 6-phosphate (Harsman et al., 2016), suggesting a function in sugar homeostasis and/or signalling in the context of pathogen defence. Preliminary data indicate that increased resistance in *pgm oep40* is established in the early biotrophic interaction with *C. higginsianum* and correlates with changes in cell wall composition, rescuing the *pgm* cell wall deficit, as well as with the overaccumulation of defence-related compounds (salicylic acid and camalexin). Of note, loss of *PGM* and *OEP40* have additive effects in increasing resistance against the biotrophic powdery mildew fungus *Erysiphe cruciferarum*, which depends on provision of sugar by its host. In order to reveal the mechanism of the *pgm* hypersusceptibility suppression in *pgm oep40* against *C. higginsianum*, we conducted transcriptomics and carbohydrate analyses, including nucleotide sugar measurements and cell wall fractionation to further elucidate the cell wall phenotype.

Together, our data indicate that metabolite transport through *OEP40* might be required for cell wall-related phenotypes in *pgm* and that defects in *OEP40* stimulate defence signalling. We will present data from genetic analyses to reveal contributions of sugar transport across the chloroplast envelope and of specific defence pathways to *oep40*-dependent pathogen resistance.

S19 T5

Characterisation of polygalacturonases in the interaction of parasitic plants and phytopathogens with their host plant

W. Häger<sup>1</sup>, T. Bawin<sup>2</sup>, K. Krause<sup>2</sup>, T. Hamann<sup>1</sup>

<sup>1</sup>Norwegian University of Science of Technology, Department of Biology, Trondheim, Norway

<sup>2</sup>The Arctic University of Norway, Department of Arctic and Marine Biology, Tromsø, Norway

Plant cell walls (PCWs) represent the first line of defence against biotic and abiotic stresses. Besides conferring stability and protection, PCWs can be dynamically modified during plant development or in response to environmental stimuli. Some stimuli are remarkably similar between intrinsic plant-derived and external plant-threatening processes: PCW-degrading enzymes – among them pectin-hydrolysing polygalacturonases (PGs) – target PCW polysaccharides and are produced by both plants and microbial phytopathogens. While plant PGs are required for processes such as PCW remodelling during development, microbial PGs macerate the PCW during plant infection.

Parasitic plants grow into host plants and establish a vascular connection with the help of PCW degrading enzymes to obtain water and/or nutrients from host plants. Such action implies that parasitic plants combine both PCW remodelling and PCW maceration during host plant infection. Combining *in silico* and *in vitro* methods, we compared PGs derived from *Arabidopsis thaliana*, phytopathogenic fungi and the parasitic plant *Cuscuta campestris* to identify PGs enabling us to I) characterise differences between microbial and plant-derived PGs, II) investigate if/how such differences result in diverging PG activities, and III) what activities and characteristics PGs have that *C. campestris* produces during host plant infection.

S20 T1

Engineering an Exo70 integrated domain of a barley NLR for improved blast resistance

I. Saado<sup>1</sup>, H. J. Brabham<sup>2</sup>, J. W. Bennett<sup>1</sup>, A. H. C. Lam<sup>3</sup>, M. J. Moscou<sup>4</sup>, J. C. D. Concepcion<sup>5</sup>, M. Banfield<sup>1</sup>

<sup>1</sup>John Innes Centre, Biochemistry and Metabolism, Norwich, United Kingdom

<sup>2</sup>The Sainsbury Laboratory, Norwich, United Kingdom

<sup>3</sup>John Innes Centre, Cell and Developmental Biology, Norwich, United Kingdom

<sup>4</sup>USDA-ARS Cereal Disease Laboratory, University of Minnesota, Minnesota, MN, United States

<sup>5</sup>Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria

Plants depend on their innate immune system to detect and respond to invading organisms. Plant genomes are equipped with a diverse array of receptors on the cell surface as well as intracellular immune receptors known as nucleotide-binding leucine-rich repeat (NLR) proteins. These receptors can recognize non-self molecules, often in the form of effector proteins translocated from the invading pathogen. Upon direct or indirect recognition, NLR receptors initiate signaling cascades resulting in a cell death, limiting the spread of infection. The discovery of unconventional domains integrated in NLRs that bind pathogen effectors and modulate immunity has emerged as a new route for engineering NLR proteins to improve disease resistance<sup>1</sup>.

The Pii-1 and Pii-2 NLR protein pair from rice (*Oryza sativa*) recognizes the AVR-Pii effector from the fungal blast pathogen *Magnaporthe oryzae* through the host factor OsExo70<sup>2,3</sup>. Previous research uncovered a novel Exo70 integrated domain in the barley NLR protein, RGH2<sup>4</sup>. Through biophysical and biochemical approaches, we show that the sensor NLR RGH2 can bind AVR-Pii from both wheat and rice blast pathogens, albeit with lower affinity compared to the rice OsExo70F3 protein. To improve this binding affinity, we engineered a modified version of RGH2, termed RGH2+, by introducing structure-guided mutations at the RGH2-Exo70 interface to mimic the binding interface of OsExo70F3. The resulting RGH2+ protein shows an enhanced affinity/activity towards AVR-Pii using multiple assay systems. Encouraged by these findings, we generated transgenic barley lines expressing the RGH2+ NLR under its native promoter. Disease infection assays showed reduced virulence with pathogen strains expressing the AVR-Pii effector. This outcome highlights the potential of engineering NLR proteins as an effective strategy for improving disease resistance in crops.

References:

- 1.Grund, Elisabeth, et al. "Plant NLRs with integrated domains: unity makes strength." *Plant physiology*, 2019.
- 2.Fujisaki, Koki, et al. "Rice Exo70 interacts with a fungal effector, AVR-Pii, and is required for AVR-Pii-triggered immunity." *The Plant Journal*, 2015.
- 3.De la Concepcion, J. C.; et al. "A Blast Fungus Zinc-Finger Fold Effector Binds to a Hydrophobic Pocket in Host Exo70 Proteins to Modulate Immune Recognition in Rice." *Natl. Acad. Sci.*, 2022.
- 4.Brabham, Helen J., et al. "An ancient integration in a plant NLR is maintained as a trans-species polymorphism", 2018.

S20 T2

Imaging of plant calcium-sensor kinase conformation monitors real time calcium decoding *in vivo*A. Liese<sup>1</sup>, B. Eichstädt<sup>2</sup>, S. Lederer<sup>1</sup>, S. Matschi<sup>1</sup>, J. A. Feijó<sup>3</sup>, W. Schulze<sup>4</sup>, K. Konrad<sup>5</sup>, T. Romeis<sup>1,6</sup><sup>1</sup>Leibniz Institute of Plant Biochemistry Halle, Biochemistry of Plant Interactions, Halle a. d. Saale, Germany<sup>2</sup>Free University, Dahlem Center of Plant Sciences, Berlin, Germany<sup>3</sup>University of Maryland, Department of Cell Biology & Molecular Genetics, College Park, MD, United States<sup>4</sup>University of Hohenheim, Plant Systems Biology, Hohenheim, Germany<sup>5</sup>Julius Maximilians University Wuerzburg, Molecular Plant Physiology and Biophysics, Würzburg, Germany<sup>6</sup>Martin-Luther-University Halle-Wittenberg, Biology, Halle a. d. Saale, Germany

The family of calcium-dependent protein kinases (CDPK) represent important calcium-decoding enzymes in plants. Acting as dual-function calcium sensors and protein kinase effectors, CDPKs detect calcium signals and convert them into substrate phosphorylation. Here we developed the so-called *CPKaleon*, a Förster resonance energy transfer (FRET)-based biosensor for assessing the calcium binding-induced conformational change of CDPKs. Calcium-dependent conformational change measured by FRET reflects the kinase enzymatic activity allowing a real time recording of enzyme activation and inactivation. The 34 *A. thaliana* CDPKs strongly differ with respect to their calcium-dependency of kinase activity. To address the specificity of CDPK-mediated decoding of calcium signals and signatures, we characterized homologous Arabidopsis CDPKs with distinct calcium-sensitivities: highly calcium-sensitive *Arabidopsis thaliana* AtCPK21 and rather calcium-insensitive AtCPK23. By combining the assessment of conformational change with kinase activity measurements, and including proteoforms that carry single amino acid variations, we uncover the impact of a single auto-phosphorylation site on the calcium-dependent conformational change. Moreover, the parallel monitoring of CDPK-FRET and the intracellular calcium-concentration allows a simultaneous assessment of calcium encoding and decoding *in planta*. Thus, *in vivo* CPKaleon imaging allows to decipher different calcium regulation mechanisms and provides a promising tool to investigate CDPK function *in situ in planta* in real time.

S20 T3

## Structural differences in functional relatives over the panproteome of barley

A. Souza Câmara<sup>1</sup>, V. Henrique Rabesquine Nogueira<sup>1</sup><sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genebank Department, Gatersleben, Germany

In these exciting times of protein structure prediction by artificial intelligence, we explore the diversity of proteoforms that have evolved with barley. The pangenome of barley gathers 76 barley genotypes (including wild, landraces and cultivars) with completely sequenced and annotated genomes. They amount to more than 3 million coding genes. But making sense of such large-scale data is not an easy task. We focus on the MATE and ALMT families [1,2,3] - aluminium-activated transporters in plants, responsible for protecting the roots from aluminium toxicity in the soil - to assess how structurally different these functional relatives can be. They sum up to more than 4600 homologs and paralogs, or copies and proteoforms, that suggest mechanisms of structural and functional evolution. We predicted their structures using AlphaFold2 [4] and analysed them regarding sequence and structure. We found three levels of structural differences between them, mostly not obvious from sequence. They may differ in number and type of structural domains, forming functional dimers or pseudimers; in preference for conformational state; or just in creative structural embellishments, but still under some constrictions. Not randomly arranged across subfamilies and genotypes, these specific structural variances can now be more easily linked to function. We come to some general observations, like some regions of the protein being more susceptible to structural variances than others (loops placed in the inner or the outer side of the cell). And we point to specific residues present in some subfamilies that possibly shift the energy barrier between conformational states of opening and closing the protein transport channel. The insights we present are only possible due to the natural abundance of proteoforms existing in the panproteome of barley. While we expect much more can be taken from panproteomes, we cherish these as guiding first steps into this blossoming field.

## References

- [1] Liu, J., et al. *The Plant Journal* 57.3 (2009): 389-399.  
 [2] Yokosho, K., et al. *Plant and Cell Physiology*, 57.5 (2016): 976-985  
 [3] Du, Z., et al. *BMC Ecology and Evolution* 21 (2021): 1-14.  
 [4] Jumper, J. *Nature* 596.7873 (2021): 583-589.

S20 T4

**Effect of changes in different AGO2 proteoforms on antiviral protection in *Arabidopsis thaliana***  
 S. Gago-Zachert<sup>1</sup>, M. Xiang<sup>1</sup>, F. Müller<sup>1</sup>, A. Winkler<sup>1</sup>, S. Fay<sup>1</sup>, T. Gursinsky<sup>1</sup>, R. Golbik<sup>1</sup>, S. E. Behrens<sup>1</sup>  
<sup>1</sup>Martin Luther University Halle-Wittenberg, Halle a. d. Saale, Germany

Viral infections are one of the most important challenges to plant health and are mainly counteracted by the plant RNA silencing machinery. Argonaute (AGO) proteins are important effectors of the RNA silencing pathway and mediate the sequence-specific degradation of viral RNAs in a process involving small interfering RNAs. In *Arabidopsis thaliana*, AGO1 and AGO2 are the major antiviral proteins<sup>1,2</sup>. In the present work, we identified the most representative AGO2 proteoforms in the collection of 1135 *Arabidopsis* genomes<sup>3</sup> using the SNPstar web tool<sup>4</sup>. We characterized the catalytic activity of the proteoforms *in vitro* using an established system of cytoplasmic extracts of cultivated *N. tabacum* cells<sup>5,6</sup> and analyzed the response of different accessions carrying the identified proteoforms to infections with *Cucumber mosaic virus* (CMV), a very important and widely distributed plant pathogen that also infects wild populations of *A. thaliana*. In addition, and to evaluate potential differences in the antiviral effects of the different AGO2 proteoforms in the same genetic background, we generated transgenic lines by complementing the null *ago2-1* mutant<sup>2</sup> with constructs containing the genes encoding the selected AGO2 proteoforms under the control of the regulatory regions of the Col-0 ecotype.

Our results indicate that accessions carrying the different AGO2 proteoforms exhibit different susceptibilities to the infection with CMV. In addition, the complemented lines showed a better survival and were able to recover from the infection, resulting in a higher seed yield compared to control plants. Differences in the success of the distinct accessions to CMV infection may be due the result of the different catalytic properties of the identified proteoforms, which may have been selected based on their adaptive advantages in different environments.

References

1. Morel JB, Godon C, Mourrain P, Béclin C, Boutet S, Feuerbach F, Proux F, Vaucheret H. (2002). *Plant Cell*, 14(3):629-39.
2. Harvey JJ, Lewsey MG, Patel K, Westwood J, Heimstädt S, Carr JP, Baulcombe DC. (2011) *PLoS One*, 6(1):e14639.
3. 1001 Genomes Consortium (2016) *Cell*, 166(2):481-491.
4. Welcome to SNP2Prot - Plant Proteoform Diversity (uni-halle.de)
5. Gursinsky T, Schulz B, Behrens S-E. (2009) *Virology*, 390, 250–260.
6. Gago-Zachert S, Schuck J, Weinholdt C, Knoblich M, Pantaleo V, Grosse I, Gursinsky T, Behrens S-E. (2019) *Nucleic Acids Res.*, 47, 9343–9357.

S20 T5

**Structural studies of the transmembrane domain of ethylene receptor ETR1**  
 B. Ruffer<sup>1</sup>  
<sup>1</sup>Institute of Molecular Photosynthesis, Heinrich-Heine-Universität, Düsseldorf, Germany

The plant hormone ethylene and its signaling pathway have been highly conserved for over 450 million years. Membrane-bound receptor kinases are the key to the ethylene signaling pathway, which is responsible e.g., for fruit ripening, defense reactions, and senescence processes. So far, the molecular structure has been resolved only for most of their cytosolic part (HK-RD), while the precise structure of the transmembrane part (TMD) carrying the ethylene binding site is still unknown. Hence, we used EPR spectroscopy and LCP crystallization to get detailed insights into the structure of the TMD. In addition, a combination of biochemical and plant experiments was used to gain more information about the binding of a copper cofactor, which is essential for high-affinity binding of the plant hormone to the receptor (Azhar et al. 2023). The EPR experiments (Kugele et al., 2022) indicate higher flexibility in the TMD than expected from computational models by Schott-Verdugo et al. (2019) or the artificial intelligence (AI) program *AlphaFold2* (Jumper et al., 2021). In the LCP crystallization trails, we obtained several crystals, which were subjected to X-ray analysis (Ruffer et al., 2024). If the structure of the TMD can be solved using these approaches, novel inhibitors of ethylene signaling may be identified. The coordination centre of the copper cofactor and the ethylene binding pocket can be analyzed in these structures in detail. Furthermore, high-resolution structures of the TMD will also allow detailed insights into how the copper cofactor is transferred from metallochaperones ATX1, CCH, and RAN1 to the copper active site in the ETR1-TMD. Additionally, the signal transduction mechanism leading to the conformational change and activation of the signaling cascade can be explained.

References

- Schott-Verdugo, S. et al. (2019) *Sci. Rep.* 9: 8869.  
 Azhar, B.J. et al. (2023) *PNAS* 120: e2215195120.  
 Kugele A. (2022) *RSC Adv.* 12: 7352-7356  
 Jumper, J. et al. (2021) *Nature*, 596: 583-589.  
 Ruffer, B. et al. (2024) *Biomolecules*, 14: 375

S21 T1

How do different ploidies adapt? A case of *Arabidopsis* in a non-extreme edaphic environmentS. Celestini<sup>1</sup>, V. Konečná<sup>2</sup>, F. Kolář<sup>1</sup><sup>1</sup>Charles University, Department of Botany, Prague, Czech Republic<sup>2</sup>ETH Zurich, Institute of Integrative Biology, Zurich, Switzerland

Understanding how organisms adapted to the environment is important in order to predict future evolutionary outcomes. Whole-genome duplication (WGD), a common macro-mutation in plants, is thought to affect the tempo and modes of evolutionary processes. By theory, the additional set of chromosomes in polyploid organisms may mask or dilute alleles in a heterozygous state, diminishing the efficiency of purifying and positive selection and slowing-down fixation. Conversely, heightened heterozygosity, in concert with an increased number of crossing-over events, might help polyploids to experiment and recombine in more disparate haplotypes following new evolutionary trajectories. How different ploidies of the same species differ in their genetic evolution in natural populations has, however, not been addressed so far. In order to fill in this gap, we analyzed resequenced genomes of 76 populations of diploid-autotetraploid *Arabidopsis arenosa* from sites characterized for locally sampled substrate and uncovered ploidies' differences in adaptation to non-extreme and common siliceous and calcareous soil types. We identified a set of genes associated with ion transport and homeostasis repeatedly selected across the *A. arenosa* range, but only partially overlapping between ploidies. Notably, at these candidate loci polyploids retain a higher variation, given by a lower fixation rate. In addition, due to polysomic masking selection struggles targeting de-novo mutations and rather sources adaptation from standing variation. Yet, contrary to diploids, tetraploid individuals exploit their alleles' accessibility and are finally able to adapt thanks to genes that are more central in the gene co-expression network, affecting a higher number of downstream fitness-related traits. Taken together, our results suggest that tetraploids successfully adapt to their environment thanks to their higher genetic flexibility which compensates for the slower effect of positive selection.

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## Fragrant Phylogenies: Decoding Daffodil scents with GC-IMS

T. Böhnert<sup>1</sup>, F. Losch<sup>1</sup>, M. Weigend<sup>1</sup><sup>1</sup>University of Bonn, Bonn Institute of Organismic Biology, Bonn, Germany

Floral volatiles are crucial drivers of natural selection, yet broad studies examining their evolutionary context are rare. Traditional methods for studying floral scent, such as Gas Chromatography-Mass Spectrometry (GC-MS), are hampered by high costs, complex sample preparation, and time-consuming analysis, limiting their use in large-scale evolutionary studies. In this study, we demonstrate the potential of a new alternative method, Gas Chromatography coupled Ion Mobility Spectrometry (GC-IMS). GC-IMS is a promising novel approach in floral ecology, offering faster and more resource-efficient analyses suitable for broad-scale investigations, permitting comprehensive sampling in short time periods. To showcase this method, we investigated the floral volatile profiles of *Narcissus* (Amaryllidaceae), a genus notorious for its strong floral scents, in a phylogenetic context using two complementary next-generation sequencing approaches. Our data reveal strong phylogenetic signals for the presence of key floral volatiles, alongside rapid shifts in overall scent profiles, reflecting evolutionary lineages on the one hand and a correspondence to morphological shifts on the other. Some floral volatiles are conserved within clades, while others exhibit rapid evolutionary changes that do not strictly follow phylogenetic trajectories. These findings highlight the dynamic nature of floral scent evolution and the influence of both genetic and ecological factors. The phylogenetic analysis, derived from ddRADseq and target capture sequencing of over 50 *Narcissus* taxa spanning all major infrageneric taxa, revealed well-supported clades and high-resolution relationships among species, while also highlighting complex signatures of reticulate evolution. Floral volatile emission patterns in these species include key compounds such as (*E*)- $\beta$ -Ocimene, Linalool, Benzyl acetate, and Lilac aldehyde. This study integrates advanced phylogenetic methods with innovative scent analysis techniques and illustrates the utility of GC-IMS in large-scale evolutionary and ecological studies. It results in a comprehensive picture of the evolution and diversity of floral scent within the genus *Narcissus*.



S21 T3

Testing different strategies to efficiently assemble organellar and nuclear plant genomes – a case study on  
*Ranunculus auricomus* (Ranunculaceae)

K. Karbstein<sup>1,2</sup>, S. Tomasello<sup>2</sup>, N. Wagner<sup>2</sup>, T. Xie<sup>3</sup>, B. H. Barke<sup>2</sup>, C. Pätzold<sup>2,4</sup>, J. P. Bradican<sup>2</sup>, M. Preick<sup>5</sup>, A. Himmelbach<sup>6</sup>  
N. Stein<sup>6</sup>, I. Irisarri<sup>7,8</sup>, B. Pucker<sup>9</sup>, J. de Vries<sup>7</sup>, A. Papantonis<sup>3</sup>, E. Hörandl<sup>2</sup>

<sup>1</sup>Max Planck Institute for Biogeochemistry, Department of Biogeochemical Integration, Jena, Germany

<sup>2</sup>University of Göttingen, Department of Systematics, Biodiversity and Evolution of Plants (with Herbarium), Göttingen, Germany

<sup>3</sup>University Medical Center Göttingen, Institute of Pathology, Göttingen, Germany

<sup>4</sup>Senckenberg Naturhistorische Sammlungen, Dresden, Germany

<sup>5</sup>University of Potsdam, Institute for Biochemistry and Biology, Potsdam, Germany

<sup>6</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany

<sup>7</sup>University of Göttingen, Department of Applied Bioinformatics, Göttingen, Germany

<sup>8</sup>Leibniz Institute for the Analysis of Biodiversity Change (LIB), Centre for Molecular Biodiversity Research, Hamburg, Germany

<sup>9</sup>TU Braunschweig, Institute of Plant Biology, Braunschweig, Germany

Questions: Currently, the reliable reconstruction of large and complex genomes from non-model plants remains a challenge in terms of laboratory effort and cost, as well as assembly quality<sup>1-3</sup>. This often hinders the study of evolutionarily complex groups of species. The large polyploid *Ranunculus auricomus* complex (Ranunculaceae) is an angiosperm model system for the study of apomixis, reticulate evolution, and biogeography<sup>4-7</sup>. However, no plastid, mitochondrial, or high-quality nuclear genomes are available. This has limited phylogenomic, ecological, and taxonomic analyses. Methods: Here, we tested different Illumina short-read, Oxford Nanopore Technology (ONT) or PacBio (HiFi) long-read, and hybrid assembly strategies. We used the diploid species *R. cassubicifolius*, a sexual diploid progenitor of the complex, and selected the best assemblies in terms of completeness, contiguity, and BUSCO quality scores. Results: We first assembled the plastid (156 Mbp; 78 genes) and mitochondrial (1,183 Mbp; 39 genes) genomes using Illumina and hybrid-based strategies, respectively. Using all available plastomes in the NCBI database, we discuss gene evolution within Ranunculaceae. For the nuclear genome, the best of 12 strategies represents a PacBio read assembly, polished three times with filtered PacBio and Illumina reads, and then grouped into 8 chromosomes by Hi-C. We obtained a complete and scaffolded genome of 2.65 Gbp in size, with 95% complete BUSCO genes found, a median chromosome length of 355 Mbp, and approximately 50,000 annotated genes. Conclusions: The genomic information presented here helps to improve phylogenomic analyses in this species complex and will enable advanced functional, evolutionary, and biogeographic analyses for the *R. auricomus* complex, the genus, and beyond in Ranunculaceae in the future<sup>8-10</sup>.

References

- <sup>1</sup> Rhie et al. 2022, Doi: 10.1038/s41586-021-03451-0
- <sup>2</sup> Zimin & Salzberg 2022, Doi: 10.1371/journal.pcbi.1009860
- <sup>3</sup> Pucker et al. 2022, Doi: 10.1017/qpb.2021.18
- <sup>4</sup> Karbstein et al. 2020, Doi: 10.1002/tax.12365
- <sup>5</sup> Karbstein et al. 2021, Doi: 10.1111/mec.15919
- <sup>6</sup> Karbstein et al. 2022, Doi: 10.1111/nph.18284
- <sup>7</sup> Hodač & Karbstein et al. 2023, Doi: 10.3390/biology12030418
- <sup>8</sup> He & Hörandl 2022, Doi: 10.3389/fpls.2022.976765
- <sup>9</sup> Hörandl 2024, Doi: 10.1093/aob/mcae044
- <sup>10</sup> Karbstein et al. 2024, Doi: 10.1016/j.tree.2023.11.002

S21 T4

## Landscape effects on the spatial distribution of genetic lineages in a widespread, generalist herb

D. Albach<sup>1</sup>, M. Daubert<sup>1</sup><sup>1</sup>Carl von Ossietzky-Universität, Oldenburg, Germany

The majority of landscape genetic studies to this day has focused on animals, often mammals of high conservation interest. The studies conducted in plants have found a significant to no effect of landscape features on the genetic structuring of the respective species. Previously, no differentiation was found between populations of the generalist, widespread herb *Veronica chamaedrys* (Plantaginaceae) growing in grasslands under different management regimes. We suspect however that grassland management changes on too short timescales to affect the species and that factors inhibiting or facilitating gene flow may reveal themselves at larger geographic scales. We sampled populations along a 500 km transect from the German North Sea coast to the central German xeric zone at the lee of the Harz mountains. At each location we characterized the surrounding vegetation via Ellenberg values. Moisture and soil reaction numbers showed the strongest geographic trends with the steepest change occurring at the southern margin of the Harz mountains. We expect these marked changes to be recognizable in genotyping-by-sequencing data. The species turned out to be much rarer in the north German plain than anticipated. This could be either due to a historical lack of suitable habitat as the region used to be characterized by extensive wetlands or due to a recent extinction of populations as a result of agricultural intensification. Yet, semi-natural and natural habitats were sampled preferentially. Should populations north of the central uplands prove not to show signs of isolation, it could indicate a buffering function of gardens and public green spaces as *V. chamaedrys* can be frequently found in parks and mature lawns.

S21 T5

## Hidden promiscuity explains duckweed diversity and evolution

A. Stepanenko<sup>1,2</sup>, P. T. Hoang<sup>1,3</sup>, J. Fuchs<sup>1</sup>, V. Schubert<sup>1</sup>, I. Schubert<sup>1</sup><sup>1</sup>The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany<sup>2</sup>Institute of Cell Biology and Genetic Engineering, National Academy of Sciences of Ukraine, Kyiv, Ukraine<sup>3</sup>Dalat University, Biology Department, Dalat, Viet Nam

The cosmopolitan, organ-reduced, aquatic monocot duckweed family (Lemnaceae) harbors the fastest growing angiosperms. It comprises 36 currently recognized species (Bog et al. 2020) within the 5 genera *Spirodela*, *Landoltia*, *Lemna*, *Wolffia* and *Wolffiella*. Duckweeds' easy to harvest biomass is suitable as food source for livestock, fish and humans, for wastewater remediation, for protein farming and biofuel generation. During the last decade, these features have fueled significant commercial interest in duckweeds, and made them a novel model for plant development, genome and stress biology (for review see Acosta et al. 2021).

Unambiguous identification of, and establishing phylogenetic relationship between (mainly vegetatively propagating) accessions of three (*Lemna*, *Wolffia*, *Wolffiella*) of the five duckweed genera are difficult because of limited morphological differences, overlap and apparent intra-specific variability of genetic features (Bog et al. 2019). For several related species, classification based on morphological features, and even barcoding of plastid markers, is not reliable. Moreover, chromosome number and genome size vary remarkably between clonal accessions assigned to distinct species (Hoang et al. 2022).

Albeit no single approach allowed unambiguous species assignment in several cases, applying multiple cytogenomic and genetic approaches identified interspecific hybridisation and ploidy variation as reason(s) for the gradual variability between accessions of the genus *Lemna*. These approaches may now be extended to further resolve phylogenetic and taxonomic position of other similar but not identical duckweed accessions of genera *Wolffia* and *Wolffiella*.

An open question remains whether, in spite of short-term advantages, clonal propagation of triploids and dihaploid hybrid accessions eventually represent an evolutionary dead end, or whether dihaploid hybrids can return to sexuality via spontaneous chromosome doubling in the course of reticulate evolution.

## References

Acosta K et al. 2021, *Plant Cell*, doi:10.1093/plcell/koab189Bog M et al. 2020, *Taxon*, doi:10.1002/tax.12188Bog M et al. 2019, *Frontiers in Sustainable Food Systems*, doi:10.3389/fsufs.2019.00117Hoang PTN et al. 2022 *Plants*, doi:10.3390/plants11202674

P 001

*Chlamydomonas reinhardtii* alpha-amylase 2 is activated by glutamine

L. Scholtysek<sup>1</sup>, A. Poetsch<sup>2</sup>, E. Hofmann<sup>3</sup>, A. Hemschemeier<sup>1</sup>

<sup>1</sup>Ruhr University Bochum, Photobiotechnology, Bochum, Germany

<sup>2</sup>Ruhr University Bochum, Plant Biochemistry, Bochum, Germany

<sup>3</sup>Ruhr University Bochum, X-ray structure analysis of proteins, Bochum, Germany

Carbon (C) and nitrogen (N) are the most abundant elements in living cells, and coordinating the use of C- and N-containing building blocks for growth is vital for cellular function. Starch is the major C storage compound in plants and algae, whereas the first assimilation product of N, glutamine (Gln), is both a donor of amino groups and recognized as a signal for the N status.

We characterized alpha-amylase 2 (AMA2) from *Chlamydomonas* on the biochemical level and were particularly interested in the enzyme's N-terminal aspartate kinase–chorismate mutase–tyrA (ACT) domain. ACT domains are often fused with catalytic domains and are known to bind small molecules, upon which they regulate the associated enzymatic function. A fusion between an alpha-amylase and an ACT domain has, to our knowledge, not been reported before, and the presence of the ACT domain suggested that the enzyme might be allosterically regulated.

Recombinant AMA2 showed the expected enzymatic activity on starch as a substrate, with an optimal activity at pH 8. As has been shown for several starch metabolism enzymes, AMA2 appears to be regulated through thiol-/disulfide chemistry, because its activity decreased strongly in the presence of the thiol oxidant diamide. ACT domains often mediate the oligomerization of proteins, and AMA2 indeed forms a dimer, but a monomer when its N-terminus is absent in an alpha-amylase domain-only AMA2 variant that we termed delta-AMA2. When we screened effects of proteinogenic amino acids, we found that Gln increased the activity of AMA2, but not of delta-AMA2, suggesting that Gln binds to the ACT domain and thereby stimulates the enzyme's activity. AMA2 variants with amino acid exchanges within the ACT domain showed altered activity profiles, and the exchange of a conserved leucine resulted in a loss of Gln sensitivity.

Integrating these results, we hypothesize that AMA2 might be one of the regulatory hubs that coordinate C- and N metabolism in *Chlamydomonas*, and perhaps in other algae. The pH optimum and sensitivity to thiol oxidation of AMA2 suggest that it is active in the illumination period, when *Chlamydomonas* cells grow in size and synthesize large amounts of C- and N-containing cellular compounds. The activating effect of Gln on AMA2 might help the cells to accelerate the release of C skeletons from starch when high levels of Gln signal abundance of assimilated N, resulting in optimized growth.

P 002

How do plants overcome the saturation of sugars due to the restricted sucrose transport in leaves?

S. Naganawa-Kinoshita<sup>1</sup>, K. Taki<sup>2</sup>, T. Suzuki<sup>3</sup>, T. Kinoshita<sup>2,4</sup>, I. Finkemeier<sup>1</sup>

<sup>1</sup>Universität Münster, Institute of Plant Biology and Biotechnology, Münster, Germany

<sup>2</sup>Nagoya University, Graduate School of Science, Nagoya, Japan

<sup>3</sup>Chubu University, College of Bioscience and Biotechnology, Kasugai, Japan

<sup>4</sup>Nagoya University, Institute of Transformative Bio-Molecules, Nagoya, Japan

Plants harvest the energy from the sunlight to assimilate atmospheric carbon dioxide via photosynthesis and produce carbohydrates such as sugars. Plant scientists have dedicated significant effort towards developing plants with enhanced photosynthesis capabilities that will produce biomass. However, when sugars accumulate beyond the storage or transport capacity in leaves, photosynthesis-associated genes are downregulated to reduce carbon assimilation. While various sugar signalling pathways have been proposed to control this downregulation, the molecular mechanism of how plants cope with excessive sugar accumulation in photosynthetically active leaves remains elusive.

Here, we employed *Arabidopsis thaliana* mutants that possess a leaky allele of *sucrose-proton symporter 2* (*suc2*), resulting in the accumulation of sugars in photosynthetically active leaves. In a new suppressor screen, we isolated novel mutants with larger biomass in the *suc2* mutant background. The physiological characteristics of the *suc2* mutant and the revertant, such as the starch accumulation pattern and photosynthesis performance, will be presented, and the hypothetical scenario of how the plants found a way to recover from the saturation of sugars will be discussed.

P 003

**A kinetic model for temperature effects on the non-photochemical quenching process in tomato**

Q. H. Nguyen<sup>1,2</sup>, T. von Bismarck<sup>3</sup>, U. Armbruster<sup>4,1</sup>, A. Matuszyńska<sup>5,1</sup>

<sup>1</sup>CEPLAS - Cluster of Excellence on Plant Sciences, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

<sup>2</sup>Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

<sup>3</sup>Jan IngenHousz Institute, Wageningen, Netherlands

<sup>4</sup>Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

<sup>5</sup>RWTH Aachen University, Aachen, Germany

Crop plants experience dynamic environmental conditions throughout their life cycle. Thus, to predict crop photosynthetic performance, we need to consider environmental factors, e.g. fluctuations in light and temperature. The combined effect of these factors can be systematically approached using mathematical models, in complement with current experimental evidence. Here, we present a coarse-grained temperature-dependent kinetic model of photosynthesis that focuses on the photosystem II and the high energy-dependent quenching (qE) component of non-photochemical quenching (NPQ). We extended an existing NPQ model [1] to account for enzymatic activity variations at different temperatures by the Arrhenius equation. For this equation, the required activation energies have been parameterized in line with the available literature. Simulation of steady-state lumen pH and the plastoquinol (PQ) pool redox state were improved (i.e. closer to the reported physiological range of photosynthesis complexes) via parameterization. In a second iteration, the model has been used to test alternative hypotheses regarding the quenching mechanism. Specifically, we implemented new quenching methods for qE and the activity of the ion channel KEA3. We compared the model simulation results with the chlorophyll fluorescence data collected from tomato (*Solanum lycopersicum*) grown in semi-controlled field conditions. The final model was able to predict variations from the tomato photosynthetic parameters dataset as a function of temperature and light acclimation state. Thus, the presented results set the ground for future work toward quantifying the combined dynamic effects of light and temperature on photosynthesis.

P 004

**Identification and 3D modeling of gene regulatory networks that determine leaf anatomy and physiology in C3-C4 intermediate Brassicaceae**

J. M. Valderrama-Martín<sup>1</sup>, M. Melzer<sup>2</sup>, U. Schlüter<sup>1</sup>, A. Weber<sup>1</sup>

<sup>1</sup>Heinrich-Heine University (Düsseldorf), Germany, Institute of Plant Biochemistry, Düsseldorf, Germany

<sup>2</sup>Leibniz Institute of Plant Genetics and Crop Plant Research, Physiology and Cell Biology, Gatersleben, Germany

The oxygenation reaction of the Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) lead to the formation of 2-phosphoglycolate (2-PG), which is detrimental to the plant as it inhibits the function of up to three enzymes within the Calvin-Benson cycle. (Eisenhut et al., 2019). In plants, C4 photosynthesis has enabled a reduction in the occurrence of photorespiration by incorporating various biochemical and anatomical adaptations that include a carbon concentration mechanism and a specific leaf anatomy referred to as Kranz anatomy (Sague et al., 2012). Certain plant species have been identified as natural intermediates in the evolutionary transition from C3 to C4 photosynthesis (Schlüter and Weber, 2016). These species exhibit CO<sub>2</sub> compensation points that are intermediate between those of typical C3 and C4 plants (Schlüter et al., 2017). C3-C4 intermediates maintain high CO<sub>2</sub> levels around RubisCO by restricting the photorespiratory pathway in mesophyll cells and limiting glycine decarboxylase (GDC) activity in bundle sheath cells (Yu, 2020). Moreover, these plant presents intermediate leaf anatomical features that often includes several C4-like traits, such as well-developed bundle sheath cells, increased vein density, a higher number of organelles with a centripetal arrangement, and a greater frequency of plasmodesmata (Sague et al., 2014; Yu, 2020). Despite these findings, the development of the unique leaf anatomy in C3-C4 intermediates and the gene-regulatory mechanisms governing this process remain unknown. In this regard, the five independent evolutionary origins of the characteristic C3-C4 leaf anatomy, biochemistry, and physiology within the Brassicaceae family provide a unique opportunity to investigate these regulatory networks. Overall, we combined different microscopy techniques to identify discrepancies at the leaf anatomy level not only between C3 and C3-C4 intermediate species but also along the development of the leaves.

References

1. Eisenhut et al., 2019. *New Phytol* 223: 1762-1769
2. Sage et al., 2012. *Annu Rev Plant Biol* 63:19-47
3. Schlüter and Weber, 2016. *Plant Cell Physiol* 57: 881-889.
4. Schlüter et al., 2017. *J Exp Bot* 68: 191-206
5. Yu, 2020. *Plant Physiol* 182: 453-454.

P 005

A versatile outdoor-protocol to acquire photosynthesis of microalgae using stable isotope ratios  
S. Gindorf<sup>1</sup>, D. Remias<sup>1</sup>, K. Schott<sup>2</sup>, A. Watzinger<sup>2</sup>

<sup>1</sup>Paris Lodron University Salzburg, Department of Environment and Biodiversity, Salzburg, Austria  
<sup>2</sup>BOKU University, Department of Forest- and Soil Sciences, Vienna, Austria

The role of microalgae living in atypical habitats for global CO<sub>2</sub> sequestering is still unknown. Photosynthetic data of communities from open soils, tree bark, wet rocks or melting snow and ice surfaces are scarce, likely because of apparent methodological constraints in assessing carbon uptake rates at such substrates. Therefore, we developed a feasible protocol for acquiring the short-time photo-assimilation, dedicated for application in liquid medium at exposed or remote sites. We aimed for a solution combining a low-cost approach, sufficient accuracy and logistic simplicity.

By applying a dosage of isotope-labelled sodium bicarbonate (NaH<sup>13</sup>CO<sub>3</sub>) into microcosms, no bulky instrumentation is needed during field work. As a case habitat, we tested psychrophilic microalgae living in melting snow and on glacier surfaces (cryoflora blooms). In detail, borosilicate bottles were filled with melted snow or slush. Three replicates were either darkened or exposed to ambient irradiation, both were kept at habitat temperatures. Assays were initiated by addition of the label and the incubation was terminated with Lugol solution (iodine). Cells were then harvested onto glass fibre filters and dried prior to analysis.

Total carbon and C isotope ratios were measured with an Isotope Ratio Mass Spectrometer coupled to an elemental analyser (EA-IRMS), and the uptake was reported in relation to the total carbon mass of the sample and per incubation time.

Our preliminary data clearly confirms that cryoflora caused by green or golden-brown algae is photosynthetically active despite living at temperatures around 0 C. While uptake numbers assessed with field blooms ranged from 507±18 to 1560±245 µg<sup>13</sup>C / g organic carbon / h, values obtained from cultured strains were significantly higher, despite irradiation could have been lower. This discrepancy points out that field measurements are inevitable to assess the true impact of microalgae like cryoflora on the local and global carbon cycle.

P 006

Localization and Dynamics of the Thylakoid Ascorbate Peroxidase (tAPX) in the Thylakoid Membrane of *Arabidopsis thaliana*

H. Hitaj<sup>1</sup>, E. Reifschneider<sup>1</sup>, S. Stolze<sup>2</sup>, H. Nakagami<sup>2</sup>, M. Baier<sup>1</sup>

<sup>1</sup>Freie Universität Berlin, BCP (Plant Physiology), Berlin, Germany

<sup>2</sup>Max Planck Institute for Plant Breeding Research, Plant Proteomics and Mass Spectrometry Group, Köln, Germany

Thylakoid ascorbate peroxidase (tAPX) serves a plethora of pivotal roles against photooxidative stress, in scavenging of chloroplast reactive oxygen species, in stress signaling as well as in immunity control (Seiml-Buchinger *et al.*, 2022, van Buer *et al.*, 2019, Griebel *et al.*, 2022). Moreover, it does so with a higher efficiency when compared to its counterpart stromal ascorbate peroxidase.

We explore the distribution and characteristics of tAPX by examining its abundance and diurnal turnover. Furthermore, we compare protein levels in solubilized thylakoid membranes using gel electrophoresis and Western blotting techniques.

Moreover, we investigate the impact of tAPX micro-environment on supracomplex regulation and its involvement in photoprotection and signaling. For a refined analysis, we explore the protein environment of tAPX through chemical crosslinking and immunopurification to identify potential interaction partners using mass spectrometry. Additionally, we perform Y2H assays to elucidate the protein-protein interactions and analyze T-DNA insertion lines to assess interactor-dependent tAPX accumulation and allocation.

This multifaceted approach provides a comprehensive understanding of thylakoid membrane dynamics and the regulatory role of tAPX in plant physiology. By elucidating the intricate mechanisms underlying tAPX function and regulation, our study not only enhances our understanding of plant stress response but also contributes to the broader knowledge of plant adaptation mechanisms in fluctuating environmental conditions.

References

Seiml-Buchinger V. *et al.* (2022). doi: <https://doi.org/10.1093/plphys/kiac355>

van Buer J, *et al.* (2022). doi: <https://doi.org/10.1038/s41598-019-39838-3>

Griebel *et al.* (2022). doi: <https://doi.org/10.1094/MPMI-11-21-0283-FI>

P 007

**Adaptive potential of the Leaf Economics Spectrum in the Brassicaceae**

L. Chausson Garate<sup>1</sup>, J. M. Valderrama-Martín<sup>1</sup>, U. Schlüter<sup>1</sup>, A. Weber<sup>1</sup>

<sup>1</sup>Institutes für Biochemie der Pflanzen, HHU Düsseldorf, Düsseldorf, Germany

Carbon concentrating mechanisms (CCM) refer to the diverse mechanisms plants have evolved to maximize the efficiency of CO<sub>2</sub> assimilation in photosynthesis, avoiding or compensating the photorespiratory losses derived from the oxygenase activity of RuBisCO. C<sub>4</sub> photosynthesis is the utmost example of carbon fixation efficiency. Plants displaying intermediate carbon compensation points (CCP) between C<sub>4</sub> and C<sub>3</sub> species (10–40 ppm) have been described, termed as C<sub>3</sub>-C<sub>4</sub> intermediates. These plants achieve carbon concentration via the photorespiratory glycine shuttle, a CCM that requires fewer and less complex modifications than C<sub>4</sub> photosynthesis. Convergent evolution of the photorespiratory glycine shuttle indicates a substantial improvement of carbon economy under certain environmental conditions.

Trade-offs between resource allocation to growth, reproduction and survival are key in determining plant ecological strategies and evolution. The Leaf Economics Spectrum (LES) is a set of six leaf traits that provides a quantitative basis for evaluation of interspecific differences across ecosystems based on leaf trait trade-offs. Variation in several LES subtraits between C<sub>3</sub> and C<sub>3</sub>-C<sub>4</sub> intermediate species has already been reported in the *Brassicaceae*, linked to differences in physiology and genome structure. Interspecific variance in the LES across environmental conditions can facilitate the identification of correlations between photosynthesis type and fitness in the *Brassicaceae*. The family of the *Brassicaceae*, where C<sub>3</sub>-C<sub>4</sub> intermediates do not encounter C<sub>4</sub> relatives, defines an excellent framework for addressing this question. Overall, a combination of morphological features, together with molecular and physiological data, will be employed to elucidate the interspecific differences in the LES across various environmental conditions in a panel of species of the *Brassicaceae*.

Key words: *Brassicaceae*, Carbon Concentrating Mechanisms, C<sub>3</sub>-C<sub>4</sub> intermediates, Leaf Economics Spectrum, evolution, ecophysiology.

P 008

**Regulation of mitochondrial malate dehydrogenase by protein modifications and interacting proteins**

Y. Stichweh<sup>1</sup>, F. Kotnik<sup>1</sup>, J. Eirich<sup>1</sup>, J. Giese<sup>1</sup>, K. Zheng<sup>1</sup>, M. Schwarzländer<sup>1</sup>, M. D. P. Martinez<sup>2</sup>, V. G. Maurino-Larcher<sup>2</sup>, I. Finkemeier<sup>1</sup>

<sup>1</sup>Universität Münster, Institut für Biologie und Biotechnologie der Pflanzen, Münster, Germany

<sup>2</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Institute of Cellular and Molecular Botany, Bonn, Germany

Malate dehydrogenase (mMDH) is a pivotal enzyme in the mitochondrial tricarboxylic acid (TCA) cycle, supplying oxaloacetate for citrate synthesis, particularly during heterotrophic growth. Due to the reversible nature of the malate-to-oxaloacetate conversion, mMDH also functions in a reverse flux mode of the TCA cycle during photoautotrophic growth. Our previous research revealed that the activity of mMDH1 is modulated by reversible lysine acetylation. Through the generation of recombinant mMDH1 with site-specifically incorporated acetyl-lysines, we investigated the impact of these modifications on enzyme activity. Acetylation at K169, K170, and K334 was found to diminish the oxaloacetate reduction activity of mMDH1, whereas K334 acetylation notably enhanced malate oxidation activity (Balparda et al., 2022). Additionally, mMDH1 was observed to undergo phosphorylation at S23 during nocturnal periods (Giese et al. 2023, Zhang et al. 2021). Considering the dynamic nature of these post-translational modifications across various cell types, growth conditions, and time points, our objective is to identify the interplay of protein modifications and regulatory proteins interacting with mMDH1. For this, we employ pull-down methods and proximity labeling utilizing the biotin ligase Turbo-ID, fused to the open reading frame of mMDH1 and stably integrated into *Arabidopsis*.

References

Balparda M, Elsässer M, Badia MB, Giese J, Bovdilova A, Hüdig M, Reinmuth L, Eirich J, Schwarzländer M, Finkemeier I, Schallenberg-Rüdinger M, Maurino VG (2022) Acetylation of conserved lysines fine-tunes mitochondrial malate dehydrogenase activity in land plants. *Plant J.* 109(1):92-111

Giese J, Eirich J, Walther D, Zhang Y, Lassowskat I, Fernie AR, Elsässer M, Maurino VG, Schwarzländer M, Finkemeier I (2023) The interplay of post-translational protein modifications in *Arabidopsis* leaves during photosynthesis induction. *Plant J.* 116(4):1172-1193

Zhang Y, Giese J, Kerbler SM, Siemiatkowska B, Perez de Souza L, Alpers J, Medeiros DB, Hinch DK, Daloso DM, Stitt M, Finkemeier I, Fernie AR (2021) Two mitochondrial phosphatases, PP2c63 and Sal2, are required for posttranslational regulation of the TCA cycle in *Arabidopsis*. *Mol Plant.* 14(7):1104-1118

P 009

Biochemical and physiological characterization of dual specific N-acetyltransferases in plastids of Arabidopsis

J. Mühlenbeck<sup>1</sup>, J. Eirich<sup>1</sup>, J. Sindlinger<sup>2</sup>, D. Schwarzer<sup>2</sup>, I. Finkemeier<sup>1</sup>

<sup>1</sup>Universität Münster, Institut für Biologie und Biotechnologie der Pflanzen, Münster, Germany

<sup>2</sup>University of Tübingen, Interfaculty Institute of Biochemistry, Tübingen, Germany

Protein N-acetylation is a common modification found in proteomes of various organisms. This process typically involves N-terminal acetyltransferases and lysine acetyltransferases that transfer acetyl groups from acetyl-Coenzyme A to the respective amino groups. N-acetylation is particularly abundant on proteins in plastids, where the majority of proteins are imported from the cytosol and undergo cleavage of their N-terminal signal peptides. In a recent study, eight dual-specific N-acetyltransferases (GNATs) have been discovered in plastids of Arabidopsis (Bienvenut et al. 2020). However, the specific NATs and KATs responsible for these acetylation processes still need to be characterised for their *in vivo* substrate specificities and physiological roles. Here, we employed an *in vitro* approach, utilizing high-performance liquid chromatography assays alongside synthetic peptides labelled with fluorophores to assess the activities and substrate specificities of selected GNATs in more detail. Furthermore, we utilized N-terminal CoA-conjugated peptide probes (CoA-N) to enrich active GNATs from plant extracts. For this purpose, we tested two different NCoA probes, previously shown to favour the binding of human NAA80 (CoA-N-Glu) and NAA10 (CoA-N-Ala), respectively (Sindlinger et al. 2022; Eirich et al., 2023). Pull-down experiments were conducted, followed by label-free LC-MS/MS quantification using *Arabidopsis thaliana* cell lysates. For selected GNATs enriched in these pull-down analyses, phenotypic analyses of single and double knock-out mutants were performed.

References

Bienvenut, Willy V.; Brünje, Annika; Boyer, Jean-Baptiste; Mühlenbeck, Jens S.; Bernal, Gautier; Lassowskat, Ines et al. (2020): Dual lysine and N-terminal acetyltransferases reveal the complexity underpinning protein acetylation. In *Molecular systems biology* 16 (7), e9464. DOI: 10.15252/msb.20209464.

Sindlinger J, Schön S, Eirich J, Kirchgäßner S, Finkemeier I, Schwarzer D. Investigating Peptide-Coenzyme A Conjugates as Chemical Probes for Proteomic Profiling of N-Terminal and Lysine Acetyltransferases. *Chembiochem*. 2022 Sep 5;23(17):e202200255. doi: 10.1002/cbic.202200255.

Eirich J, Sindlinger J, Schön S, Schwarzer D, Finkemeier I. Peptide CoA conjugates for in situ proteomics profiling of acetyltransferase activities. *Methods Enzymol*. 2023;684:209-252. doi: 10.1016/bs.mie.2022.09.005.

P 010

Shedding light on protein acetylation in chloroplasts - A new family of plastid-localized acetyltransferases

A. Brünje<sup>1</sup>, J. Eirich<sup>1</sup>, J. B. Boyer<sup>2</sup>, P. Heinkow<sup>1</sup>, U. Neumann<sup>3</sup>, M. Konert<sup>4</sup>, A. Ivanauskaitė<sup>4</sup>, J. Seidel<sup>5</sup>, S. I. Ozawa<sup>6</sup>

W. Sakamoto<sup>6</sup>, T. Meinzel<sup>2</sup>, D. Schwarzer<sup>5</sup>, P. Mulo<sup>4</sup>, C. Giglione<sup>2</sup>, I. Finkemeier<sup>1</sup>

<sup>1</sup>Universität Münster, Münster, Germany

<sup>2</sup>Université Paris-Saclay, Paris, France

<sup>3</sup>Max Planck Institute for Plant Breeding Research, Köln, Germany

<sup>4</sup>University of Turku, Turku, Finland

<sup>5</sup>Universität Tübingen, Tübingen, Germany

<sup>6</sup>University of Okayama, Kurashiki, Japan

Plants are exposed to a constantly changing environment, which requires fast acclimation strategies. Post-translational modifications (PTMs) of proteins allow cells to rapidly respond to varying environmental conditions, thereby having the potential of altering localization, interactions or enzymatic activities. Protein acetylation is one of the most abundant co- and post-translational modifications in eukaryotes, extending its occurrence to chloroplasts within vascular plants. Specific acetyltransferases catalyze the acetylation of the amino groups of internal lysine residues or protein N-termini by transferring acetyl-groups donated by the metabolite acetyl-CoA. Recently, a novel plastidial enzyme family comprising eight acetyltransferases that exhibit dual lysine and N-terminus acetylation activities was unveiled in Arabidopsis. Among these, GNAT1, GNAT2, and GNAT3 reveal notable phylogenetic proximity forming a subgroup termed NAA90.

Here, I will focus on GNAT1, which is closely related to the state transition acetyltransferase GNAT2. In contrast to GNAT2, GNAT1 did not prove essential for state transitions and displayed no discernible phenotypic difference compared to the wild type under high light conditions, while *gnat2* mutants were severely affected. However, co-immunoprecipitation coupled to mass spectrometry revealed a robust interaction between GNAT1 and GNAT2, as well as a significant association of GNAT2 with GNAT3 - the third acetyltransferase within the NAA90 subfamily. This study unveils the existence of at least two acetyltransferase complexes within chloroplasts, whereby complex formation might have a critical effect on the fine-tuning of the overall acetyltransferase activities. These findings introduce a novel layer of regulation in acetylation-dependent adjustments in plastidial metabolism.

P 011

Characterization of C<sub>3</sub>-C<sub>4</sub> intermediate *Diplotaxis* speciesJ. Peter<sup>1</sup>, U. Schlüter<sup>1</sup>, A. Weber<sup>1</sup><sup>1</sup>Institute of Plant Biochemistry, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

The *Diplotaxis* genus belongs to the Brassicaceae family and is of interest for agronomy and photosynthetic research. A prominent species from this genus is *Diplotaxis tenuifolia*, otherwise known as rocket or arugula. Some species in the *Diplotaxis* genus evolved a specialized carbon concentrating mechanism known as C<sub>3</sub>-C<sub>4</sub> intermediate photosynthesis. C<sub>3</sub>-C<sub>4</sub> intermediate traits are of scientific interest as they are perceived as interim stages in the evolutionary progression towards C<sub>4</sub> photosynthesis. Since C<sub>4</sub> photosynthesis is more efficient compared to the more common C<sub>3</sub> photosynthesis, insights into the developmental adjustments of these traits are highly interesting. The differentiation and decreased autonomy of mesophyll and bundle-sheath cells around the leaf vein is a fundamental principle of C<sub>3</sub>-C<sub>4</sub> photosynthesis, separating CO<sub>2</sub> fixation and carbohydrate synthesis between both cell types. Initially, we aim to describe characteristics of a wide range of C<sub>3</sub> and C<sub>3</sub>-C<sub>4</sub> intermediate *Diplotaxis tenuifolia* accessions, focusing on the bundle sheath architecture as well as the metabolite shuttle between mesophyll and bundle-sheath cells. Selected *Diplotaxis tenuifolia* accessions are currently characterized at our institute and will be subject to next-generation sequencing, thus building an extensive meta-genomic resource. These datasets will be supplemented with Deep Learning predictions using the *Helixer* and *Predmoter* pipelines. We further aim to employ high-resolution single-cell transcriptomics to unravel the cell-specific expression dynamics underlying the C<sub>3</sub>-C<sub>4</sub> intermediate development in *Diplotaxis*. Here, we will specify on the role of mesophyll and bundle-sheath cells in carbon and nitrogen homeostasis and unravel the underlying gene regulatory networks involved in the basic principles of C<sub>3</sub>-C<sub>4</sub> intermediate photosynthesis. This data will reveal key events in the evolution of C<sub>4</sub> photosynthesis, even beyond the *Diplotaxis* genus, and highlight targets for crop breeding research.

P 012

## The function of N-terminal acetylation of plastid precursor proteins

P. Möllenbeck<sup>1</sup>, J. Grimmer<sup>2</sup>, D. S. Stolle<sup>1</sup>, S. Baginsky<sup>1</sup><sup>1</sup>Ruhr-Universität Bochum, Plant Biochemistry, Bochum, Germany<sup>2</sup>Martin-Luther University Halle-Wittenberg, Institute of Biochemistry and Biotechnology, Halle a. d. Saale, Germany

Chloroplast functionality requires the post-translational import of plastid-destined nuclear-encoded proteins. Chloroplast precursor protein availability in the cytosol and import into the plastid is tightly regulated to maintain chloroplast biogenesis and functionality, respectively. One of these regulatory mechanisms is the co-translational modification of the precursor proteins by N-terminal acetylation (NTA). NTA is a common protein modification and associated with the coordination of proteome stability. Thus, NTA is suggested to determine the half-life of chloroplast precursor proteins in the cytosol. We aim to further investigate and unravel the role of NTA and search for potential new players in the fate of chloroplast precursors. We therefore use *Arabidopsis thaliana* mutants with reduced N-terminal acetyltransferase A (NatA) complex function, to perform protein import analysis with native and non-acetylated chloroplast precursor substrates. Furthermore, the effects of precursor stability on the biogenesis of chloroplasts and their photosynthetic performance in plastid protein import-deficient plant lines will be investigated.

P 013

## Chloroplast positioning and its role in metabolic acclimation to a changing environment

S. Bagshaw<sup>1</sup>, A. Kitashova<sup>1</sup>, T. Naegele<sup>1</sup><sup>1</sup>LMU München, Plant Evolutionary Cell Biology, Planegg-Martinsried, Germany

Combined cold and high light exposure causes an imbalance between available light energy and thermodynamic constraints on enzyme activity in plants. This imbalance between photochemical and biochemical reactions can be mitigated through metabolic reprogramming. Chloroplast positioning has been found to minimize the photodamage from high light conditions and it has also been found to affect both photosynthetic and sucrose cycling rates during cold acclimation. However, the effect of chloroplast localization on intercompartmental interaction and metabolic acclimation to cold and high light conditions in *Arabidopsis thaliana* remains unclear. In this study, we show that chloroplast positioning is central to the acclimation of the sugar metabolism to cold and high light. Moreover, the application of a novel benchtop subcellular hexose phosphate quantification method in combination with kinetic modelling suggested that chloroplast localization is linked to subcellular distribution of hexose phosphates. These findings demonstrate the importance of subcellular metabolite measurements as whole cell analyses may mask subcellular dynamics contributing to the cold and high light acclimation phenotype. In summary, subcellular dynamics of hexose phosphates are affected by chloroplast positioning and, thus, contribute to metabolic acclimation to a changing environment.



P 015

Compartment-specific metabolic acclimation in *Arabidopsis thaliana*

V. Brodsky<sup>1</sup>, T. Nägele<sup>1</sup>

<sup>1</sup>Ludwig-Maximilian-University Munich, Biology, München, Germany

Dynamics of plant-environment interactions shape plant survival and fertility. Acclimation, which is the reversible physiological adjustment to such environments, represents a multigenic trait that enables reprogramming of metabolism and photosynthesis. Research in this domain is constantly challenged in its ability to predict *in situ* environmental circumstances from strictly controlled growth conditions and selected genotypes. Additionally, the concept of natural variability within the genome of *Arabidopsis thaliana* introduces another layer of complexity to the generation of new hypotheses. Current knowledge is scarce on the subcellular metabolic organization of a plant cell, although it is becoming increasingly evident that this information could explain intricacies of the metabolic phenotype between populations of different habitats of *A. thaliana* as well as reveal the acting processes driving cold acclimation.

Here, results of a study are presented in which four natural accessions of *Arabidopsis thaliana*, originating from Europe, were subjected to low, non-freezing temperatures and increased irradiance. Experiments were performed on single grown leaf rosettes and densely grown *Arabidopsis* plants to study the effect of different growth conditions on acclimation capacities. Comparison between these two approaches revealed substantial differences in regulation of metabolism, ranging from carbon uptake to subcellular compartmentation of central metabolites. Photosynthetic efficiency was determined together with carbon assimilation rates which showed a significant degree of natural variation. Regulation of the primary and secondary metabolism were affected in an irradiance-dependent manner. Overall, accessions from different habitats spread over Europe were found to cope with stress differently.

These findings suggest strong effects of cellular and subcellular responses to low temperature in natural accessions of *Arabidopsis*, thus emphasizing on the role of metabolic evolution as a key player in the understanding of the effects of (sub)-cellular metabolic organization on cold acclimation.

P 016

Towards fluorescent protein-based biosensing of thylakoid lumen pH

M. T. T. Hoang<sup>1</sup>, A. P. Cislighi<sup>1,2</sup>, K. Zheng<sup>1</sup>, K. Busch<sup>2</sup>, M. Schwarzländer<sup>1</sup>

<sup>1</sup>Institute for Plant Biology and Biotechnology, University of Münster, Münster, Germany

<sup>2</sup>Institute of Integrative Cell Biology and Physiology, University of Münster, Münster, Germany

The pH within the thylakoids is essential for photosynthesis. As part of the photosynthetic light reactions, proton translocation across the thylakoid membrane generates a proton motive force that consists of a pH gradient ( $\Delta\text{pH}$ ) and an electrical gradient ( $\Delta\Psi$ ) to drive ATP synthase. While genetically encoded pH biosensors have been instrumental in dissecting pH dynamics of different plant cell compartments, *in vivo* pH biosensing in the subcompartments of the chloroplast had remained challenging. We recently established pH biosensing in response to photosynthetic activity and revealed a major impact on pH dynamics not only in the stroma but also in the cytosol and the mitochondria. While the observed pH dynamics clearly mirror photosynthetic proton pumping, measuring the *bona fide* pH gradient across the thylakoid membrane as the major determinant of the proton motive force, requires monitoring pH in the thylakoid lumen. Yet, establishing luminal pH monitoring by genetically-encoded biosensors presents unique challenges, such as import across three membrane systems, low pH values that may be adopted in the light, silencing of sensor expression and direct vicinity of photosynthetic pigments. To address those challenges and to optimize luminal pH biosensing in tobacco and *Arabidopsis* leaves, we have generated a collection of constructs that include different pH biosensors, signal peptides and promoters. Initial experiments show that illumination induces an inverse response between luminal and stromal targeted pH sensors, which provides evidence for correct subcellular targeting. I will present my recent progress in developing luminal pH sensors while highlighting remaining challenges.

P 017

**Elevating embryo energy status: A novel approach to improve storage starch biosynthesis in dicotyledonous plants**R. Chamansara<sup>1</sup>, M. Raorane<sup>1</sup>, B. H. Junker<sup>1</sup><sup>1</sup>Martin Luther University of Halle-Wittenberg, Pharmazie, Halle a. d. Saale, Germany

Starch constitutes one-third of the human daily diet. With the ever-growing population and climate change posing threats to food security, optimizing crop nutritional value and yield is paramount. Cereal monocots (Poaceae) are efficient starch producers compared to dicots, this advantage stems from a cytosolic ADP-glucose pyrophosphorylase (AGPase) and a plastidial nucleotide sugar transporter (NST) in cereals. The pathway utilizes readily available ATP and recycles the byproduct pyrophosphate (PPi) into a nucleotide triphosphate (NTP), maximizing energy efficiency. Conversely, dicots rely solely on plastidial AGPase, necessitating ATP import into plastids and incurring energy loss (-21.7 kJ/mol) during PPi breakdown. Previous studies suggest, in heterotrophic storage organs, sucrose synthase (SuSy) can catalyze formation of ADP-glucose as well as UDP-glucose. Thereby SuSy could be an alternative source of ADP-glucose other than the rate limiting AGPase enzyme. However, the absence of a plastidial NST in dicots restricts ADP-glucose import into the plastids, hindering its utilization for starch biosynthesis. Inspired by the cereal model, we investigated the hypothesis that a dicot model system (*Pisum sativum*) expressing the barley *HvNST1* could facilitate the movement of cytosolic ADP-glucose into plastids, bypassing the rate-limiting and energy-costly plastidial AGPase. Initial experiments revealed elevated ADP-glucose levels and a higher ADP-glucose/UDP-glucose ratio in transgenic pea embryos compared to the wild type control. Despite reaching total starch content comparable to the WT control, transgenic lines exhibited earlier onset of starch accumulation, which could be attributed to the higher adenylated energy charge (AEC) observed in the transgenic lines compared to the WT. To understand the impact of *HvNST1* on starch biosynthesis, we will employ qRT-PCR to track expression changes in key pathway genes (*SuSy*, *AGPase*, *PGM* and *HvNST1*) and enzyme activity assays, including SuSy substrate preference clarifying role of SuSy in producing ADP-glucose. Finally, dynamic <sup>13</sup>C-glucose labeling in embryos will reveal how *HvNST1* alters metabolic fluxes thus elucidating the effect of increased energy status on starch biosynthesis.

P 018

**GL11 is a novel chloroplastic protein involved in photoprotection**M. Balparda<sup>1</sup>, J. M. Böhm<sup>1</sup>, Y. Zaplatnikov<sup>1</sup>, J. Ellieroth<sup>1</sup>, V. G. Maurino-Larcher<sup>1</sup><sup>1</sup>University of Bonn, IZMB - Molekulare Pflanzenphysiologie, Bonn, Germany

Chloroplasts, mitochondria and the cytosol contain systems for scavenging reactive carbonyl species (RCS), which at high levels pose a threat to cellular integrity. RCS such as methylglyoxal and glucosone, are by-products of sugar metabolism and photosynthesis, and their production is increased under biotic stress. The primary defense mechanism against RCS involves the glyoxalase (GLX) system, which consists of two enzymes, glyoxalase I (GLXI) and glyoxalase II (GLXII), working in sequence. In Arabidopsis, three gene loci encode active GLXI and eight gene loci encode GLXI-like (GL) proteins. GL proteins share only 17-25% identity with active GLXI, suggesting an evolutionary and functional divergence. Subcellular localization studies showed that one of these GL proteins (cGL11) is localized in the chloroplast and Golgi. cGL11 is co-expressed with chloroplastic proteins involved in carotenoid degradation and photoprotection mechanisms. Phylogenetic analysis revealed that cGL11 homologs are found in proteobacteria, cyanobacteria and viridiplantae, suggesting an exclusive function in photosynthetic eukaryotes. Homozygous knockout mutants of cGL11 exposed to high light develop prominent yellowish stripes at the base of young rosette leaves, show reduced photosynthetic efficiency, and increase non-photochemical quenching. In addition, under this condition the mutant plants exhibit significantly increased zeaxanthin levels compared to the wild type, suggesting a potential role for cGL11 in enhancing photoprotection mechanisms. Expression of cGL11 in the knock-out background restored wild-type characteristics. In conclusion, cGL11 is a novel chloroplastic protein involved in photoprotection with potential applications in improving plant stress tolerance and crop yield.

P 019

**Eukaryote-specific assembly factor AT2G48070 is required for efficient assembly of Photosystem II**

J. C. Dávila Frantzen<sup>1,2</sup>, S. Kappel<sup>3</sup>, U. Armbruster<sup>1,2</sup>

<sup>1</sup>Heinrich-Heine-Universität Düsseldorf, Institute of Molecular Photosynthesis, Düsseldorf, Germany

<sup>2</sup>CEPLAS - Cluster of Excellence in Plant Science, Heinrich-Heine University, Düsseldorf, Germany

<sup>3</sup>Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

Photosynthesis stands as a cornerstone in biology, driving the fixation of CO<sub>2</sub> and production of molecular oxygen, thereby sustaining all aerobic life on Earth. The splitting of H<sub>2</sub>O to release molecular oxygen is carried out by the Photosystem II (PSII). PSII, a protein complex composed of multiple subunits located in the thylakoid membrane of chloroplasts and cyanobacteria, harnesses light energy via chlorophyll-binding proteins to drive this reaction. The assembly of PSII follows a specific order and is carried out by a number of assembly factors. Removing a critical assembly factor can significantly decrease the amounts of active PSII, leading to severely damaged plants<sup>1</sup>.

This work focuses on the characterization of the eukaryote-specific assembly factor AT2G48070. Our recent studies have shown that *Arabidopsis thaliana* lines lacking AT2G48070 show a severe defect in growth and pigmentation. Spectroscopic and immunological analysis reveal this is caused by a drastic reduction in the concentration of PSII subunits. AT2G48070 is strongly co-regulated with plastid ribosomal proteins on transcript level, which may suggest that it is involved in co-translational assembly processes. Moving forward, our research aims to employ different biochemical analyses to better understand the molecular mechanisms underlying AT2G48070-mediated PSII assembly.

<sup>1</sup> Keller, J.M., Frieboes, M.J., Jödecke, L., Kappel, S., Wulff, N., Rindfleisch, T., Sandoval-Ibanez, O., Gerlach, I., Thiele, W., Bock, R. and Eirich, J. (2023). Eukaryote-specific assembly factor DEAP2 mediates an early step of photosystem II assembly in *Arabidopsis*. *Plant Physiology*, 193(3), pp.1970-1986.

P 020

**Light changes promote distinct responses of plastid protein acetylation marks in the model plant *Arabidopsis thaliana***

J. Eirich<sup>1</sup>

<sup>1</sup>WWU, Münster, Germany

Protein acetylation is a key co- and post-translational modification in various organisms. How different types of acetylation respond to environmental stress is still unknown. A member of the newly discovered family of plastid acetyltransferases (GNAT2) in plants, which is featuring both lysine- and N-terminal acetyltransferase activities, was used to obtain a holistic multi-omics acetylation-dependent view of the acclimation of *Arabidopsis thaliana* to short-term light changes. Both yield and coverage of the N-terminal acetylome remain unchanged in wild-type and *gnat2*-knockout backgrounds after two hours of exposure to high light or darkness. Similarly, no differences in transcriptome or adenylate energy charge were observed between the genotypes under the tested light conditions. In contrast, the GNAT2-associated lysine acetylome turned out to be sensitive to light changes suggesting unique strategies of plant acclimation for quick responses to environmental changes involving lysine but not N-terminal GNAT2-mediated acetylation activity.

We used a multiplexing approach and optimized ionmobility settings for full proteome and lysine acetylome profiling.

P 021

Facultative Crassulacean Acid Metabolism in *Talinum fruticosum*H. Göhlmann<sup>1</sup>, A. Weber<sup>1</sup><sup>1</sup>Heinrich Heine University, Plant Biochemistry, Düsseldorf, Germany

Crassulacean acid metabolism (CAM) is one of the most water-use-efficient modes of photosynthesis. CAM plants employ a temporal separation of primary and secondary carbon assimilation. The stomates are open at night so that CO<sub>2</sub> can enter the cells to be assimilated by PHOSPHOENOLPYRUVATE CARBOXYLASE (PEPC) and stored as malic acid in the vacuole. This leads to an increase in acidification, which can be measured by titration. During the day, the stomates are closed, resulting in reduced water loss from evaporation, and malic acid is released from the vacuole in the form of CO<sub>2</sub> to be refixed by RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXIDASE (RuBisCO).

Facultative CAM species usually rely on C<sub>3</sub> photosynthesis and only switch to CAM when exposed to stresses such as drought. One example of such a species is *Talinum fruticosum*, a tropical herbaceous dicot. *T. fruticosum* can facultatively and reversibly reallocate its resources from C<sub>3</sub> to CAM under drought. Upon rewatering, the plant will switch back to C<sub>3</sub> mode. Previously, RNA-seq was performed over a time course of drought and exogenous application of abscisic acid (ABA). The plant was found to undergo a major reprogramming of its transcriptome to change the form of metabolism.

We will analyse gene co-expression networks from previous RNA-seq datasets to find putative transcription factors for PEPC. Genome sequencing and assembly revealed that *T. fruticosum* possesses six PEPC orthologs, four of which are found on one contig, indicating a tandem duplication event. We propose that there are CAM and C<sub>3</sub>-specific orthologs. Knowing how certain genes are regulated may hint at specific components of the CAM pathway. To elucidate the turnover of carbon-storage pools, photosynthetic pigments, and proteins, we will employ <sup>13</sup>CO<sub>2</sub> labelling during a time course of a C<sub>3</sub> to CAM transition and calculate the energetics cost for the formation of new metabolites and proteins. An understudied aspect is the vegetative phase change (VPC), the transition from juvenile to adult and vegetative to reproductive stages, and how it affects the transition from C<sub>3</sub> to CAM. We will look at aspects such as the FW/DW ratio and specific leaf area to determine whether leaf or plant age affects the metabolic transition.

P 022

## Light changes promote distinct responses of plastid protein acetylation marks

J. Eirich<sup>1</sup>, I. Finkemeier<sup>1</sup><sup>1</sup>WWU, Münster, Germany

Protein acetylation is a key co- and post-translational modification. How different types of acetylation respond to environmental stress is still unknown. A member of the newly discovered family of plastid acetyltransferases, which is featuring both lysine- and N-terminal acetyltransferase activities, was used to obtain a holistic multi-omics acetylation-dependent view of the acclimation of plants to short-term light changes.

We investigated the role of acetylation in the plant's response to changes in light intensity using mass spectrometry-based proteomic and acetylome profiling. We grew WT and *gnat2* plants under the same conditions and subjected them to high light, darkness, or standard growth conditions for two hours. This analysis revealed that the different types of acetylations, catalysed by GNAT2 in the chloroplast, distinctively respond to changes in light conditions.

Under high light treatment, the *gnat2* mutant showed a more pronounced de-regulation in the lysine acetylome, with 50 acK sites up-regulated compared to only nine acK sites in the WT. The *gnat2* mutant specifically downregulated diverse anabolic reactions and upregulated the base excision repair pathway in response to short-term high light treatment. The analysis also showed that the *gnat2* mutant had a more pronounced de-regulation in the lysine acetylome under darkness, with 7 acK sites significantly changed compared to only 2 acK sites in the WT.

Furthermore, the analysis revealed that plastid NTA yield did not significantly change under different light conditions. However, the *gnat2* mutant displayed downregulation of transcripts involved in translation-related pathways under darkness, suggesting that GNAT2 might be involved in the light-dependent control of translation.

In conclusion, our study highlights the importance of lysine acetylation in the plant's response to changes in light intensity and suggests that plastid K- and N-terminal acetylations may respond differently to environmental or developmental stimuli. Our research provides valuable insights into the role of lysine acetylation in the plant's response to changes in light intensity and the interplay between genetic and environmental factors by mass spectrometry-based acetylome profiling.

P 023

Functional interaction of STN7/8 and pCK2 in photosynthetic acclimation

T. Demmig<sup>1</sup>, A. Rödiger<sup>1</sup>, T. Pfannschmidt<sup>2</sup>, S. Baginsky<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Biochemistry of plants, Bochum, Germany

<sup>2</sup>Leibniz University Hannover, Plant physiology, Hannover, Germany

In plant chloroplasts, protein kinases regulate photosynthetic acclimation by phosphorylation of thylakoid membrane proteins allowing rapid short-term acclimation to changing light conditions. This type of phosphorylation control is mediated by the light-regulated kinases STN7/STN8 at the thylakoid membrane system. Recent data suggested furthermore that STN7 may be involved in long-term acclimation affecting chloroplast and nuclear gene expression (Schönberg et al., 2017, Longoni and Goldschmidt-Clermont, 2021). The plastid kinase originally identified as a regulator of plastid gene expression is plastid casein kinase 2 (pCK2) that phosphorylates RNA binding proteins and components of the transcription apparatus (Rödiger et al., 2021). We have generated the triple mutant *stn7/stn8/pck2* and characterized it phenotypically and biochemically. Our goal is to unravel functional crosstalk between these three protein kinases in photosynthetic acclimation. Since the *stn7/stn8/pck2* phenotype is severe we hypothesized cooperativity between the different chloroplast kinases in the regulation of chloroplast functions. Phosphoproteome analyses revealed cooperation in the phosphorylation of at least two proteins of the thylakoid membrane system, i.e. PsbH and PSI-P.

P 024

Climate change, Photosynthesis and Advanced biofuels

A. Kumar<sup>1</sup>

<sup>1</sup>University of Rajasthan, Jaipur 302004, Department of Botany and Biotechnology, Jaipur, India

Anthropogenic increase in greenhouse gases has resulted in unprecedented increase in global warming. The Synthesis Report (SYR) of the IPCC Sixth Assessment Report (AR6) summarizes the state of knowledge of climate change, its widespread impacts and risks, and climate change mitigation and adaptation. Projected changes of annual maximum daily maximum temperature, annual mean total column soil moisture and annual maximum 1-day precipitation at global warming levels of 1.5°C, 2°C, 3°C, and 4°C relative to 1850–1900 are projected by IPCC. The use of fossil fuel contributes to 80 percent of the global warming and energy transition from fossil fuels to green energy is required to check the global warming. Biomass continues to be one of the most practical and suitable sources towards the production of renewable fuels for the future. Photosynthesis is going to be affected due to climate change and there will be need for climate resilient crops to withstand adverse climatic conditions. Recent efforts have focused on the development of renewable alternatives to fossil fuels, and cellulosic biomass has great potential to contribute to the demand for liquid fuel. We started work on biofuels after a meeting with Professor Melvin Calvin during a Photobiology conference at Colorado Springs, USA in 1980. This led to work on *Euphorbia lathyris* L., *Euphorbia antisyphilitica*, *Euphorbia caudicifolia*, *Pedilanthus tithymaloides* var. *green*, *Calotropis procera* and *Jatropha curcas* with support Department of Biotechnology, Govt of India. Agrotechnology was developed for two selected hydrocarbon yielding plants *Calotropis procera* and *Euphorbia antisyphilitica* and *Jatropha curcas*. In our previous book (Biofuels: Greenhouse Gas Mitigation and Climate Change), we discussed establishing bio-based hydrocarbon production from cheap feedstocks, lowering the cost of developing efficient and robust microbial cell factories, and establishing more efficient routes for biomass hydrolysis to sugars for fermentation. (Climate Change, Photosynthesis and Advanced Biofuels: The Role of Biotechnology in the Production of Value-added Plant Bio-products | SpringerLink). It is proposed to review status of renewable energy for climate change mitigation

P 025

*Sarcandra glabra*: characterization of BAHD hydroxycinnamoyltransferases and phenolic acids  
P. Bömeke<sup>1</sup>, M. Petersen<sup>1</sup>

<sup>1</sup>Philipps-Universität Marburg, Pharmazeutische Biologie und Biotechnologie, Marburg, Germany

BAHD hydroxycinnamoyltransferases (HCTs) play a crucial role in the biosynthesis of many natural products in plants by catalysing the acylation of hydroxyl or amine groups. The resulting esters, such as caffeoylshikimate (CS), chlorogenate (CA) or rosmarinate (RA), provide the basic units for lignification or protect against pathogens and UV radiation. All three phenolic acids are present in *Sarcandra glabra* (Sg; Chloranthaceae) which is used in TCM against arthritis [1]. Pharmaceutical effects may come from RA, which is well known for its anti-inflammatory properties. RA derives from L-phenylalanine and L-tyrosine: the former is converted to 4-coumaroyl-CoA (4c-CoA), whereas the latter results in 4-hydroxyphenyllactate (pHPL) [2]. A member of the BAHD HCT family, rosmarinate synthase (RAS), connects both units, and the product, 4c-pHPL is further transformed into RA after double hydroxylation [3]. Investigation on SgHCTs will provide insights in the metabolism of phenolic acids in this medicinal plant.

Five HCTs were identified in the transcriptome of Sg. Total RNA was isolated, converted to cDNA, and the sequences amplified by PCR. The enzymes were heterologously expressed in *E. coli*, and purified extracts were used to find suitable substrates. Michaelis-Menten kinetics were analysed to find the potentially best substrate.

All characterized HCTs accept 4c- and caffeoyl-CoA as hydroxycinnamoyl donor. The structurally most similar enzymes, SgHCT-A and SgHCT-C, were both able to acylate shikimate (shik) and quinate (quin). SgHCT-A had a high catalytic efficiency, and clearly favoured shik over quin, while the reverse preference was found in tests with SgHCT-C. SgHCT-E accepted quin exclusively, but only to a very low extent, and formed crypto-CA instead of the expected CA. SgHCT-D was able to acylate pHPL. The expression of SgHCT-F was successful, but testing several substrates did not lead to any activity.

In summary, three SgHCTs play a key role in the biosynthesis of CS and CA derivatives, while the production of RA is mediated by the fourth. To elucidate the connection between the *in vitro* activities and the occurrence of phenolic acids, the content of CS, CA and RA will be assessed and expression analysis performed.

#### References

- [1] Zeng Y et al. (2021) Front Pharmacol 12:652926
- [2] Petersen M (2013) Phytochem Rev 12:207–227
- [3] Petersen M (2016) Phytochem Rev 15:699-727

P 027

An insight of anti - psoriatic potential of *Wrightia tinctoria* leaf phytochemicals  
D. Thankappan<sup>1</sup>, A. Muttanolla<sup>1</sup>, V. Narayangari<sup>1</sup>, S. R. Kanade<sup>1</sup>

<sup>1</sup>University of Hyderabad, Department of Plant Sciences, Hyderabad, India

The plant *Wrightia tinctoria* is renowned in Ayurveda for its medicinal properties and widely used the traditional medical practitioners in India to treat various ailments like, inflammation, skin diseases and wounds. In Siddha and Ayurvedic system of medicine it is used to treat psoriasis which is a chronic skin hyper proliferative disease. Psoriasis is an auto immune, complex genetic disorder characterized by abnormal keratinocyte differentiation, hyper proliferation, and immune infiltration into the dermis and epidermis. Psoriasis shows relapse and can only be temporarily quenched when appropriately treated. The therapeutic method employed mainly includes synthetic drugs which induce side effects such as cardiovascular disease. Hence traditional therapies have gained a greater momentum than the conventional methods. In light of this, leaf powder of *Wrightia tinctoria* was subjected to Soxhlet extraction with 80% methanol to isolate active compounds. The different fractions obtained were diethyl ether, ethyl acetate and aqueous fractions with composition of alkaloids, phenolics, flavonoids, anthraquinones and terpenoids on phytochemical analysis. Total phenolics in diethyl ether, ethyl acetate and methanolic fractions found to be 3.4 mg; 2.88 mg and 3.4 mg Gallic Acid Equivalents (GAE)/ g of extract and the total flavonoid content found to be 11.96 mg; 19.53 mg and 15.11 mg of quercetin Equivalents (QE)/ g of extract respectively. LCMS analysis of the fractions and data analysis with Plant sync database and enrichment analysis with MetaboAnalyst 6.0 indicated the presence of various kind of phenolics, alkaloids, indoles, terpenoids, flavonoids and quinolines. Cytotoxicity evaluation confirmed that the diethyl ether fraction is more cytotoxic than the other two fractions with an IC<sub>50</sub> value of 12.02µg in HEK 293 cell lines and 28.66 µg in HaCaT cell lines. The anti-psoriatic effect of the extracts was studied on *in vitro* imiquimod induced psoriasis model on HaCaT cell lines. Microscopic observation clearly exhibited morphological differences when treated with different concentrations of plant extract and the results were validated by gene expression analysis. Henceforth we conclude that our research foresees the investigation on the phytochemical constituents of the plant *Wrightia tinctoria* and its potential on treating psoriasis.

Keywords: *Wrightia tinctoria*, Psoriasis, HaCaT cells, Anti-proliferation

P 028

First insights into alternative trait combinations of desiccation tolerant plants

E. Zokov<sup>1</sup>, L. Bondi<sup>1</sup>, J. Köhler<sup>1</sup>, S. Porembski<sup>1</sup>

<sup>1</sup>University of Rostock, Rostock, Germany

Photosynthesis is one of the main processes affected by drought stress. Drought impacts productivity in plants in general, but desiccation tolerant plants differ from other plants in their ability to lose up to 90% of their cellular water content and resume normal growth after rehydration while keeping their leaves (Farrant et al., 2007). Desiccation tolerant species possess different traits, allowing them to cope with desiccation (Marks et al., 2021). To the best of our knowledge, no study has focused so far on investigating different trait combinations and their consequences on plant functioning. In this study, we tested the effects of drought on photosynthesis of four desiccation tolerant plant species, three being closely related within a family (Velloziaceae) and an outgroup species (Cyperaceae). We conducted a desiccation experiment, measuring the stomatal conductance and pigment composition to describe decreases in productivity as well as changes in chlorophyll and anthocyanin content to depict alternative ways to cope with drought. Irrespective of the systematic position, the analysed species showed different rates of decreases in gas exchange. In addition, two Velloziaceae and the Cyperaceae species dismantled their photosynthetic apparatus during desiccation, whereas one Velloziaceae species kept its chlorophyll. Plants keeping their chlorophyll are expected to suffer from higher photo-oxidative damage while desiccating, hindering the recovery of photosynthetic activity after rehydration. However, keeping the chlorophyll was compensated with a high accumulation of anthocyanins to mitigate the negative effects of drought. Despite their different traits, all four species occur under similar conditions regarding the exposure to high solar radiation. This might indicate the existence of alternative trait combinations causing species to exhibit a similar fitness in a given ecological condition. Our results suggest that responses to drought stress in desiccation tolerant plants are not dependant on only one trait combination within one pathway, but an array of traits and their inherent trade-offs.

References

Farrant, J. M. (2007). Mechanisms of desiccation tolerance in angiosperm resurrection plants. *Plant desiccation tolerance*, 51-90.

Marks, RA, Farrant, JM, McLetchie, DN & VanBuren, R. (2021). Unexplored dimensions of variability in vegetative desiccation tolerance. *American Journal of Botany*. 108(2):1–13.

P 029

Assessing the effects of glucosinolates and their different breakdown product types on the rhizosphere bacterial community

A. Hielscher<sup>1</sup>, E. C. M. Chroston<sup>1</sup>, N. Bziuk<sup>2,3</sup>, E. J. Stauber<sup>1</sup>, B. M. Ravindran<sup>1,4</sup>, K. Smalla<sup>3</sup>, U. Wittstock<sup>1</sup>

<sup>1</sup>TU Braunschweig, Institute of Pharmaceutical Biology, Braunschweig, Germany

<sup>2</sup>Graz University of Technology, Institute for Environmental Biotechnology, Graz, Austria

<sup>3</sup>Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

<sup>4</sup>Saaten-Union Biotec GmbH, Leopoldshöhe, Germany

Specialized metabolites help plants to interact with their environment. When glucosinolates, a group of thioglucosides found in the Brassicales, get hydrolyzed by co-occurring myrosinases, a mixture of bioactive products is formed. According to the classical model of the “mustard oil bomb”, these products act as a defense against herbivores and pathogens upon tissue disruption. However, glucosinolates also get hydrolyzed in intact tissue. The functions and mechanisms of the involved breakdown pathways are not well understood. Roots of *Arabidopsis thaliana* contain aliphatic and indolic glucosinolates and express three nitrile-specifier proteins (NSP1, NSP3, NSP4) as well as several myrosinases. As a consequence, nitriles are formed as the major breakdown products in root homogenates next to toxic isothiocyanates and derived products. In order to better understand the metabolic network of glucosinolate breakdown product formation in roots and its biological roles, we develop mutant *A. thaliana* lines with disrupted pathways of either breakdown or breakdown and synthesis of glucosinolates. For biological evaluation, we compare the bacterial rhizosphere community of these lines with that of wildtype plants.

We have previously constructed the *nsp134* mutant which is unable to form nitriles upon glucosinolate hydrolysis in roots. Although bacterial diversity was unchanged, its rhizosphere bacterial community composition was strikingly different from that of wildtype plants. This suggests that glucosinolates are hydrolyzed in the intact root and nitriles are exported to the rhizosphere. In contrast, lack of aliphatic glucosinolates affected both alpha and beta-diversity. To further dissect the contributions of structural types of glucosinolates and breakdown products, we have crossed the *nsp134* mutant with glucosinolate biosynthesis mutants (*myb28 myb29*, no aliphatic glucosinolates; *myb34 myb51 myb122*, no indolic glucosinolates). Chemical analysis of *myb34 myb51 myb122 nsp1 nsp3 nsp4* demonstrated that this line is deficient in indolic glucosinolates and unable to form nitriles upon glucosinolate hydrolysis in roots. The line is presently being used to study its rhizosphere microbiota in comparison to its parental lines and the wildtype.

P 030

## Understanding the Protein Quality Control of Antennae Complex Proteins Under Heat and High-Light-Cold stress

D. Ghosh<sup>1,2</sup>, -. Twinkle<sup>1,2</sup>, V. Dogra<sup>1,2</sup><sup>1</sup>CSIR- Institute of Himalayan Bioresource Technology, Palampur, INDIA, Biotechnology, Palampur, India<sup>2</sup>Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India

Abiotic stresses, including high light (HL) and high temperature negatively affect photosynthesis and cause drop in crop yields. Chloroplasts, in addition to driving photosynthesis, act as environmental sensors. Both HL and temperature fluctuations, augment reactive oxygen species (ROS) generation in the photosynthetic apparatus to impose oxidative damage. Photosynthesis is composed of light and dark reactions. During this process, pigment-protein complexes [Photosystem I and Photosystem II (PSI and PSII)], which are composed of their respective Reaction centers (RCI and RCII) and Light harvesting Complex proteins (LHCI and LHCII)], harvest light energy of sun and channel it through electron transport chain to generate ATP to run Calvin cycle to produce energy. ROS generated during such stresses damage several biomolecules including photosynthetic proteins resulting in inhibition to photosynthetic process. Out of both photosystems, PSII is more susceptible to oxidative damage, which attracts biotechnological engineering approaches to develop resilience. Several efforts have been done to understand protein quality control (PQC) of reaction center (RC) proteins, but reports regarding PQC of LHC proteins are rare. Here, we report the damage and degradation of some of the components of LHC proteins of PSII (LHCII) and their mechanism of turnover under above said stress conditions in *Arabidopsis thaliana*.

Key words: Abiotic stresses, Reactive oxygen species (ROS), Chloroplast, Light Harvesting Complex (LHC), Protein quality control (PQC)

P 031

## Biosynthetic Pathway Discovery in Plants Based on Omics Data Integration

F. Wolters<sup>1</sup><sup>1</sup>Wageningen University and Research, Plant Sciences, Wageningen, Netherlands

Specialized plant metabolism has evolved a plethora of biochemicals that provides a largely unexplored resource for drug discovery and agrochemicals. In plants, the genomic organization of biosynthetic pathways assembled to a higher level of complexity compared to bacteria and fungi. Hence, discovery efforts based on comparative approaches require the development of similarity metrics accounting for this complexity, preferably followed by automated pathway annotation. To this end, integration of genomics, transcriptomics, and metabolomics data is needed. However, paired omics datasets are unavailable for most plant species.

We profiled a bio-panel of 17 species in the mustard family (*Brassicaceae*) including well studied crops and wild relatives via tissue-specific paired Liquid Chromatography-Mass Spectrometry and whole transcriptome sequencing. From this set, we determined eight species based on metabolic distance to generate time-series metabolomics and transcriptomics datasets covering treatments with plant hormones (abscisic acid, jasmonic acid, salicylic acid) *in vitro*. To identify orthologous pathway modules across species, we will develop a phylogenetic metric that will be tested and validated on generated omics datasets, accounting for polyploidy and sub-genome dominance.

Deciphering biosynthetic pathway evolution will accelerate natural product discovery and provide new resources for synthetic and systems biology in plant sciences.



P 032

Molecular basis of phytochelatin synthase function in pathogen-triggered indole glucosinolate metabolism in the Brassicaceae family

J. Lalak-Kańczugowska<sup>1</sup>, M. Piślewska-Bednarek<sup>1</sup>, S. Bugaj<sup>1</sup>, P. Bednarek<sup>1</sup>

<sup>1</sup>Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan, Poland

Phytochelatin synthase (PCS) has an established function in plant tolerance to heavy metal ions through phytochelatin synthesis. Recent research has uncovered a fascinating duality of PCS1 from *Arabidopsis thaliana*, revealing it to be a moonlighting protein. *AtPCS1* contributes to the pathogen-triggered PENETRATION2 (PEN2) pathway, which is a key player in indole glucosinolate (IG) metabolism. This IG metabolic pathway is of utmost importance for the immunity of *A. thaliana* against many filamentous pathogens. Despite our comprehensive understanding of the mechanisms underlying PCS contribution to PC synthesis and heavy metal ion tolerance, the precise molecular mechanisms governing *AtPCS1* function in PEN2 myrosinase-mediated IG metabolism, remains largely enigmatic. It has been only shown that this function is strictly separated from PC biosynthesis.

In our current study, we would like to determine molecular basis of PCS1 function in IG metabolism. To achieve this, we address the question if PCS1 orthologs from closely related with *A. thaliana* species representing Camelinaeae tribe, such as *Camelina sativa* and *Capsella rubella*, which lost the capacity to produce and metabolize IGs, can contribute to IG activation. To answer to this question we expressed these *PCS1* orthologs as well as in the *pcs1* mutant line under the native *AtPCS1* promoter. Generated lines have been validated with western blot analysis and tested with targeted metabolic analyses for complementation of the biochemical phenotype. This included accumulation of IG metabolism products, indol-3-ylmethyl amine (I3A) and raphanusamic acid (RA), in flagellin epitope (*flg22*)-sprayed leaves, which is strongly compromised in *pcs1* mutant. Our analysis revealed that *pcs1* mutant complemented with *AtPCS1* produces efficiently I3A and RA upon *flg22* treatment. In contrast, we did not detect accumulation of these compounds in lines expressing tested *PCS1* orthologs despite detection of corresponding proteins in selected transgenic lines during western blot analysis. This indicated that unlike *AtPCS1*, the investigated *PCS1* ortholog proteins are not functional in IG metabolism. Overall, these findings might allow us to identify amino acid residues that are indispensable for the investigated novel function of *PCS1*.

P 033

Comparative analysis of *Taraxacum* hybrids with focus on the rubber biosynthesis

J. Wiemann<sup>1</sup>, N. van Dam<sup>2</sup>, C. Schulze Gronover<sup>1</sup>, D. Prüfer<sup>1,2</sup>

<sup>1</sup>Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Münster, Germany

<sup>2</sup>University of Münster, Institute of Plant Biology and Biotechnology - IBBP, Münster, Germany

Natural rubber (NR) is an important biopolymer which cannot be substituted through synthetic rubber due to its superior properties. The high demand for NR is mainly covered by the tropical rubber tree *Hevea brasiliensis*. The dependence on the rubber tree harbors some risks, therefore the establishment of alternative rubber crop is a focus of research. One such rubber crop is a dandelion species originally found in Kazakhstan, *Taraxacum koksaghyz*, which produces high amounts of high-quality NR in its roots. Although it is already cultivated in temperate regions, there are still some challenges and room for improvement for profitable use as a rubber crop such as the relatively small biomass compared to related species like the common dandelion *T. officinale*. *T. officinale* develops a larger biomass but no significant amount of natural rubber. Hybridization of closely related species has been used as a breeding method for many modern crop varieties with the goal to breed hybrids which combine the best traits of both species. Although F1-hybrids of *T. koksaghyz* and *T. officinale* have a biomass equal to or even exceeding the common dandelion, they contain very little NR. Research into *T. koksaghyz*, *T. officinale* and their hybrids aims to understand the differences in NR production between these plants. The main component of NR the isoprenoid poly(*cis*-1,4-isoprene), is synthesized and stored in specialized organelles called rubber particles (RPs) which are produced in the latex of specialized root cells called laticifers.

In the presented work, RPs and NR related factors are investigated in detail in a comparative analysis of inter- and intraspecific dandelion hybrids using transcriptomic, proteomic and metabolomic approaches with the aim to shed light on the regulation of NR biosynthesis which supports the breeding efforts to establish dandelions as valuable rubber crops.

P 034

The GAMEs cluster and specialized metabolism of the bittersweet nightshade *Solanum dulcamara* by genomic and transcriptomic analyses

B. Laker<sup>1</sup>, R. A. Anaia<sup>2,3</sup>, D. Wulf<sup>1</sup>, N. van Dam<sup>2,3,4</sup>, A. Bräutigam<sup>1</sup>

<sup>1</sup>Bielefeld University, Computational Biology, Bielefeld, Germany

<sup>2</sup>Friedrich Schiller University Jena, Institute of Biodiversity, Jena, Germany

<sup>3</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Molecular Interaction Ecology, Leipzig, Germany

<sup>4</sup>Leibniz Institute of Vegetable and Ornamental Crops, Plant Biotic Interactions, Großbeeren, Germany

Steroidal alkaloids are commonly found in members of the Solanaceae family, such as the crop plants tomato (*Solanum lycopersicum*) and potato (*S. tuberosum*). These specialized metabolites and their glycosylated variants are poisonous and taste bitter, thereby constituting a chemical defense mechanism against various pests and pathogens. A member of the Solanaceae family, the bittersweet nightshade *S. dulcamara*, forms differentiated glycoalkaloid profiles between individual plants<sup>1</sup>. During the biosynthesis of steroidal glycoalkaloids in Solanaceae, the glycoalkaloid metabolism (GAME) enzymes are the key players to synthesize the different glycoalkaloids<sup>2,3</sup>. Two clusters that contain GAME enzymes were identified in *S. lycopersicum* and *S. tuberosum* on chr07 and on chr12<sup>2</sup>.

In this study, the genome sequence assembly of *S. dulcamara* accession TW12<sup>1</sup> was constructed using Oxford Nanopore long reads. All genes were functionally annotated with a particular focus on specialized metabolism, and the GAME clusters known from tomato and potato were searched. In *S. dulcamara*, the GAME clusters are located on chr06 and chr10. Even though, *S. dulcamara* has significantly larger clusters compared to *S. lycopersicum*, the clusters are highly syntenic between these Solanaceae family members. As different *S. dulcamara* chemotypes show altered levels and classes of glycoalkaloids, they are a perfect model to further understand the glycoalkaloid production in correlation to the GAME enzyme concentrations. Leaf transcriptomes with and without addition of methyl jasmonate reveal the responses of GAMEs and of specialized metabolism in general. Adjusting the levels of specific GAME enzymes, especially in Solanaceae plants, can enhance fruit quality for human consumption and increase the natural resistance in these crops.

References

1. Calf, O. W., Huber, H., Peters, J. L., Weinhold, A. & van Dam, N. M. Glycoalkaloid composition explains variation in slug resistance in *Solanum dulcamara*. *Oecologia* **187**, 495–506 (2018).
2. Itkin, M. *et al.* Biosynthesis of antinutritional alkaloids in solanaceous crops is mediated by clustered genes. *Science* **341**, 175–179 (2013).
3. Cárdenas, P. D. *et al.* The bitter side of the nightshades: Genomics drives discovery in Solanaceae steroidal alkaloid metabolism. *Phytochemistry* **113**, 24–32 (2015).

P 035

Comparative genomics reveals a conserved biosynthetic gene cluster in withanolide-producing *Solanaceae* species

N. Choudhary<sup>1</sup>, R. Friedhoff<sup>1</sup>, S. E. Hakim<sup>2,3</sup>, K. Malhotra<sup>2</sup>, J. Peng<sup>2,3</sup>, A. Bueltemeier<sup>2,3</sup>, A. Arafa<sup>2,3</sup>, J. Franke<sup>2,3</sup>, B. Pucker<sup>1</sup>

<sup>1</sup>Institute of Plant Biology & BRICS, TU Braunschweig, Plant Biotechnology and Bioinformatics, Braunschweig, Germany

<sup>2</sup>Institute of Botany, Leibniz University Hannover, Hannover, Germany

<sup>3</sup>Centre of Biomolecular Drug Research, Leibniz University Hannover, Hannover, Germany

Genes responsible for producing specialized metabolites are often physically clustered in genomes, a phenomenon commonly observed in bacteria and fungi. This gene cluster organization is rare in plants and some have been identified in the members of the Solanaceae family. Withanolides, a group of steroidal lactones derived from 24-methylenecholesterol, are found in several members of Solanaceae. Known for their proven anti-cancer properties, withanolides are key active components of *Withania somnifera* (Ashwagandha), a highly valued medicinal plant in traditional Indian medicine (Ayurveda). Until now, only the first enzyme specific for the biosynthesis of withanolides (24-ISO) has been elucidated, limiting its potential for drug development. In this study, we produced a high-quality genome sequence of *Withania somnifera* (2.8 Gb in size) with a contig N50 of 71.32 Mb. Through synteny analysis with other Solanaceae genome sequences and co-expression analysis, we identified a conserved biosynthetic gene cluster in all withanolide-producing species. This cluster includes 24-ISO and other genes associated with specialized metabolism. This study paves the way towards biotechnological production of withanolides for medicinal purposes.

P 036

The role of acetohydroxyacid synthase in the biosynthesis of pyrrolizidine alkaloids

M. Ebeling<sup>1</sup>, A. Engelhardt<sup>1</sup>, D. Ober<sup>1</sup>

<sup>1</sup>Christian-Albrechts University to Kiel, Botanical Institute and Botanic Gardens, Biochemical Ecology and Molecular Evolution, Prof. Dr. Dietrich Ober, Kiel, Germany

Pyrrolizidine alkaloids (PAs) are a group of toxic secondary metabolites which are synthesized by various unrelated plant families. Their sporadic occurrence has led to the hypothesis that they have evolved several times independently in the evolution of angiosperms. Current data suggest that there are at least seven independent origins for PA biosynthesis.

From a chemical point of view PAs are characterized by a bicyclic necine base which is esterified to one or more necic acids. The necic acids vary widely between individual PAs and PA types and are often used to classify the 600 currently known structures for PAs. Our focus lies on the so called lycopsamine-type PAs, which are characterized by a C<sub>7</sub> necic acid, an uncommon aliphatic necic acid containing seven carbon atoms. The aim of the project is to further elucidate the biosynthesis of the lycopsamine-type PAs in *Symphytum officinale* (Boraginaceae) and *Eupatorium cannabinum* (Asteraceae). Since our current understanding of the biosynthetic pathway is very limited we are focusing on the acetohydroxyacid synthase (AHAS). This enzyme is typically involved in the branched-chain amino acid biosynthesis but it has been shown that an isoform of this enzyme is involved in the biosynthesis of the PA-specific C<sub>7</sub> necic acid. We propose to call this isoform the C<sub>7</sub>-acid synthase (C<sub>7</sub>HAS).

Currently we are analyzing the tissue specificity and subcellular localization of the C<sub>7</sub>HAS in *S. officinale* via immunolocalization. In addition, the AHAS from primary metabolism and C<sub>7</sub>HAS in PA biosynthesis from *E. cannabinum* are biochemically characterized via a novel GC-MS based assay developed in our work group. The comparison of kinetic data and substrate specificity with a previously characterized C<sub>7</sub>HAS from *S. officinale* is most informative for a better understanding of the parallel evolutionary origin of these PA-specific enzymes.

P 037

Different flavonoid biosynthesis R2R3-MYB transcription factors recognize distinctive elements in the *chalcone synthase* promoter

L. Sielmann<sup>1</sup>, R. Stracke<sup>1</sup>, B. Weisshaar<sup>1</sup>

<sup>1</sup>Bielefeld University, Faculty of Biology, Bielefeld, Germany

Biological processes can be regulated on several levels. As the first step in gene expression, transcriptional regulation is of great importance. Proteins involved in transcriptional regulation are called transcription factors (TFs) and can either activate or repress the recruitment of the RNA polymerase by binding to *cis*-acting elements, thereby enabling or suppressing the initiation of transcription.

One of the largest and most abundant TF families is the group of MYB proteins [1], with R2R3-MYBs forming the largest group within this family. R2R3-MYBs have been shown to regulate various processes, including flavonoid biosynthesis.

Flavonoid biosynthesis in *Arabidopsis thaliana* leads to the formation of three metabolic subgroups: flavonols, anthocyanins and proanthocyanidins. The different branches of flavonoid biosynthesis have been shown to be regulated by different R2R3-MYBs. While MYB12-like proteins regulate the flavonol branch without any known cofactors, the anthocyanin and proanthocyanidin branches are regulated by TF complexes, comprising a MYB, bHLH and a WD40 repeat protein, called MBW complexes [2].

Although the branches are regulated by different TFs or TF complexes, several structural genes are likely to be regulated by both, single TFs and TF complexes. To identify specific *cis*-acting elements corresponding to different MYBs or MBW complexes, we chose the first structural gene of flavonoid biosynthesis: *chalcone synthase*. We identified an *in planta* minimal promoter required for the production of all flavonoid subgroups. By modifying specific regions of this minimal promoter, we identified *cis*-acting elements which correspond to the different R2R3-MYB types.

To identify specific regulons for the different R2R3-MYB TFs, we generated a collection of multiple *myb knock-out* mutants, shutting down one, two or three branches of flavonoid biosynthesis at the regulatory level, and performed RNA-seq analysis.

References

1 Pucker B, Pandey A, Weisshaar B, Stracke R (2020) The R2R3-MYB gene family in banana (*Musa acuminata*): Genome-wide identification, classification and expression patterns. PLOS ONE 15(10): e0239275

2 Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L (2010) MYB transcription factors in Arabidopsis. Trends Plant Sci. 15(10):573-81

P 038

Unraveling the volatile vocabulary of maize – Characterization of two terpene synthases involved in maize volatile production

S. M. Riedl<sup>1</sup>, C. Schaff<sup>1</sup>, J. Degenhardt<sup>1</sup>

<sup>1</sup>Institute of Pharmacy, Halle a. d. Saale, Germany

Maize, an essential agricultural crop, is an excellent model to investigate the biosynthesis of terpenes that are involved in the indirect defense of the plant. *Zea mays* produces a blend of volatile terpenes after herbivore attack, which are able to attract herbivore enemies like specific parasitic wasps<sup>1</sup>. The volatile blend consists mostly of mono-, sesqui-, and homoterpenes, including the tertiary alcohols linalool, nerolidol and geranylinalool. The key enzymes of terpene biosynthesis are terpene synthases (TPS), which convert the prenyl diphosphate precursors geranyl diphosphate (GDP), farnesyl diphosphate (FDP), and geranylgeranyl diphosphate (GGDP) into a highly diverse set of terpene compounds. The maize genome contains approximately 40 putative TPS genes, several of which have been characterized<sup>2</sup>. We cloned two of the remaining uncharacterized TPS genes from the inbred lines B73 and A638 for functional characterization in a bacterial expression system and by transient transformation of *N. benthamiana*. The first terpene synthase had the structural features of a monoterpene synthase and produced only a single monoterpene, the acyclic olefin ocimene in the presence of the GDP substrate. The second TPS lacks the signaling peptide usually associated with mono- and diterpene synthases. The enzyme converts GDP into the tertiary monoterpene alcohol linalool, FDP into the corresponding sesquiterpene alcohol (*E*)-nerolidol, and likewise GGDP into (*E,E*)-geranylinalool. The *in planta* function of this enzyme is most likely that of a (*E*)-nerolidol synthase in the cytoplasm, but localization studies are needed for confirmation. This enzyme differs from the previously identified maize terpene synthase TPS2, which has the same activity but contains a signal peptide and is expressed in the chloroplast<sup>3</sup>. Since genetic evidence shows that TPS2 is still responsible for the production of volatile (*E*)-nerolidol, we would like to understand the role of this newly characterized terpene synthase.

References

1. Turlings et al. (1990), *Science*, 250, 1251–1253
2. Köllner et al. (2023), *BMC Genomics*, 24:744
3. Richter et al. (2016), *The Plant Cell*, 28, 2651-2665

P 039

Elucidation of early withanolide biosynthesis using an engineered *Nicotiana benthamiana* platform

A. Bülteimeier<sup>1,2</sup>, K. Malhotra<sup>1</sup>, S. E. Hakim<sup>1,2</sup>, N. Choudhary<sup>3</sup>, J. Peng<sup>1,2</sup>, A. Arafa<sup>1,2</sup>, B. Pucker<sup>3</sup>, J. Franke<sup>1,2</sup>

<sup>1</sup>Leibniz University Hannover, Institute of Botany, Hannover, Germany

<sup>2</sup>Leibniz University Hannover, Centre of Biomolecular Drug Research, Hannover, Germany

<sup>3</sup>TU Braunschweig, Institute of Plant Biology & BRICS, Braunschweig, Germany

Withanolides are a class of highly oxidised steroids. They are derived from phytosterols and produced by several species of the Nightshade family, e.g., from the genera *Withania* and *Physalis*. Owing to their range of bioactivities, such as anti-inflammatory and anti-proliferative, withanolides are pharmacologically promising compounds. However, their abundance in plants is low, preventing the exploitation of this potential. Elucidating their biosynthesis would pave the way for heterologous production of withanolides.

*Nicotiana benthamiana* is a suitable heterologous host for plant biosynthetic pathways, since it allows for the rapid and flexible co-expression of gene candidates. However, it does not produce 24-methylsterol (1), the last confirmed precursor of withanolides. So far, this limited the application of *N. benthamiana* in the investigation of withanolide biosynthesis.

In my poster presentation, I will illustrate how we applied metabolic engineering to overcome the issue of limited precursor availability. Employing this platform, we discovered the first three oxidative steps of withanolide biosynthesis, leading to formation of the characteristic side chain lactone ring.

This work will facilitate the elucidation of the full withanolide biosynthetic pathway. Ultimately, this will enable the production of increased amounts of withanolides, accelerating pharmacological studies on these specialised metabolites.

References

1. Knoch, E. et al. Third DWF1 paralog in Solanaceae, sterol  $\Delta$ 24-isomerase, branches withanolide biosynthesis from the general phytosterol pathway. *Proc. Natl. Acad. Sci. U.S.A.* 2018, 115 (34), E8096-E8103.

P 040

Cytochrome P450 dependent hydroxylation in the biosynthesis of phenolic compounds in *Anthoceros agrestis*,  
*Marchantia polymorpha*, *Physcomitrium patens* and *Chara braunii*

C. Kentrath<sup>1</sup>, M. Petersen<sup>1</sup>

<sup>1</sup>Philipps Universität Marburg, Institut für pharmazeutische Biologie und Biotechnologie, Marburg, Germany

After leaving behind an underwater environment where buoyancy made plants float up and the water itself acted as UV-screen, plants had to find new ways to fulfil these tasks on the dry land. Phenolic specialized metabolites like lignin, flavonoids and coumarins would emerge to fill in these roles in land plants. Lignin gives the needed structure and flavonoids and other phenolic compounds could act as protection against pathogens and UV-screens [1].

The biosynthesis of these phenolic compounds starts with the aromatic amino acids L-phenylalanine and L-tyrosine in the phenylpropanoid pathway. The deamination is followed by the enzymatic hydroxylation of *trans*-cinnamic acid in position 4 to *p*-coumaric acid, catalysed by the enzymes cinnamic acid 4-hydroxylase (C4H) and NADPH:cytochrome P450 reductase (CPR). C4H is encoded by genes belonging to the CYP73 family and requires CPR in close proximity to transfer electrons from NADPH to the cytochrome [2]. Later steps in this pathway require further hydroxylation reactions that are often postulated to be performed by cytochrome P450s as well. Genes encoding enzymes responsible for 3-hydroxylation belong to the CYP98 gene family [3].

The aim of this work is to find potential candidate genes from the CYP73 and CYP98 families in the land-living bryophytes *Anthoceros agrestis* (*Aa*), *Marchantia polymorpha* (*Mp*) and *Physcomitrium patens* (*Pp*) and the water-living streptophyte alga *Chara braunii* (*Cb*). Comparing the sequences of the genes as well as the enzymatic capabilities, the preferred substrates and the differences in these enzymes might give a hint on the enzymes that were necessary to evolve life on the dry shores.

Putative CYP73 and CYP98 genes from *Aa*, *Mp* and *Pp* have been transferred into *Saccharomyces cerevisiae* and the encoded proteins expressed and characterized with different substrates. The affinity for the proposed main substrates has been quantified in Km values and the enzymatic activity has been monitored as Vmax to make the enzyme activities comparable.

*Cb* gene candidates only show vague similarities with known genes encoding CYP73 or CYP98 [4]. However, if homologs for the respective enzymes from *Aa*, *Mp* or *Pp* were found in *Cb*, it might help to understand the origin of these enzymes.

References

- [1] Cheynier V et al. (2013) Plant Physiol Biochem 72:1-20
- [2] Denisov I et al. (2007) Biol Chem 282:7066-7076
- [3] Alber A et al. (2019) Plant J 99:924-936
- [4] de Vries J et al. (2017) Plant Cell Physiol 58:934-945

P 041

Elucidation of herbivore-induced volatile terpenoid formation in leaves of Japanese orange cherry (*Idesia polycarpa*)

M. Panagoulas<sup>1</sup>, M. Bach<sup>1</sup>, M. Fernández-Giro Muñoz<sup>1</sup>, M. Krischke<sup>1</sup>, N. D. Lackus<sup>1</sup>

<sup>1</sup>Julius-Maximilians-University Würzburg, Pharmaceutical Biology, Würzburg, Germany

In response to herbivory, plants produce a variety of volatile organic compounds that are released into the environment. These volatiles can mediate the direct defense response of the plant by being repellent or toxic, or the indirect defense by attracting natural predators. In order to gain first insights into the defense mechanisms of the ornamental tree species *Idesia polycarpa* (Japanese orange cherry, *Salicaceae*), we conducted volatile collections to determine the formation of volatiles in leaves upon herbivory by the generalist gypsy moth (*Lymantria dispar*) caterpillars. The herbivore-induced volatile bouquet of *I. polycarpa* leaves is characterized by the emission of several mono- and sesquiterpenes, including the homoterpene (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT). To gain a better understanding of the biosynthesis of volatile terpenoids in *I. polycarpa*, we performed a comparative analysis of herbivore-induced and control leaves using modern transcriptomic approaches. *De novo* assembly and gene expression analysis of the obtained transcriptomes allowed the identification of putative class I terpene synthases as well as a cytochrome P450 monooxygenase in *I. polycarpa*, which are tentatively involved in the respective terpene biosynthesis. The corresponding candidate genes were cloned and heterologously expressed in *E. coli* and *Nicotiana benthamiana* to study the enzymatic activity of the identified candidates *in vitro* and *in planta*. Thus, we were able to characterize seven terpene synthases capable of catalyzing the formation of major components of the terpenoid volatile bouquet, including (*E*)- $\beta$ -ocimene,  $\alpha$ -pinene,  $\beta$ -pinene, 1,8-cineole, linalool, germacrene D, (*E,E*)- $\alpha$ -farnesene, (*E*)-nerolidol, and several other sesquiterpenes. Furthermore, the identified P450 candidate catalyzed the formation of the homoterpene DMNT with the substrate (*E*)-nerolidol *in vitro* and *in planta*. Thus, our work enabled the identification and characterization of *I. polycarpa* class I terpene synthases and the reconstitution of the corresponding herbivore-induced terpene profile in a heterologous host.

P 042

**Disrupted Steryl Ester Biosynthesis Alters Metabolome and Impacts Phenotypes in Tomato Seeds and Fruits**

N. Laibach<sup>1</sup>, J. M. Lopez-Tubau<sup>2</sup>, A. Burciaga-Monge<sup>2</sup>, S. Alseekh<sup>3,4</sup>, C. Deng<sup>2</sup>, A. R. Fernie<sup>3,4</sup>, A. Ferrer<sup>2,5</sup>, T. Altabella<sup>2,5</sup>

<sup>1</sup>Rhine-Waal University of Applied Sciences, Life Sciences, Kleve, Germany

<sup>2</sup>Center for Research in Agricultural Genomics, Department of Molecular Genetics, Barcelona, Spain

<sup>3</sup>Max-Planck-Institute of Molecular Plant Physiology, Potsdam, Germany

<sup>4</sup>Center of Plant Systems Biology and Biotechnology, Plovdiv, Bulgaria

<sup>5</sup>University of Barcelona, Faculty of Pharmacy and Food Sciences, Barcelona, Spain

Steryl esters (SEs) stored in cytoplasmic lipid droplets play a crucial role in maintaining the balance of free sterols (FS) within cell membranes during plant development and stress responses. Special enzymes called sterol acyltransferases, particularly phospholipid:sterol acyl-transferase (PSAT) and acyl CoA:sterol acyltransferase (ASAT), synthesize SEs by transferring fatty acids from phospholipids and acyl-CoA molecules to FS, respectively. Recently, we demonstrated that the tomato CRISPR/Cas9 double knock-out mutant *slpsat1 x slsat1* has an early germination phenotype (Burciaga-Monge et al., 2022). In this study, we investigated how this mutation affects the sterol profiles of both seeds and fruits, and how these changes influence the overall metabolite composition of these tomato organs. We also report an additional fruit phenotype showing an increased resistance of the *slpsat1 x slsat1* mutant to *Botrytis cinerea*. A detailed analysis encompassing primary metabolites, semi-polar secondary metabolites, and lipids revealed significant alterations in the global metabolic profiles of mutant seeds and fruits compared to wild-type plants. Interestingly, some metabolic changes, like a notable rise in various dipeptides, were observed in both organs. However, other changes, such as increased antioxidative metabolism and reduced triacylglycerides, were specific to fruits. Our datasets suggest that *slpsat1 x slsat1* mutant metabolism of dry seeds is already at the status similar to seed imbibition, clearly contrasting the wildtype. This indicates a potential for premature ripening or even seed senescence. Additionally, the observed metabolic shifts suggest alterations in the levels of plant hormones gibberellins and abscisic acid, which might explain the early germination phenotype. Furthermore, combining this metabolomic data with our previous transcriptomic analysis (gene expression) of dry and imbibed seeds revealed that disrupted SE biosynthesis also triggers changes in sulfur metabolism and pathways related to phagosomes (cellular compartments involved in breakdown). These alterations could be linked to the observed senescence phenotypes. In conclusion, our findings provide valuable insights into how SE biosynthesis influences the metabolic processes that govern fruit health and, consequently, seed development.

P 043

**Gene clustering in barley reveals cryptic oxidative rearrangement in gramine biosynthesis**

S. Leite Dias<sup>1</sup>, L. Chuang<sup>2,3</sup>, S. Liu<sup>2,3</sup>, B. Seligmann<sup>2</sup>, F. L. Brendel<sup>1</sup>, B. Chavez<sup>1</sup>, R. E. Hoffie<sup>4</sup>, J. Kumlehn<sup>4</sup>, J. Wolf<sup>2</sup>, A. Bülteimeier<sup>2,3</sup>  
M. Herde<sup>5</sup>, C. P. Witte<sup>5</sup>, J. D'Auria<sup>1</sup>, J. Franke<sup>2,3</sup>

<sup>1</sup>Leibniz Institute for Plant Genetics and Crop Plant Research (IPK), Department of Molecular Genetics, Seeland/OT Gatersleben, Germany

<sup>2</sup>Leibniz University Hannover, Institute of Botany, Hannover, Germany

<sup>3</sup>Leibniz University Hannover, Centre of Biomolecular Drug Research, Hannover, Germany

<sup>4</sup>Leibniz Institute for Plant Genetics and Crop Plant Research (IPK), Department of Physiology and Cell Biology, Seeland/OT Gatersleben, Germany

<sup>5</sup>Leibniz University Hannover, Department of Molecular Nutrition and Biochemistry of Plants, Hannover, Germany

Gramine is an alkaloid found in barley and other Poaceae members that protects them against insects and affects their palatability to ruminants. The biochemical basis for the formation of gramine from the amino acid tryptophan has remained unresolved. In our study, we identified a gene cluster in barley containing two genes, encoding a previously reported N-methyltransferase as well as a cytochrome P450 monooxygenase, which we named AMI synthase (AMIS) [1]. Via a recently developed fluorescence detection method for tryptophan-derived allelopathic compounds [2] we assessed the production of gramine, upon the integration of these two genes, in heterologous species *Nicotiana benthamiana*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae*, and constituted the necessary genetic complementation to reactivate gramine biosynthesis in the barley variety Golden Promise that does not produce it. To further characterize CYP76M57 in its natural host, we mutated the gene by Cas endonuclease technology in the cultivar Tafeno, which prevented the production of gramine in the plant. Based on in vitro experiments with yeast microsomes, we demonstrated that CYP76M57 performs a cryptic oxidative rearrangement of tryptophan to an iminium intermediate. Taken together, our findings revealed how the gramine scaffold is generated from a simple amino acid. The discovery of the genetic basis of gramine formation enables access to breeding initiatives that aim to reduce pesticide use and harness the biological activity of gramine for barley cultivation.

References

- [1] S. L. Dias *et al.*, "Biosynthesis of the allelopathic alkaloid gramine in barley by a cryptic oxidative rearrangement," *Science*, vol. 383, no. 6690, pp. 1448–1454, Mar. 2024, doi: 10.1126/science.adk6112.
- [2] S. Leite Dias *et al.*, "A New Fluorescence Detection Method for Tryptophan- and Tyrosine-Derived Allelopathic Compounds in Barley and Lupin," *Plants*, vol. 12, no. 10, Art. no. 10, Jan. 2023, doi: 10.3390/plants12101930

P 044

Functional analysis of the cytochrome P450 71B subfamily in *Arabidopsis thaliana*

L. Nicol<sup>1</sup>, T. Müller<sup>2</sup>, C. Böttcher<sup>3</sup>, E. Glawischmig<sup>1</sup>

<sup>1</sup>TU Munich, Straubing, Germany

<sup>2</sup>TU Munich, Freising, Germany

<sup>3</sup>JKI, Berlin, Germany

The expansion of gene families via duplication is a characteristic feature of plant genomes and presents a major challenge for assigning biological functions using single-gene knockouts. Here, we present a strategy for analyzing the biological functions of highly expanded enzyme families in *A. thaliana*, using the cytochrome P450 subfamily 71B as an example. This subfamily contains 32 expressed genes, and only a few are functionally characterized. Using CRISPR/Cas9-based gene editing we have individually deleted four gene clusters (up to 83 kb in size) and generated a septuple mutant of the non-cluster genes. Metabolite compositions are being systematically analyzed from a range of different tissues and stress conditions via UHPLC/Q-TOF-MS. Preliminary results indicate the enzymes play a role in the biosynthesis and modification of metabolites involved in defense and/or communication. Through the analysis of partial deletion and complementation lines, and heterologous expression in *S. cerevisiae* and *N. benthamiana* we aim to assign biological functions to specific genes.

P 045

Identification and characterisation of desulfoglucosinolate sulfotransferases from *Tropaeolum majus*

K. Hartelt<sup>1</sup>, M. von Barga<sup>2,1</sup>, R. Friedhoff<sup>3</sup>, B. Pucker<sup>3</sup>, I. El-Awaad<sup>4,1</sup>, U. Wittstock<sup>4,1</sup>

<sup>1</sup>TU Braunschweig, Institute of Pharmaceutical Biology, Braunschweig, Germany

<sup>2</sup>Utrecht University, Department of Biology, Microbiology, Braunschweig, Germany

<sup>3</sup>TU Braunschweig, Institute for Plant Biology & BRICS, Braunschweig, Germany

<sup>4</sup>TU Braunschweig, Center of Pharmaceutical Engineering (PVZ), Braunschweig, Germany

Glucosinolates are sulphur-containing plant specialized metabolites. They are found in the order Brassicales including members of the Brassicaceae. Certain glucosinolates, among them benzylglucosinolate, can have positive health effects, for example through the antimicrobial and cancer-preventative properties of their breakdown products upon myrosinase-catalysed hydrolysis. There is a strong interest in producing glucosinolates for further evaluation and medical applications.

Heterologous production of benzylglucosinolate in microbial and plant hosts using genes isolated from *Arabidopsis thaliana* has frequently resulted in the accumulation of the penultimate intermediate desulfobenzylglucosinolate and low yields of benzylglucosinolate[1]. To overcome this bottleneck in heterologous production, our aim is to test enzymes, especially sulfotransferases (SOTs), from other glucosinolate-producing plants for their ability to increase the flow through the pathway. No SOTs from plants other than *A. thaliana*[2] and *Brassica napus*[3] have been characterised on the molecular level so far. We have chosen the benzylglucosinolate-accumulating species *Tropaeolum majus* (Tropaeolaceae) to identify and characterise desulfoglucosinolate SOTs.

We have generated a highly continuous genome sequence of *T. majus* using nanopore sequencing (N50 >35 Mbp). A phylogenetic analysis of predicted gene models supported by publicly available transcriptomic data[4] led to the identification of a pool of eight genes encoding proteins with homology to the *A. thaliana* SOTs. The closest three were cloned from cDNA, heterologously expressed in *E. coli*, purified, and analysed for SOT activity. So far, two SOTs catalysed the conversion of desulfobenzylglucosinolate to benzylglucosinolate. Enzyme properties such as substrate specificity and kinetics will be compared with those of the *A. thaliana* homologues used previously for heterologous benzylglucosinolate production. We anticipate that exchanging *A. thaliana* SOTs by more efficient enzymes in metabolic engineering efforts for future heterologous glucosinolate production would lead to an increased yield.

References

- [1] Petersen et al. (2018), J. Integr. Plant Biol. 60(12):1231–1248.
- [2] Klein et al. (2006), FEBS Journal 273(1):122–136.
- [3] Hirschmann and Papenbrock (2015) Plant Physiology and Biochemistry 91:10–19.
- [4] Jensen et al. (2012), Molecular Plant 5(5): 984–992.

P 046

Chasing histochemical and ultrastructural changes in petal tissues to provide a better understanding of scent volatiles synthesis and emission in tropical flowers

A. Mitra<sup>1</sup><sup>1</sup>Indian Institute of Technology Kharagpur, Agricultural and Food Engineering Department, Kharagpur, India

Studying histochemistry of plant tissues is often considered as an old-fashioned approach to address any scientific question(s) in plant biology. However, appropriate selection of histochemical techniques coupled with microscopic analyses can generate wealth of information especially on temporal accumulation of volatile metabolites in petals and their subsequent release in the environment, which can be measured from tissue internal pool or from floral headspace as by GC-MS. Investigation on localization and temporal accumulation of floral volatiles can help in generating a hypothesis on possible emission mechanism. Further, scant information is available on ultrastructural changes that occur inside the cells and facilitate the scent volatiles emission from floral organs especially from petals. Thus, carefully selected transmission electron microscopic (TEM) images of petal tissues throughout floral lifespan can provide new insights on the changes at cellular levels prior to, during, and after scent emission from the floral tissues. In addition to floral volatiles analyses, this presentation aims to showcase a range of histochemical and TEM analyses of floral tissues to provide better understanding of temporal emission of scent volatiles in selected tropical flowers. To detect the localization of volatiles in its floral tissue for subsequent emission, histochemical analyses were performed using light microscopy. Histological studies on *Morinda citrifolia* flowers showed the presence of inverted urn shaped cells on the adaxial surface of petals. It has been revealed that cuticular layer appears to be responsible for volatiles emission. Similar phenomenon has been observed in *Gardenia jasminoides* and *G. carinata* flowers. The spatio-temporal accumulation pattern of primary metabolites, which provide precursors for scent volatiles formation have been studied in *M. citrifolia*, *G. jasminoides* and *G. carinata* flowers through histochemical and TEM analysis. Similar analyses in petals of different *Plumeria* spp. revealed distinct variations in epidermal cell shapes with dense cellular content rich in terpenoids. Primary metabolites, such as carbohydrates and proteins have also been histo-localized on temporal basis, indicating their active participation in floral volatile biosynthesis. The ultrastructural analyses demonstrated that organelle-rich epidermal cells and thick cuticular layers display an active physiological role in volatile synthesis and emission.

P 047

Identification of the intermediates and the mechanisms involved in the crosstalk of isoprenoid biosynthesis pathways

J. M. Al-Mousawi<sup>1</sup>, M. Raorane<sup>1</sup>, T. Rahpeyma<sup>2</sup>, P. Nick<sup>2</sup>, B. H. Junker<sup>1</sup><sup>1</sup>Martin-Luther University Halle-Wittenberg, Institute of Pharmacy, Pharm. Biology, Biosynthesis of Active Substances, Halle a. d. Saale, Germany<sup>2</sup>Karlsruhe Institute of Technology, Botanical Institute, Molecular Cell Biology, Karlsruhe, Germany

The significance of plant-based isoprenoids and terpenes has been proven repeatedly in recent years. An abundance of pharmaceutical applications of individual isoprenoids and the medicinal plants containing these compounds has been described. These include sedative, antiviral, antimicrobial, anti-inflammatory and antineoplastic properties. Therefore, the insight into the biosynthetic pathways of isoprenoids is crucial for advancing our comprehension of their production and potential applications.

Currently, there are two individual isoprenoid pathways described in plants, each yielding the isoprene monomers IPP and DMAPP. The multi-organellar MVA pathway synthesizes isoprenoids out of FPP, while the plastidial MEP pathway produces isoprenoids from GPP. Traditionally, these pathways have been perceived as entirely independent of each other; however, recent research has challenged this exclusivity.

Our research has already shown and quantified the crosstalk during monoterpene and sesquiterpene production in plants of the Lamiaceae family. Additionally, several studies have found evidence for the existence of a crosstalk between the compartments of the MVA and MEP pathway, where a unidirectional transport from IPP from the plastid to the cytosol was observed. Whether this transport is due to a specific, not yet uncovered transporter, tubular extensions of a specific organelle, or a not yet postulated mechanism of transport still remains to be determined. Overall, the precise details of the exchanged intermediates remain elusive.

To study this subcellular exchange of metabolites, we work with heterotrophic Tobacco BY-2 cell cultures. They are a proven model system, in which the crosstalk has already been observed. The primary objective of establishing a Linalool producing BY-2 cell line has been achieved, which will now enable us to study the crosstalk between both isoprenoid pathways. To this end, we shall perform microscopic analysis utilizing fluorescent markers and organelle dyes alongside labelled precursors of the terpene pathways. To corroborate the findings, <sup>13</sup>C – Metabolite Flux Analysis with labelled sugars will be conducted to quantify the changes in the metabolic fluxes.

Therefore, this study may reveal new functional aspects of organelle interaction and provide the fundament for further research about the relation of metabolic processes by organelle shape and form.



P 048

*Coffea canephora* Kaurene Hydroxylases Catalyze Key Steps to Cafestol Biosynthesis

M. Frey<sup>1,2</sup>, G. Antoine<sup>3,4</sup>, U. Bathe<sup>5</sup>, R. Falke<sup>1</sup>, L. Meink<sup>6,1</sup>, O. Frank<sup>7</sup>, C. Dawid<sup>7</sup>, A. Hassanin<sup>8,9</sup>, M. D. Davari<sup>8</sup>, T. Joet<sup>3</sup>  
S. Dussert<sup>3</sup>, A. Tissier<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry, Department of Cell and Metabolic Biology, Halle a. d. Saale, Germany

<sup>2</sup>Martin-Luther-University Halle-Wittenberg, Institute of Pharmacy, Pharmaceutical Biology and Pharmacology, Halle a. d. Saale, Germany

<sup>3</sup>University of Montpellier, DIADE, IRD, CIRAD, Montpellier, France

<sup>4</sup>University of North Texas, Department of Biological Sciences and BioDiscovery Institute, Denton, TX, United States

<sup>5</sup>University of Florida, Department of Horticultural Science, Gainesville, FL, United States

<sup>6</sup>Martin-Luther-University Halle-Wittenberg, Institute of Pharmacy, Pharmaceutical Biology and Pharmacology, Halle a. d. Saale, Germany

<sup>7</sup>Technical University of Munich, Chair of Food Chemistry and Molecular Sensory Science, Freising, Germany

<sup>8</sup>Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Halle a. d. Saale, Germany

<sup>9</sup>Assiut University, Department of Pharmacognosy, Faculty of Pharmacy, Assiut, Egypt

From its origin in Ethiopia over Yemen to Europe and the rest of the world, coffee has made a remarkable journey to the globalized product it is today. The global market for coffee products was an estimated 116 bn \$ in 2023, with a market share of 70% arabica and 30% robusta coffee, that are produced from the beans of the closely related species *Coffea arabica* and *Coffea canephora*. Coffee beans contain a complex blend of natural products that contribute to its flavor and its health-promoting effects. Among these are cafestol and kahweohl, two kaurene-type diterpenes. Kaurene is also a central precursor to the gibberellic acid family of plant hormones. Here, we report key genes for the biosynthesis of cafestol and kahweol in *Coffea canephora* up to kaurenoic acid with two possible branching points in the pathway towards the gibberellins. Furthermore, we characterize three kaurene hydroxylases from the CYP76D subfamily of cytochrome p450 enzymes that accept kaurenoic acid as a substrate. Using docking experiments, we give insight into the structural enzymatic environment for the oxidation of the kaurene backbone and convergent evolution of kaurene 3 $\beta$ -hydroxylases from rice and coffee.

P 049

Investigation on BAHD Acyltransferases in *Neoblechnum brasiliense*

M. Ufland<sup>1</sup>, M. Petersen<sup>1</sup>

<sup>1</sup>Phillips-Universität Marburg, Institut für Pharmazeutische Biologie und Biotechnologie, Marburg, Germany

*Neoblechnum brasiliense* (Desv.) Gasper & V.A.O. Dittrich is a fern of the Blechnaceae family, which is one of 26 families of the order Polypodiales, one of the biggest order of leptosporangiate ferns. *N. brasiliense* is native to the warm and humid subtropical forests of South America. Up to now, the Blechnaceae is the only family among ferns that contain rosmarinic acid, an ester formed of caffeic acid and 3,4-dihydroxyphenyllactic acid (Petersen & Simmonds 2003).

BAHD acyltransferases are found in fungi and plants. After terrestrialization the number of BAHD copies increased from 2-5 BAHD copies to 50-200 BAHD copies in angiosperms. These acyltransferases play a significant role in molecular adaptation to land, as four biopolymers (lignin, sporopollenin, suberin, cutin) are needed and BAHDs are active in all of the respective biosynthetic pathways. BAHD acyltransferases catalyze the formation of an ester or amide by transferring a CoA-activated acid moiety to an acceptor (alcohol or amine). BAHD acyltransferases from clade 5 are part of the phenylpropanoid pathway in plants, which is involved in the formation of the above-mentioned products like lignin or suberin but also chlorogenic or rosmarinic acid (Moghe et al. 2023).

Several genes for hydroxycinnamoyltransferases (HCT) from clade 5 could be found in *N. brasiliense* by RACE-PCR. The transcriptome of *Struthiopteris spicant* (L.) F.W. Weiss from the 1kp database (<https://db.cngb.org/onekp/>) served as a template for designing RACE primers. The identified sequences show a high similarity to *S. spicant* genes but a low similarity to genes from flowering plants. The enzymes were heterologously synthesized in *E. coli* and kinetically characterized.

NbHCT1, NbHCT2 and NbHCT12 are hydroxycinnamoyl-CoA:shikimate HCTs that accept a broad range of cinnamoyl derivatives as donors and shikimate, quinate and 3-hydroxyanthranilate (only NbHCT1) as acceptors. NbHCT4 is a hydroxycinnamoyl-CoA:quininate HCT that accepts shikimate and quinate as acceptors. NbHCT4 is responsible for the formation of chlorogenic acid, which could be found in extracts of *N. brasiliense*. All enzymatically formed products were verified via LC-MS/MS against an external standard.

References

Moghe et al. (2023) Annu Rev Plant Biol 74:165–194

Petersen & Simmonds (2003) Phytochemistry 62:121–125

P 050

### The 3D chromosomal organisation of plant biosynthetic gene clusters

H. W. Nützmann<sup>1</sup>, G. Jervis<sup>1</sup>, J. Bishop<sup>2</sup>, J. Taylor<sup>2</sup>, D. Doerr<sup>3</sup>, S. Fernández Valverde<sup>4</sup>, E. Wegel<sup>5</sup>, M. Di Stefano<sup>6</sup>, A. Osbourn<sup>5</sup>

<sup>1</sup>University of Exeter, Biosciences, Exeter, United Kingdom

<sup>2</sup>University of Bath, Life Sciences, Bath, United Kingdom

<sup>3</sup>Heinrich Heine University, Düsseldorf, Germany

<sup>4</sup>University of New South Wales, Sydney, Australia

<sup>5</sup>John Innes Centre, Norwich, United Kingdom

<sup>6</sup>Institut de Génétique Humaine CNRS, Montpellier, France

Co-localisation of functionally related but non-homologous genes in so called gene clusters is a rare yet reoccurring phenomenon in eukaryotic genomes. Gene clusters encoding essential information for development, immunity and metabolism are present in both lower and higher eukaryotes. Recent discoveries have unveiled genetic clustering as a common feature of plant specialised metabolism and have established the genetic basis for the biosynthesis of plant derived compounds of major nutritional and pharmacological importance.

The genes within biosynthetic gene clusters are typically co-expressed in specific plant organs and in response to environmental triggers. Here, we show that these clustered pathways genes are characterized by distinct chromatin signatures associated with cluster repression and activation. By Hi-C, polymer simulations and high-resolution microscopy we further show that active and repressed gene clusters are embedded in spatially distinct chromosome domains with variable positioning within the nuclear environment.

The presented results reveal the complex chromosomal architecture surrounding biosynthetic gene clusters and form the basis for future studies to better understand its role in defining the regulation and evolution of plant specialised metabolic pathways.

P 051

### Learning more about the complex machinery involved in glucosinolate breakdown in *Arabidopsis thaliana*

L. Behnsen<sup>1</sup>, C. Seeling<sup>1</sup>, A. Backenköhler<sup>1</sup>, K. Meier<sup>1</sup>, E. J. Stauber<sup>1</sup>, U. Wittstock<sup>1</sup>

<sup>1</sup>Technische Universität Braunschweig, Institut für Pharmazeutische Biologie, Braunschweig, Germany

The glucosinolate-myrosinase system is a well-studied defense system from plants of the order Brassicales. Upon tissue disruption glucosinolates get hydrolysed by myrosinases leading to an instable aglycone. This aglycone can be rearranged to an isothiocyanate that can be toxic for pathogens or herbivores. Alternatively, in the presence of specifier proteins the aglycone can be converted to other products like simple nitriles. In the model plant *Arabidopsis thaliana* in accession Columbia-0 there are five genes encoding for nitrile-specifier proteins (NSP1-NSP5) and six genes encoding for classical myrosinases (TGG1-TGG6; TGG3 and TGG6 are pseudogenes). Alongside these classical myrosinases, there is also a sister clade of 16 beta-glucosidases (BGLU18-BGLU33) which are referred to as atypical myrosinases. These BGLUs are expressed in different organs and during different developmental stages of the plant and could be involved in pathways of glucosinolate turnover without tissue disruption. Only few of these atypical myrosinases have been characterized biochemically or with respect to their biological functions.

To learn more about the possible involvement of atypical myrosinases in glucosinolate breakdown pathways, we have expressed *A. thaliana* TGG and BGLU cDNAs in *Pichia pastoris* using expression constructs for secretion of the heterologous proteins. The enzymes were subjected to substrate screening using glucosinolate mixtures present in extracts of *A. thaliana* and/or to reactions with pure glucosinolates. These experiments have been complemented by analyses of glucosinolate levels in various *A. thaliana* lines with T-DNA insertions or indel mutations due to CRISPR/Cas9 genome editing in *BGLU* genes. As *NSPs* are coexpressed with specific *BGLUs* in roots, seeds and seedlings of *A. thaliana*, we are also studying the effects of *NSP* knockouts on *BGLU* gene expression. First results will be presented.

P 052

How plants eliminate an apparent toxic by-product - Identification and functional characterization of the C4-ketone products of homoterpene biosynthesis in *Zea mays*

C. M. Jochimsen<sup>1</sup>, C. Schaff<sup>1</sup>, J. Degenhardt<sup>1</sup>

<sup>1</sup>Martin-Luther-University Halle-Wittenberg, Institute for Pharmacy, Halle a. d. Saale, Germany

Homoterpenes, such as the C11-compound (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), are commonly found volatiles emitted by many plant species. Despite their common occurrence, a functional role of DMNT has been identified in only a few interactions between plants and animals [1]. Our previous work has elucidated the biosynthetic pathway of DMNT in *Zea mays*, which proceeds in two enzymatic steps. First, the terpene synthase TPS2 catalyzes the formation of the tertiary C15-alcohol (*E*)-nerolidol from the ubiquitous farnesyl diphosphate (FDP) substrate. The subsequent oxidative degradation of (*E*)-nerolidol is catalyzed by a cytochrome P450 monooxygenase, CYP92C5 [2]. While this oxidative elimination step has not been characterized in detail, the formation of a C4 fragment has been postulated. We identified this by-product of homoterpene biosynthesis and aim to determine its fate *in planta*.

CYP92C5 was heterologously expressed in *Saccharomyces cerevisiae* and the enzyme was incubated with the substrate (*E*)-nerolidol *in vitro*. The butane derivatives methyl vinyl ketone (MVK) and methyl ethyl ketone (MEK) were detected. While MEK was stable under *in vitro* conditions, MVK was converted to MEK. This conversion was also observed in plant systems and is probably catalyzed by reductase activities [3]. It is necessary for plants to eliminate MVK due to the toxicity of the  $\alpha,\beta$ -unsaturated carbonyl structure. We identified putative reductases in *Zea mays* with an expression pattern similar to that of *cyp92c5*. To determine whether these reductases catalyze the detoxification step, activity assays of these candidate enzymes are being performed.

References

1. Tholl, D., et al., *The biochemistry of homoterpenes--common constituents of floral and herbivore-induced plant volatile bouquets*. *Phytochemistry*, 2011. **72**(13): p. 1635-46.
2. Richter, A., et al., *Characterization of Biosynthetic Pathways for the Production of the Volatile Homoterpenes DMNT and TMTT in Zea mays*. *Plant Cell*, 2016. **28**(10): p. 2651-2665.
3. Mano, J.i., et al., *Protection against Photooxidative Injury of Tobacco Leaves by 2-Alkenal Reductase. Detoxication of Lipid Peroxide-Derived Reactive Carbonyls*. *Plant Physiology*, 2005. **139**(4): p. 1773-1783.

P 053

Molecular and biochemical investigations of acyltransferases and esterases in the formation of phenolic compounds in *Anthoceros agrestis* and *Mesotaenium endlicherianum*

J. Marks<sup>1</sup>, M. Petersen<sup>1</sup>

<sup>1</sup>Philipps-Universität Marburg, Institut für Pharmazeutische Biologie und Biotechnologie, Marburg, Germany

The focus of this work primarily revolves around the hornwort *Anthoceros agrestis* of the division Anthocerotophyta. The ancestors of Bryophytes are speculated to be the first plants to make the step onto dry land<sup>1</sup>. To thrive in this new environment a myriad of adaptations were necessary. Our work focuses on phenolic compounds, like chlorogenic and rosmarinic acid (RA). Phenolic compounds have proven to possess UV- and pathogen-protective properties, which makes them prime indicators for potentially crucial adaptations in early land plants<sup>2</sup>. Furthermore, phenolic metabolites are essential in the formation of lignin, which is an important molecule in the stabilization of the plant and to uphold homeostasis due to the lack of external water.

Previous research revealed that *Anthoceros agrestis* is capable of producing large quantities of RA, up to 5% of its dry weight<sup>3,4</sup>. However, the final step in the biosynthesis of RA still has to be investigated and is a key part of this research. To achieve this goal multiple different approaches had to be considered. Primarily the heterologous expression of a promising enzyme family, the serine carboxypeptidase-like acyltransferases (SCPL), and crude protein extract experiment were conducted to elucidate alternative enzymatic reactions that catalyse the final step of this metabolic pathway.

The second major field of interest examines caffeoyl shikimate esterases (CSE), which are involved in the production of caffeoyl moieties, that feed into reactions downstream the phenylpropanoid pathway. High activity in these enzymes may hint to an increased demand for these types of phenylpropanoid molecules in early land plants relative to algae like *Mesotaenium endlicherianum*.

References

- <sup>1</sup> Konrat M, Shaw AJ, Renzaglia KS (2010) *Phytotaxa* 9:5-10
- <sup>2</sup> Clé C, Hill LM, Niggeweg R, Martin CR, Guisez Y, Prinsen E, Jansen MA (2008) *Phytochemistry* 69:2149-2156
- <sup>3</sup> Vogelsang K, Schneider B, Petersen M (2005) *Planta* 223:369-373
- <sup>4</sup> Pezeshki S (2016) Biosynthese von Kaffeesäuremetaboliten im Ackerhornmoos *Anthoceros agrestis* und im Kleinen Blasenmützenmoos *Physcomitrella patens*. Doctoral Thesis, Philipps-Universität Marburg

P 054

Prediction and Validation of Terpene Synthase Functions in *Tanacetum vulgare*M. Hildebrandt<sup>1</sup>, B. Laker<sup>1</sup>, M. Eisenhut<sup>1</sup>, S. Tschikin<sup>1</sup>, D. Ziaja<sup>1</sup>, R. Jakobs<sup>1</sup>, C. Müller<sup>1</sup>, A. Bräutigam<sup>1</sup><sup>1</sup>Bielefeld University, Bielefeld, Germany

Terpenoids are the largest and most diverse class of plant specialized metabolites, of which many are ecologically and economically important. A local population of *Tanacetum vulgare* is able to produce many different terpenoids with substantial standing variation raising the question of how this high level of chemodiversity evolves and is maintained. To understand the evolutionary patterns, we determined the products of a representative number of terpene synthase (TPS) candidates. We sequenced 24 transcriptomes and one genome of *T. vulgare*, assembled and annotated, and correlated the TPS abundance to metabolite levels to determine the candidates. Biochemical tests confirmed the predictions, demonstrating that transcript abundance can predict product abundance. Phylogenetic analyses suggest that in *T. vulgare* at least one type of TPS evolved de novo while others are already present in other Asteraceae. RNA-seq data suggests that different TPS at varying expression levels are present in one chemotype, suggesting they are not allelic but reside in different loci. We tested if alignments were able to determine the amino acid residues relevant for substrate specificity but were unable to pinpoint relevant amino acids. We also tested if homology modeling revealed obvious, large changes within the active site and were again unable to identify large changes. We thus conclude that subtle changes, likely away from the active site, alter the active site sufficiently to change the product. Currently, we use the genome of a *T. vulgare* individual with a chemotype dominated by beta-thujone to trace the evolutionary history of the TPS loci identified in biochemical analyses in this single chemotype. Our quantitative, population-level RNA-seq approach demonstrates its suitability for large-scale mining for new specialized metabolism enzymes for biotechnological applications based on chemodiverse plant populations.

P 055

## Production of bioactive compounds from plant cell suspension cultures in bioreactors

L. Meink<sup>1</sup>, B. H. Junker<sup>1</sup>, M. Raorane<sup>1</sup><sup>1</sup>Martin-Luther-University, Institute of Pharmacy, Pharmaceutical Biology, Biosynthesis of Active Ingredients, Halle a. d. Saale, Germany

Plants possess a robust immune system with a myriad of defence molecules to enhance their chemical immunity against various stresses. These molecules are also interesting and profitable for human organisms. Many of these bioactive molecules are functional directly as drugs or used as precursors for semi-synthetic modifications. Among these natural products that provide medical benefits for an organism, terpenes play major roles.

The lack of basic research on the synthetic pathways is currently the biggest problem in the *in vitro* production of plant secondary metabolites. Their chemical structure and the specific stereochemical requirements makes chemical synthesis not economically feasible. Semi-synthetic methods have been developed with the help of metabolic engineering combined with organic chemistry, but these have achieved only limited success.

Another alternative source of terpenes are heterologous production systems. *E.coli* and yeast have shown good production potentials. However, due to lack of certain genes, efficient biosynthesis requires introduction of multiple genes. Plant cell cultures represents an alternate viable option to comprehend such a challenge. Several cell lines have been established from various plants, We use the tobacco "Bright Yellow – 2" (BY-2) cell line as a model system for our research. BY-2 cells are well studied for cell biology research for several decades and thus provides a significant source of knowledge.

The project is in the inception stage and the first steps are to establish transgenic cell lines producing various terpenes. These cell lines shall then be upscaled for large scale bioreactors. In our group we have recently established a single monoterpene producing cell line, however with low yield. The idea of producing secondary plant products in large bioreactors offers an ideal way to realize the three major goals of the economical production of secondary plant materials: high productivity, yield and concentration of the product of interest. However, except for some notable exceptions, e.g. paclitaxel in *Taxus* cell cultures, an economically relevant production of secondary plant products, using huge fermentation tanks, remains a challenge for plant biotechnologists.

Our ultimate aim is to establish BY-2 cells as a unique production platform for terpenes with a perspective for industrial application.

P 056

**<sup>13</sup>C-Metabolic flux analysis: A powerful approach to quantify light-regulated crosstalk between Isoprenoid Pathways**

S. Koley<sup>1,2</sup>, E. Grafahrend Belau<sup>1,3</sup>, M. Raorane<sup>1</sup>, R. Chamansara<sup>1</sup>, B. H. Junker<sup>1</sup>

<sup>1</sup>Martin Luther University Halle Wittenberg, Pharmacy, Halle a. d. Saale, Germany

<sup>2</sup>Donald Danforth Plant Science Center, Saint Louis, MO, United States

<sup>3</sup>Trait Genetics GmbH, Gatersleben, Germany

The plant kingdom boasts an extraordinarily diverse secondary metabolism, with over a million compounds exclusive to this life form. Among these natural products, terpenes play a significant and multifaceted role, providing numerous medical benefits. Two primary terpene biosynthesis pathways exist: the plastidic methyl erythritol 4-phosphate (MEP) pathway and the cytosolic mevalonate (MVA) pathway. Despite being studied for over a quarter of a century, these pathways still present numerous enigmas. Historically, the MEP pathway was understood to synthesize monoterpenes and diterpenes, while the MVA pathway was believed to produce sesquiterpenes and triterpenes. However, this exclusivity has since been challenged, with growing evidence suggesting a crosstalk between these two pathways. Interactions between both pathways have been documented in several plant species as well.

Complex regulatory mechanisms are known to control fluxes through these terpene pathways, with light serving as a major environmental cue for terpene accumulation. Studies in *Arabidopsis* seedlings have demonstrated that low light induces the MVA pathway, whereas exposure to relatively high light leads to MEP pathway upregulation. In some instances, light has been shown to also differentially control the expression of pathway genes, indicating that a general model of light regulation of plant isoprenoid biosynthesis is yet to be established.

This study employed isotopic tracer analysis, steady-state <sup>13</sup>C metabolic flux analysis (MFA), and pathway inhibition studies to measure the metabolic fluxes of primary and isoprenoid metabolism in a peppermint plant, under varying light conditions. Our findings provide novel insights into peppermint GT metabolism by confirming and quantifying the crosstalk between the two isoprenoid pathways toward monoterpene biosynthesis. Furthermore, a quantitative description of precursor pathways involved in isoprenoid metabolism is presented. Glycolysis was found to provide precursors for the MVA pathway. In contrast, the oxidative bypass of glycolysis fueled the MEP pathway, highlighting the prominent roles of the oxidative branch of the pentose phosphate pathway and RuBisCO. This study underscores the potential of <sup>13</sup>C-MFA to elucidate previously unquantified metabolic routes of the trichomes, thereby advancing our understanding of the action of the environmental cues on the molecular networks that regulate isoprenoid biosynthesis in plants.

P 057

**Subcellular localization of homospermidine oxidases involved in pyrrolizidine alkaloids biosynthesis**

A. Shahid<sup>1</sup>, D. Ober<sup>1</sup>

<sup>1</sup>CAU Kiel University, Kiel Germany, Botanical Institute und Botanic Gardens, Kiel, Germany

Pyrrolizidine alkaloids (PAs) are toxic secondary metabolites that are constitutively synthesized by angiosperms as defense compounds against herbivores. The basic structure of PAs includes a bicyclic necine base, common to all PAs, that is esterified with necic acids. Homospermidine synthase (HSS), a cytosolic enzyme, is the first known enzyme in PA biosynthesis. Recently, homospermidine oxidase (HSO), a copper-containing amine oxidase catalyzing the second step of necine base formation, was identified. It oxidizes both primary amino groups of homospermidine, resulting in the formation of a bicyclic structure 1-formylpyrrolizidine. This study investigates the subcellular localization of three PA-specific HSOs from Heliotropiaceae and Boraginaceae species: HSO1 from *H. indicum*, HSO1 and HSO5 from *S. officinale*, all of which have N-terminal signal sequences for the secretory pathway. The localization of HSOs involved in PA biosynthesis is important for understanding their functional role and interactions within specific cellular compartments, which is crucial for elucidating this evolutionary pathway of toxic compounds.

Three experimental approaches were employed. First, fusion constructs of GFP with signal peptides or complete open reading frames of the HSOs were transiently expressed in *N. benthamiana* leaves using *Agrobacterium*-mediated transformation, and localization was monitored by confocal laser scanning microscopy (CLSM). Secondly, hairy root transformation using *Agrobacterium rhizogenes* was used to study the stable expression of GFP-fused HSOs in hairy root of *Symphytum officinale*, by CLSM. In a third approach, subcellular localization is studied by immunolabelling after transient expression in *N. benthamiana* leaves. The infiltrated leaves sections were fixed, dehydrated, embedded in resin and sequentially immunolabelled with HSO specific primary antibodies and Alexa Fluor 488 secondary antibodies for detection under CLSM. We propose that HSOs involved in PA biosynthesis are targeted to the secretory pathway. We hypothesized that the HSOs are localized in specific structures, probably vesicles, but the exact identity of these structures has to be determined. Methods and first results will be presented and discussed.

P 058

Unraveling Tropane Alkaloid Biosynthesis in *Erythroxylum coca*B. Chavez<sup>1</sup>, P. Srinivasan<sup>2</sup>, C. Smolke<sup>2</sup>, J. D'Auria<sup>1</sup><sup>1</sup>IPK-Gatersleben, Molecular Genetics, Gatersleben, Germany<sup>2</sup>Stanford University, Bioengineering, Stanford, CA, United States

Tropane alkaloids (TAs) are heterocyclic nitrogenous metabolites found across seven orders of angiosperms, including Malpighiales (Erythroxylaceae) and Solanales (Solanaceae). Despite cocaine being an infamous tropane alkaloid from *Erythroxylum coca*, the biosynthetic pathway has remained incomplete for the past couple of decades. Using yeast as a screening platform, the missing enzymatic steps of TA biosynthesis in *Erythroxylum coca*. This work characterized a polyamine synthase along with amine oxidase-like enzymes *in vitro*, in yeast, and *in planta*, revealing that the first ring closure of TAs in *E. coca* occurs via bifunctional spermidine synthase/*N*-methyltransferases and both flavin- and copper-dependent amine oxidases. Identification of a SABATH family methyltransferase is responsible for the 2-carbomethoxy moiety characteristic of TAs from the Erythroxylaceae family. Finally, a cytochrome P450 of the CYP81A family was identified as responsible for the second tropane ring closure in *Erythroxylum coca*. These results demonstrate that tropane alkaloid biosynthesis in Erythroxylaceae and Solanaceae is polyphyletic in origin, further revealing that independent recruitment of unique biosynthetic mechanisms and enzyme classes occurred at nearly every step in the evolution of this pathway.

P 059

## Alternative splicing of RNA binding proteins from the RBP45 group is controlled by a structured mRNA motif

M. Reinhardt<sup>1</sup>, M. Sack<sup>2</sup>, Z. Weinberg<sup>2</sup>, A. Wachter<sup>1</sup><sup>1</sup>JGU Mainz, Mainz, Germany<sup>2</sup>Leipzig University, Leipzig, Germany

Alternative splicing (AS) is a widely distributed type of gene regulation among eukaryotes. It is based on the variable definition of exons and introns and results in increased transcriptome diversity. In plants, AS plays an important role in developmental processes such as photomorphogenesis and stress responses. Accordingly, a precise coordination of gene activity and AS is crucial for these processes. So far, the understanding of regulation is limited to single AS events. These regulatory mechanisms can involve the action of RNA-binding proteins (RBPs), but also *cis*-acting elements on the precursor (pre) mRNA such as structured mRNA motifs. Our study intends to provide a better understanding of the impact of structural elements on the regulation of AS in plants. Using bioinformatic strategies based on covariation, we identified the potentially structured RNA motif *45ABC*, which is highly conserved among monocots and dicots and harbors two stem loops. In *Arabidopsis thaliana*, this motif was identified in *RBP45A*, *RBP45B*, and *RBP45C*. These three homologs have two major transcript variants resulting from cassette exon inclusion or skipping. Skipping of this exon generates a splicing variant encoding the full-length protein, while its inclusion introduces a premature termination codon and a long 3' untranslated region, typical features that trigger degradation via nonsense-mediated decay. Interestingly, the *45ABC* motif encompasses the alternative splice sites, suggesting a functional relationship. Using splicing reporters for the three *RBP45* genes in transient expression assays, we demonstrated negative feedback autoregulation of their pre-mRNAs via AS, along with cross-regulation among all homologs. Disruption of the motif's structure results in an increase in the cassette exon variant, while compensatory mutations can restore this effect. Beyond their molecular functions, our findings suggest significant physiological implications. Preliminary evidence indicates that these RBPs not only promote primary root growth but also play a crucial role in abiotic stress responses. Higher-order loss-of-function mutants exhibit significantly shorter primary roots compared to wild-type plants and display reduced resilience to environmental stressors. In our ongoing research, we would like to address how the highly conserved *45ABC* element is functionally connected to the physiological functions of the corresponding genes.

P 060

**A microRNA Pair Controls Colour Change in Developing Eggplant Fruit Skin**

S. Panda<sup>1</sup>, A. Aharoni<sup>2</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry (IPB), Department of Cell and Metabolic Biology, Halle a. d. Saale, Germany

<sup>2</sup>Weizmann Institute of Science, Department of Plant and Environmental Sciences, Rehovot, Israel

Fruit pigmentation is a major signal that attracts frugivores to enable seed dispersal. In most fleshy fruit, green chlorophyll typically accumulates early in development and is later replaced by a range of pigments during fruit ripening. In many species such as grape and strawberry, chlorophyll is replaced by red anthocyanins generated through the flavonoid biosynthetic pathway. Eggplant (*Solanum melongena*) is unique in terms of pigmentation as its fruit accumulate anthocyanins starting from fruit set which are later replaced by a yellow pigment. We found that this yellow colour is the flavonoid pathway intermediate naringenin chalcone. To decipher the genetic regulation of such an extraordinary pigmentation shift, we integrated mRNA and small RNA profiling data obtained from developing eggplant fruit. We discovered that while SQUAMOSA PROMOTER BINDING-LIKE (i.e., SPL6a, SPL10, and SPL15), MYB1 and MYB2 transcription factors (TFs) regulate anthocyanin biosynthesis in early fruit development, the MYB12 TF controls late naringenin chalcone accumulation. Here we show that microRNA157 and microRNA858 negatively regulate SPLs and MYB12 expression, respectively. Taken together, our model suggests that opposing and complementary expression of microRNAs and TFs controls the pigmentation switch in eggplant fruit skin. Intriguingly, despite the distinctive pigmentation pattern in eggplant, fruit of and array of other species utilize homologues regulatory factors to control the temporal and spatial production of a different classes of pigments.

P 061

**Genome Sequence of the ornamental plant *Digitalis purpurea* reveals the molecular basis of flower traits**

J. M. Horz<sup>1</sup>, B. Pucker<sup>1</sup>, K. Wolff<sup>1</sup>, R. Friedhoff<sup>1</sup>

<sup>1</sup>TU Braunschweig, Institute of Plant Biology, AG Pucker, Braunschweig, Germany

*Digitalis purpurea* (foxglove) is a widely distributed ornamental plant and the producer of the biomedical compound digoxin. Here, we present a long-read sequencing-based genome sequence of a red flowering *D. purpurea* plant and a corresponding prediction of gene models. This genomic resource paves the way for an in-depth investigation of various flower traits of *D. purpurea*.

Structural genes of the anthocyanin biosynthesis and the corresponding transcriptional regulators were identified. The comparison of red and white flowering plants based on re-sequencing several plant genomes with long reads revealed a large insertion in the anthocyanidin synthase gene in white flowering plants that most likely renders this gene non-functional and could explain the loss of anthocyanin pigmentation.

An additional floral trait was investigated in which a large terminal flower develops at the top of the inflorescence spike. A large insertion in the ortholog of the *Arabidopsis TFL1* gene was found to be present homozygously in plants with this trait. This insertion most likely leads to a loss-of-function of this gene resulting in the large terminal flower.

P 062

## Harnessing plant transporters for sustainable agriculture

N. C. Sanden<sup>1</sup>, B. A. Halkier<sup>1</sup>, D. Xu<sup>1</sup><sup>1</sup>University of Copenhagen, Copenhagen, Denmark

The green transition dictates an urgent need to change from animal-based towards plant-based protein sources in our diet. Rapeseed is the world's third-largest oilseed crop, but the rapeseed press cake that contains 30-40% protein with an excellent amino acid composition is unexploited as plant-based protein food for human consumption due to the presence of anti-nutritional glucosinolates.

In the model plant *Arabidopsis thaliana*, we recently discovered that the funiculus, which connects the silique septum in the mother plant with the seed, is a highly active production site for seed-bound glucosinolates. Additionally, we identified three funiculus-localized transporters UMAMIT29, -30 and -31, as glucosinolate exporters in *Arabidopsis* (Xu et al., Nature, 2023). UMAMIT stands for Usually Multiple Amino acids Move In and out Transporter and was believed to be a family of only amino acid transporters. We found that UMAMIT exporters and previously identified GLUCOSINOLATE TRANSPORTERS (GTRs) importers (Nour-Eldin et al., Nature, 2012) form a transporter cascade that is both essential and sufficient for moving glucosinolates across at least four plasma membrane barriers along the seed loading route (Sanden et al., Nature Plants, 2024). Mutating both importer and exporter genes eliminates seed glucosinolates, while maintaining the defense compounds in the rest of the plant. We are currently translating this transport engineering technology to rapeseed.

Successful development of a rapeseed with low glucosinolate level in the seeds without altering the glucosinolate level in the remaining part of the plant, could enable breeding towards increased disease and pest resistance and potentially open the gene pool beyond the Bronowski that is the genetic background of all elite lines. By addressing today's farming challenge in growing rapeseed after EU's ban on selected neonicotinoids in 2013, this crop has great potential for becoming an attractive protein crop for human consumption. Development of the already-existing, locally-grown rapeseed into a novel protein crop will contribute to sustainable agriculture in the green transition.

## References

Nour-Eldin et al., Nature 488, pages531–534 (2012)

Xu et al., Nature 617, pages132–138 (2023)

Nature Plants volume 10, pages172–179 (2024)

P 063

Genome wide identification of heat shock proteins from *Prosopis cineraria* and their interaction studiesH. Sehgal<sup>1</sup>, M. Joshi<sup>1</sup><sup>1</sup>Birla Institute of Technology and Science, Department of Biological Science, Pilani, India

Innate mobility of plants restrained their potential to circumvent heat stress and required them to withstand stress through inherent defence abilities in which heat shock proteins (HSPs) played essential roles by acting as chaperones. HSPs mediate disaggregation of denatured proteins leading to protein homeostasis and their expression is regulated by heat shock transcription factors (HSFs). *Prosopis cineraria* is a phreatophyte distributed across arid and semi-arid regions of India and can tolerate high temperatures due to its adaptive physiological and biochemical mechanisms. Therefore, *P. cineraria* represents a repository of genes for abiotic stress tolerance. Two months old *P. cineraria* plants were subjected to heat stress at two different high temperatures and their genome-wide transcriptome sequencing was conducted to identify and validate the expression of HSPs with real-time qPCR. It was noted that small HSPs (sHSPs) including *HSP15.7*, *HSP17.9*, *HSP18.5*, *HSP22.7* and *HSP26.5* are major chaperone proteins upregulated immediately after heat stress. A few HSFs including *HSFA3a*, *HSFA6b* and *HSFA7a* were upregulated which control the expression of HSPs. These results provide new insight into good candidate genes for crop improvement. Identified genes were cloned from *P. cineraria* and mobilized in pCAMBIA1300-UbiP-NosT for plant transformation. To elucidate the function of sHSPs, we constructed recombinant pRSETA vectors and transformed into *E. coli* BL21 (DE3) pLysS. These sHSPs will be induced in *E. coli* and purified His-tagged sHSPs will be harvested. The interaction studies of purified sHSPs with their interactome will be carried out by mass spectrometry.



P 064

**Hiding in plain sight: Bacterial gibberellin biosynthesis**  
R. Nagel<sup>1,2</sup>, L. E. Alexander<sup>2</sup>, C. E. Stewart Jr.<sup>2</sup>, R. J. Peters<sup>2</sup>

<sup>1</sup>Universität Leipzig, Plant Physiology, Leipzig, Germany

<sup>2</sup>Iowa State University, Roy J. Carver Department of Biochemistry, Biophysics, and Molecular Biology, Ames, TX, United States

Gibberellins are essential plant hormones that regulate growth and development. Besides plants, also phytopathogenic fungi and plant-associated bacteria also produce gibberellins. The recently elucidated bacterial pathway is organised in a single operon and shows an intriguing distribution in beneficial and pathogenic bacteria, longing to the  $\alpha$ -,  $\beta$ - and  $\gamma$ -proteobacteria. Their first key reaction during gibberellin biosynthesis is catalysed by cytochromes P450 in all three kingdoms. It is the ring contraction reaction from a 6-6-6-5 to a 6-5-6-5 ring system. While the unusual nature of this reaction has long been noted, its mechanistic basis has remained opaque. Building on identification of the relevant CYP114 from bacterial GA biosynthesis, detailed structure-function studies were performed, including development of in vitro assays as well as crystallographic analyses both in the absence and presence of substrate. These structures provided insight into enzymatic catalysis of this unusual reaction, as exemplified by identification of a key role for the “missing” acid from an otherwise highly conserved acid-alcohol pair of residues. Notably, the results demonstrate that ring contraction requires dual factors, both the use of a dedicated ferredoxin and absence of the otherwise conserved acidic residue, with exclusion of either limiting turnover to just the initiating and more straightforward hydroxylation. The results provide detailed insight into the enzymatic structure-function relationships underlying this fascinating reaction and support the use of a semipinacol mechanism for the unusual ring contraction reaction.

P 065

**Investigating a bHLH transcription factor as marker and regulator of saponin biosynthesis in *Chenopodium quinoa***

M. Kollmar<sup>1</sup>, S. Otterbach<sup>1</sup>, L. John<sup>1</sup>, S. Schmöckel<sup>1</sup>

<sup>1</sup>University of Hohenheim, Physiology of Yield Stability, Stuttgart, Germany

Quinoa (*Chenopodium quinoa* Willd.) has gained global attention as a promising crop for its significant nutritional qualities and adaptedness to diverse environmental conditions, such as soil salinity and drought. Quinoa’s ability to grow under these challenging conditions makes it an interesting breeding target and underlines its potential role in safeguarding global food security in the future. Quinoa accessions are considered “bitter” when bitter-tasting saponins are present in the pericarp of the seed or “sweet”, when saponins are low. Although these triterpenoid saponins might be beneficial for the plant, they must be removed before human consumption because of their haemolytic activity and bitter flavour. Since removing the saponins is a cost and water intensive process and reduces nutritional quality of the seeds, saponin-free quinoa accessions are a valuable breeding target. Previously, a SNP in the genetic region of the transcription factor TSARL1 has been suggested to be causal for the absence of saponins and, therefore, the sweet phenotype. Here, we investigate the role of TSARL1, a bHLH transcription factor, in saponin biosynthesis. We propose that alternative splicing of TSARL1 results in a non-functional protein and subsequently downregulates the entire MVA pathway responsible for saponin biosynthesis. We used qPCR to analyze the pattern of expression for saponin biosynthesis in three “bitter” and three “sweet” quinoa accessions. Furthermore, the influence of the abundance on germination time was tested in more than 150 quinoa accessions.

Understanding the molecular mechanisms and key players in saponin biosynthesis enables the development of a marker for breeders to efficiently screen for sweet quinoa accessions.

P 066

**Oil Body Lipase-type Triacylglycerol Lipases Contribute to the Production of Fatty Acid-derived Volatiles**

V. Lebedev<sup>1</sup>, M. Bonin<sup>1</sup>, P. Scholz<sup>2</sup>, A. Müller<sup>2</sup>, M. Hädrich<sup>2</sup>, J. Sendker<sup>3</sup>, D. Staiger<sup>4</sup>, T. Ischebeck<sup>1</sup>

<sup>1</sup>University of Münster, IBBP, Münster, Germany

<sup>2</sup>University of Göttingen, Plant Biochemistry, Göttingen, Germany

<sup>3</sup>University of Münster, IPBP, Münster, Germany

<sup>4</sup>University of Bielefeld, Faculty of Biology, Bielefeld, Germany

The plant lipidome is in constant flux and highly adaptable requiring not only synthesizing enzymes but also lipases for degradation. The *Arabidopsis* genome codes for a plethora of lipases, many of which have been shown or been predicted to act on the same substrate releasing the same product. While such a redundancy on the biochemical level might appear puzzling, lipases with similar enzymatic function could be differentially expressed leading to functions in different tissues or under different environmental or developmental conditions. Furthermore, also the subcellular localization and/or interaction with other enzymes could result in differential subcellular and physiological functions. Several plant triacylglycerol lipases have been described as for example SUGAR-DEPENDENT 1 that acts at the interface of peroxisomes and lipid droplets. This enzyme is important for seedling establishment as the released fatty acids are channeled into  $\beta$ -oxidation. Here, we investigated a five-member family of triacylglycerol lipases that localize to lipid droplets. Four of the five lipases from this OIL BODY LIPASE (OBL) family showed enzymatic activity against triacylglycerol and diacylglycerol. While we could not detect a function in triacylglycerol breakdown during seedling establishment or general glycerolipid metabolism, we could collect evidence that OBLs are involved in the production of fatty acid-derived volatiles. The levels of several volatiles were increased upon overexpression in *Nicotiana benthamiana* and *Arabidopsis*, while knockout lines in *Arabidopsis* displayed reduced levels. We could also show that several helices that constitute a hydrophobic face of the enzyme are involved in targeting it to the lipid droplet surface.

P 067

**Investigating plasticity trade-offs and the role of spliceosome factors in flower size plasticity to light and temperature**

G. Andreou<sup>1</sup>, J. Hoffmann<sup>1</sup>, R. Laitinen<sup>1</sup>, Z. Nikoloski<sup>2</sup>

<sup>1</sup>University of Helsinki, Organismal and Evolutionary Biology, Helsinki, Finland

<sup>2</sup>University of Potsdam, Institute of Biochemistry and Biology, Bioinformatics, Potsdam, Germany

The acceleration of increasing global temperatures is predicted to continue. Plants, as seemingly static organisms, must sense and rapidly adapt to this change. While adaptation through natural selection is slow, phenotypic plasticity can provide a way to adapt across an individual's lifespan. Understanding flower size plasticity is agriculturally relevant to stakeholders hoping to cultivate stable plants in unstable future conditions. To this, we have previously shown that flower size plasticity to temperature is shaped by additive inheritance. Flower size is a key trait that defines the reproductive strategy of plants and is therefore assumed to be linked with fitness. We are interested in describing yield stability and trade-offs with temperature-mediated flower size plasticity by using natural variation across global accessions of *Arabidopsis thaliana*. In a similar way, we have previously shown that flowers differ in size in response to temperature and described a known flowering-time alternatively spliced gene cluster to be involved in flower size plasticity.

Since alternative splicing is regulated by the spliceosome, we reasoned that altering spliceosomal factors may also influence the degree of flower size plasticity. In this project, we investigated 12 spliceosome-associated genes and looked at mutants for their light and temperature-mediated plasticity at two ambient temperatures, 17 and 25 °C, under low (45  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and normal light (180  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) conditions. By looking at the wild-type, we saw that the largest flower size decrease of -49 %, was in response to a combination of warmer temperature and lower light. This decrease in flower size was 10 % stronger than each environmental cue alone, suggesting that light and temperature-mediated plasticities may be independently regulated. In addition, we saw 2 different spliceosome-associated gene mutants lose their plastic ability to light only. A mutant line for a component of the U1 snRNP, the first protein recruited in the assembly of the spliceosome, showed similar size flowers across all growth conditions. This line begins to describe the uncoupling of flower size plasticity and other plant trait plasticities, as the same loss of rapid adjustment was not consistently seen across leaf and flowering time traits. Altogether, these results highlight the importance of alternative splicing and its complexity for the regulation of flower size plasticity in response to temperature and light.

P 068

The JA induced emission of volatiles in herbivore attacked maize is negatively influenced by a bHLH transcription factor *tf23*

C. Schaff<sup>1</sup>, B. Athmer<sup>1</sup>, J. Degenhardt<sup>1</sup>

<sup>1</sup>MLU Halle-Wittenberg, Institute of Pharmacy, Halle a. d. Saale, Germany

The production of volatile compounds by maize after herbivore attack is mostly mediated by the jasmonate pathway. Recently, the maize analog of Arabidopsis MYC2 was identified which seems to be the master regulator of jasmonic acid production. We aim to investigate downstream transcription factors leading to the production of volatile terpenes to fully understand the regulation of this defense mechanism.

We identified a transcription factor that is highly upregulated after maize plants were treated with mechanical damage and the application of oral secretion of *Spodoptera littoralis* larvae. This transcription factor, *tf23*, shows its highest expression already half an hour after treatment and starts to decline after one hour, implicating an early role in the herbivore defense pathway. To further investigate the functional role of this bHLH factor, we generated stable overexpression lines in maize.

The overexpression lines of *tf23* show a general trend of reduced volatile emission after herbivore induction, with a significant reduction of linalool, geranylacetate, and DMNT ((*E*)-3,8-dimethyl-1,4,7-nonatriene), indicating a negative role in the regulation of herbivore defense. In accordance with this result, *in silico* analysis revealed coexpression and putative functional interactions with several jasmonate ZIM domain proteins. In addition, RNA sequencing data of our transgenic maize lines showed a high expression of ZIM14, ZIM16, and ZIM30. On the other hand, we found a significant reduction of expression of a deoxyxylulose phosphase synthase which is responsible for the production of terpenoid precursors.

These results indicate that *tf23* is a negative regulator in the signaling transduction pathway. Further experiments with a protoplast transformation system will reveal the interactions with the predicted ZIM proteins.

P 069

Post-transcriptional RNA modification (m6A) influences the hormone-mediated drought stress response in *Arabidopsis thaliana*

Y. Shoaib<sup>1</sup>, F. Chigri<sup>1</sup>, U. Vothknecht<sup>1</sup>

<sup>1</sup>University of Bonn, Biology, Bonn, Germany

Drought is considered as one of the foremost abiotic stresses that negatively affects plant's growth and development. On the molecular level, plants respond to drought stress e.g. by reprogramming transcription, post-transcriptional modifications, cellular metabolism and hormone signalling (Gupta *et al.*, 2020). Phytohormones such as salicylic acid (SA), abscisic acid (ABA) and jasmonic acid (JA) regulate a wide range of functions like growth, development and responses to environmental stresses. Moreover, post-transcriptional RNA modification (m6A) regulate a variety of developmental, stress responsive and hormonal responses in plants (Shoaib *et al.*, 2021).

In this study, the relative expression level of a m6A-regulatory drought responsive gene (*m6A-DRG*) was analysed under drought stress in *Arabidopsis thaliana* wild-type (Col-0) and in mutant lines for the JA and ABA-biosynthetic pathways through quantitative real-time PCR. The results showed that the relative expression level of *m6A-DRG* increased substantially under progressive drought stress in Col-0. Interestingly, in the JA-biosynthetic mutants (*aos1-1*, *lox6* & *jar1-11*), the expression level of *m6A-DRG* increased even more than in Col-0, while it remained unchanged in the *aba2-1* mutant, indicating that ABA is involved in the up-regulation under drought stress. Moreover, the regulation of expression of the *m6A-DRG* was investigated in 21-days-old Col-0 plants treated with exogenous ABA, JA-Ile and SA. The results showed that *m6A-DRG* was positively regulated by ABA and negatively regulated by SA, however, JA-Ile did not affect the expression level. However, JA-Ile inhibited the ABA-induced up-regulation of *m6A-DRG*. These results were confirmed by investigating the *m6A-DRG*-promoter regulation using luciferase luminescence. Additionally, phenotyping of *m6A-DRG* mutant and over-expression lines under drought stress showed that the knockout mutant is more sensitive to drought stress than the wild type, while over-expression lines better withstood the stress by regulating their stomatal aperture.

References

- Gupta, A., Rico-Medina, A., & Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. *Science*, 368(6488), 266-269
- Shoaib, Y., Hu, J., Manduzio, S., & Kang, H. (2021). Alpha-ketoglutarate-dependent dioxygenase homolog 10B, an N6-methyladenosine mRNA demethylase, plays a role in salt stress and abscisic acid responses in *Arabidopsis thaliana*. *Physiologia Plantarum*, 173(3), 1078-1089.

P 070

## Unraveling Maize's Defensive Arsenal: Insights into the Regulation of Volatile Terpene Biosynthesis

J. Telleria Marloth<sup>1</sup>, A. Richter<sup>2</sup>, C. Schaff<sup>1</sup>, J. Degenhardt<sup>1</sup><sup>1</sup>Martin-Luther-University Halle-Wittenberg, Department of Pharmacy, Halle a. d. Saale, Germany<sup>2</sup>Boyce Thompson Institute, Ithaca, NY, United States

Maize (*Zea mays*) is an important crop with a production exceeding 1.1 billion tons in 2022 worldwide (source: fao.org). Besides its use as food for humans and livestock, maize is a key biofuel source. Insect herbivores pose a major threat to maize production, and their attacks trigger various defense mechanisms, including the formation of terpenes. These terpenes are synthesized rapidly and contribute significantly to the plant's defense strategy. The mostly volatile compounds can either deter herbivores directly or attract natural predators of the herbivores, resulting in an indirect defense of the plant. The biosynthesis is regulated by complex multi-step signaling pathways, with the jasmonic acid signaling pathway, which is activated upon herbivore damage, being the most important. While maize has long served as a model organism to study plant defense responses against herbivores and fungi, many aspects of the regulation of terpene biosynthesis remain unknown. Since historical times, selective breeding has cultivated a vast genetic diversity within maize. The Nested Association Mapping 5 (NAM5) population is comprised of 26 maize lines, which represent about 85% of the overall genetic diversity. This population was used to screen Quantitative Trait Loci (QTLs) related to volatile terpene biosynthesis. The inbred line Ky21 was of particular interest since it only produces minimal amounts of terpenes after simulation of herbivore attack with jasmonic acid-mimic elicitors. A Genome-Wide Association Study (GWAS) pinpointed a QTL near a *basic-helix-loop-helix transcription factor*. Further investigation of this gene in Ky21 revealed a deletion in the coding sequence, resulting in the deletion of 41 aa in the N-terminal regulatory domain. Given that this class of transcription factors forms multimers, we used the putative interaction database STRING (<https://string-db.org>) to find potential interaction partners. Since an initial split-ubiquitin yeast-two-hybrid assay resulted in auto-activation, we established an expression system in etiolated maize protoplasts. This homologous expression system was used for bimolecular fluorescence complementation to test the interaction of the targets. Here, we present the localization of a maize bHLH transcription factor and its potential interaction partners to elucidate its role as a potential master regulator of terpene biosynthesis in maize.

P 071

## Peculiar evolution of an ancient microRNA presumably regulating class B floral homeotic genes

L. Gramzow<sup>1</sup>, C. Gafert<sup>1</sup>, G. Theißen<sup>1</sup><sup>1</sup>Matthias Schleiden Institute / Friedrich Schiller University Jena, Genetics, Jena, Germany

microRNAs (miRNAs) are small, non-coding RNAs that usually negatively regulate gene expression. A number of miRNAs have been shown to control developmental processes and their evolution is tightly linked to morphological innovations. Here, we study a miRNA, termed miR5179, that is likely involved in flower development. We elucidate that the *MIR5179* gene originated in the lineage that led to extant flowering plants (angiosperms) after extant gymnosperms branched off. Furthermore, we demonstrate that *MIR5179* has been conserved in some lineages of angiosperms for about 150 million years but has been lost independently in numerous other lineages. In all species where *MIR5179* has been conserved, class B floral organ identity genes are predicted as targets while in some species, miR5179 may also target other genes. The likely regulation of flower development genes by miR5179 is also substantiated by high conservation of the target site in flower development genes of species with *MIR5179* as compared to those in species without *MIR5179*. Hence, in some species, miR5179 seems to be of great importance as judged by the conservation of the miRNA itself and its target site. In contrast, miR5179 was dispensable in other lineages such that its gene has been lost. To understand this highly peculiar evolutionary pattern, the ultimate function of miR5179 needs to be elucidated. We provide an outlook on how we aim to achieve this goal by generating a knock-out of *MIR5179* using the CRISPR-Cas9 technique in the model grass species *Brachypodium distachyon*.

P 072

Whirly proteins regulating stress-related reprogramming of nuclear gene expression via epigenetic control

I. Mladenovic<sup>1</sup>, M. Bui Manh<sup>1</sup>, K. Humbeck<sup>1</sup>, L. Thuy Nguyen<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle-Wittenberg, Institut Biologicum, Pflanzenphysiologie, Halle a. d. Saale, Germany

Whirly proteins (WHY) are plant DNA-binding proteins which are synthesized in the cytosol and then delivered to the plastids and mitochondria. However, there is evidence that the WHY proteins can move between compartments in response to triggers from the environment. The nuclear form of WHY1 binds to specific promoter sequences of genes which are linked to the stress response and senescence [1]. WHY1 is involved in the regulation of stress responses and can act as an activator or repressor of nuclear genes. However, its function depends on interactions with other transcription factors such as those from the WRKY family or with epigenetic regulators, e.g. histone deacetylases (HD). It is also shown that WHY1 alters chromatin structure during leaf senescence [4]. From the previous work it is proved that gain- and loss-of-function of WHY1 affect ABA synthesis and signaling: mutants of *Arabidopsis thaliana* lacking WHY1 have a reduced sensitivity toward salicylic acid (SA) and abscisic acid (ABA) during germination [3]. Recently it is shown that WHY1 suppresses expression of ABA-related genes and ABA accumulation in barley [4]. Regarding all of that, WHY1 may play an important role in epigenomic reprogramming during stress and development.

References

- [1] Huang, et al. (2018) *Front. Plant Sci.* 9: 1503 ;
- [2] Humbeck, Klaus. (2013) *Plant Mol. Biol.* 82: 529-537;
- [3] Isemer, Rena, et al. *Front. Plant Sci.* 3: 283;
- [4] Manh, Minh Bui, et al. *Int. J. Mol. Sci.* 24.7: 6326;

P 073

Redox signaling to chromatin during stress responses in plants

M. Sharma<sup>1,2</sup>, M. Wrzaczek<sup>1,2</sup>, P. Kerchev<sup>3</sup>

<sup>1</sup>Biology Centre CAS, Plant Molecular Signaling, České Budějovice, Czech Republic

<sup>2</sup>University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic

<sup>3</sup>Mendel University in Brno, Brno, Czech Republic

GENERAL CONTROL NON-REPRESSIBLE 5 (GCN5) is a subunit of the evolutionary conserved Spt-Ada-Gcn5-acetyltransferase (SAGA) complex, which is involved in acetylation of lysine residue on histone H3. GCN5 regulates the expression of genes involved in development, and abiotic and biotic stress responses. In addition, GCN5 plays a crucial role in cell wall synthesis, including lignin deposition, which is linked to the response to salt stress. However, little is known about the molecular mechanism of the regulation of GCN5 activity in response salt stress. Peroxidases are important members of enzymatic antioxidant defense machinery, which regulate the redox balance of the cell and thereby affect the oxidation of phenolic compounds promoting the formation of lignin. We analysed the expression of peroxidases (PRX) under salt stress condition using qRT-PCR approach. We have found PRX71 and PRX33 to be potential candidates for further investigation of their roles under salt stress conditions. In our study, we explore the role of GCN5 mediated regulation of lignin deposition via the regulation of *PRX* genes under salt stress.

P 074

## Regulation of betalain biosynthesis by a MYB transcription factor

S. K. Vollmer<sup>1</sup>, T. S. Winkler<sup>2</sup>, G. Hensel<sup>1</sup>, M. Stetter<sup>2</sup><sup>1</sup>Heinrich Heine University Düsseldorf, Institute of Plant Biochemistry, CPGE, Düsseldorf, Germany<sup>2</sup>University of Cologne, Institute for Plant Sciences, Köln, Germany

Specialized metabolites fulfill important functions in plants, including pollinator attraction, stress response and defense. The respective biosynthetic pathways have to be tightly regulated to ensure their effectiveness. Hence, new pathways need to be integrated into a regulatory network. Betalains are specialized metabolites that have likely replaced anthocyanins in numerous species of the *Caryophyllales*. Intriguingly, no species producing both of these metabolites has been identified. The introduction of betalains and their replacement of the evolutionary-older and biochemically independent anthocyanins, allows studying the regulatory integration of novel metabolic pathways.

We study the genetic basis and regulation of betalain pigments in *Amaranthus hypochondriacus*, a betalain-producing crop with high quality genomic resources available. Genetic mapping of inflorescence color revealed a MYB transcription factor, AhMYB2. The transcription factor is a close ortholog to the anthocyanin-controlling MYB transcription factors, while anthocyanins cannot be produced in amaranth. Transcriptomic analysis and cloning of the gene revealed two splice variants of the gene (*AhMYB2.1* and *AhMYB2.2*). To investigate the function of AhMYB2, we implemented hairy root transformation for this species. Overexpression of *AhMYB2.1-GFP* but not *AhMYB2.2-GFP* led to intense red coloration of the transgenic hairy roots. This finding shows the positive regulation of the betalain production by AhMYB2.1 in grain amaranth. In addition, it has previously been shown that betalain biosynthesis is influenced by light quality and phytohormone concentration. *AhMYB2.1-GFP* overexpression in amaranth calli demonstrates that AhMY2.1 can activate the betalain pathway independent of light and phytohormone concentration. Together, these results suggest the downstream regulation of betalains by AhMYB2.1. To further investigate the regulatory control of betalains by MYB transcription factors, we study the potential direct binding of the transcription factor to the promoters of betalain enzyme genes. To regulate anthocyanins, MYB transcription factors require the interaction with two additional transcription factors, but the regulation of betalains remains unclear.

Our finding can help to understand how the new metabolites integrate into regulatory networks and how this contributed to the mutual exclusive nature of betalains and anthocyanins.

P 075

## Telescripting in Plants: A Novel Mechanism Ensuring Complete mRNA Transcription

L. Nagel<sup>1</sup><sup>1</sup>Martin-Luther Universität Halle-Wittenberg, Halle a. d. Saale, Germany

Gene expression is a tightly regulated mechanism in eukaryotes. The process from pre-mRNA to mature mRNA to translation, has to undergo several checkpoints, including splicing and mRNA cleavage followed by polyadenylation.

The U1 snRNP complex, consisting of an snRNA, an Sm-ring, and three core proteins, initiates mRNA splicing by recognizing the 5' splice site (5'SS). Studies in metazoan systems have shown that U1 snRNP has splicing-independent function: Binding 5'SS sites and association with mRNA cleavage and polyadenylation factors prevents premature cleavage and polyadenylation on alternative polyadenylation (APA) sites in intron. This enables full-length mRNA transcription; – a process called “telescripting”.

While U1 snRNP is highly conserved in eukaryotes, its functions in plant systems are not well understood. We recently found that telescripting also occurs in the model plant *Arabidopsis thaliana*, but very little is known about *cis*- and *trans*-acting factors affecting Telescripting in plants. This research aims to identify the *cis* elements necessary for preferred PAS utilization and investigates the role of telescripting in response to environmental stress. Understanding how environmental conditions affect intronic APA usage will provide valuable insights into co-transcriptional mRNA processing in plants.

P 076

Activating *cis*-regulatory elements from the histone genes of green algae and angiosperms: from characterization to application

Y. Lihanova<sup>1</sup>, R. Nagel<sup>1</sup>, T. Jakob<sup>1</sup>, S. Sasso<sup>1</sup>

<sup>1</sup>Leipzig University, Plant Physiology, Leipzig, Germany

In the past decade, our understanding of gene regulation in eukaryotes has been considerably enriched by *cis*-regulatory elements (CREs), which are non-coding transcription factor-binding DNA motifs. CREs are often assembled into *cis*-regulatory modules, CRMs, and are capable of activating or downregulating gene expression on the transcriptional level (Schmitz et al., *Plant Cell* 34, 718–741 (2022); Marand et al., *Annu. Rev. Plant Biol.* 74, 111-137 (2023)). Despite this background knowledge on CREs, their activity has not been studied in detail. Therefore, we set out to characterize the activating CREs from the histone genes of green algae and angiosperms in the genetic background of the well-studied algal model species *Chlamydomonas reinhardtii* (Lihanova et al., *Plant J.*, accepted manuscript, DOI: 10.1111/tpj.16781). Towards this end, we established a reporter assay and quantified the activity of four candidate elements using flow cytometry. Two candidates significantly upregulated the expression of a fluorescent reporter and were characterized in detail. The first CRE, called  $E_{upstr}^1$  originates from highly expressed genes of *C. reinhardtii*, including a histone gene variant, and activated two native promoters in an orientation-independent manner. The second candidate,  $E_{hist\ cons}^1$  is a CRM from the histone genes of angiosperms, which activated the  $\beta$ 2-tubulin basal promoter in *C. reinhardtii* over a distance of at least 1.5 kb. The octamer motif from this CRM was identified in the histone genes of *C. reinhardtii* and related green algae, demonstrating its high evolutionary conservation among different phyla of the plant kingdom. The thorough characterization of two active CREs in our study opens new possibilities for efficient gene expression in green algae and beyond. Our current research is focused on utilizing CREs in a new strategy to study gene function in green algae, which may become a viable alternative to conventional gene knock-out approaches.

P 077

The Arabidopsis Rna-processing Factor Serrate is Involved in Polyadenylation and U1-mediated Telescripting

M. Adler<sup>1</sup>

<sup>1</sup>Biology, General Genetics, Halle a. d. Saale, Germany

The Arabidopsis multifunctional adapter protein SERRATE (SE), plays a crucial role in various RNA maturation processes and thus regulates gene expression on different levels, including miRNA processing, mRNA splicing, direct transcription regulation of distinct intronless genes and RNA turnover through degradation. The homologous protein in metazoans, ARS2, is further known for its function in mRNA export and 3'-end formation. Within this functional framework, we provide evidence that SE is involved in polyadenylation and U1-mediated repression of premature cleavage and polyadenylation (PCPA) events, a process known as telescripting.

During this mechanism, U1 aggregates with core components of the cleavage and polyadenylation complex which, inter alia, includes CPSF-73-I, FY and CFIm68, as proposed recently by our lab (1). In here, we also provided evidence that SE is one of the U1 interactors. More recently, the mentioned polyadenylation factors were found to be interacting with SE which provides initial insights into its involvement regarding the polyadenylation process. Furthermore, Nanopore-seq revealed that specific target genes in SE-deficient mutants tend to be PCPAed, resembling the profile of u1-knockdown mutants, which may indicate a supportive role of SE in U1-mediated telescripting. Through comparative analysis of the relative expression of distinct transcription regions upstream or downstream of a selected polyadenylation site, prone to be the target of premature cleavage, we demonstrated that SE indeed has an effect in regulating PCPA events at particular loci.

We aim to establish SE as an important regulatory element in 3'-end processing and expect to complement the existing results with a future BRB-seq approach, in which we compare polyadenylation defects in hypomorphic *se* mutants and selected polyadenylation factor mutant lines. Our work will solidify SE's role as a central hub in the regulation of gene expression at multiple levels and extend its already diverse functional portfolio to an additional layer of regulation. Understanding this regulatory key element will complete our knowledge of the molecular mechanisms underlying plant adaptability.

References

1: Mangilet, A. et al. The Arabidopsis U1 snRNP regulates mRNA 3'-end processing. *bioRxiv*2023.09.19.558503, doi: 10.1101/2023.09.19.558503(2023).

P 078

**Understanding mRNA half-lives and turnover dynamics in plants**

S. Schüler<sup>1</sup>, J. Weber<sup>1</sup>, C. Schmitz-Linneweber<sup>2</sup>, S. Laubinger<sup>1</sup>

<sup>1</sup>Martin-Luther-University Halle-Wittenberg, General Genetics, Halle a. d. Saale, Germany

<sup>2</sup>Humboldt-University Berlin, Molecular Genetics, Berlin, Germany

Understanding RNA dynamics, particularly mRNA stability, is fundamental to understand gene expression regulation and cellular homeostasis in plants and may have implications for improving crop resilience and agricultural productivity. By regulating the abundance of mRNA molecules, mRNA stability influences various cellular processes, including development, stress responses, and post-transcriptional gene regulation, impacting plant growth, adaptation, and productivity in diverse environments. Our study aims to elucidate mRNA stability variations across different *Arabidopsis* accessions, mutants and stress conditions and identify the regulatory mechanisms involved. We employed the 5-EU immunoprecipitation chase (ERIC)-seq technique, an RNA pulse-chase labelling approach. This method involves incorporating 5-ethynyluridine (5-EU) into newly synthesized RNAs (pulse), followed by a chase period to track the depletion of the 5EU to infer mRNA stability. Preliminary ERIC-seq results reveal variations in mRNA stability among transcripts in different accession, suggesting that RNA stability can contribute to accession-specific gene expression. Future work will expand this analysis to include stress conditions and mutants, aiming to unravel upstream regulatory factors and further elucidate the mechanisms governing RNA stability.

References

Emese Xochitl Szabo, Philipp Reichert, Marie-Kristin Lehniger, Marilena Ohmer, Marcella de Francisco Amorim, Udo Gowik, Christian Schmitz-Linneweber, Sascha Laubinger, Metabolic Labeling of RNAs Uncovers Hidden Features and Dynamics of the *Arabidopsis* Transcriptome, *The Plant Cell*, Volume 32, Issue 4, April 2020, Pages 871–887, <https://doi.org/10.1105/tpc.19.00214>

P 079

***In vitro* reconstitution of the cytosolic redox regulatory network revealed dynamics of H<sub>2</sub>O<sub>2</sub> detoxification**

L. Vogelsang<sup>1</sup>, J. Eirich<sup>2</sup>, I. Finkemeier<sup>2</sup>, K. J. Dietz<sup>1</sup>

<sup>1</sup>University Bielefeld, Plant Biochemistry and Physiology, Bielefeld, Germany

<sup>2</sup>University of Münster, Plant Physiology, Münster, Germany

Reactive oxygen species (ROS) participate in signaling and most organelles contribute to the cellular H<sub>2</sub>O<sub>2</sub> load in a stress specific manner, e.g. chloroplasts under high light conditions or upon pathogen infection, the endoplasmic reticulum with increased demand for protein folding, mitochondria under hypoxia and subsequent re-oxygenation, or peroxisomes during photorespiration. From all these sources, H<sub>2</sub>O<sub>2</sub> reaches the cytosol where the information becomes integrated and modulated. The cytosol has been considered to be just a reducing environment with high ROS-detoxification capacity due to the high abundance of the non-enzymatic antioxidants glutathione (GSH) and ascorbate (ASC) and the presence of enzymatic antioxidants. The thiol peroxidases peroxiredoxins (PRXII, C, and D) and glutathione peroxidase-like (GPXL2 and 8) and the high number of cytosolic transmitters of the glutaredoxin (GRX) and thioredoxin (TRX) families constitute a redox network of high complexity in the cytosol.

As a novel synthetic approach for dynamic analyses, this cytosolic redox network was reconstituted with recombinant proteins according to their cellular abundance and substituted with the H<sub>2</sub>O<sub>2</sub> sensor roGFP-Orp1 or the [GSH]<sup>2</sup>/[GSSG]-redox sensor GRX1-roGFP2 in order to monitor changes in the H<sub>2</sub>O<sub>2</sub> and glutathione pools. The redox-regulated enzymes malate dehydrogenase 1 (MDH1) and glyceraldehyde-3-phosphate dehydrogenase (GAPC2) were selected as targets and the network was supplemented with NADPH and GSH. Addition of H<sub>2</sub>O<sub>2</sub> resulted in transient changes of the GSH redox state and detoxification of H<sub>2</sub>O<sub>2</sub> within minutes, demonstrating the functionality of the reconstituted network. Omitting single members or entire functional groups revealed that PRXs contribute more to detoxification than GPXLs. Mass spectrometry data showed that the loss of PRX even resulted in an altered transient redox state of GPXL2, GPXL8 and GRXC1 after H<sub>2</sub>O<sub>2</sub>-addition. Both targets MDH1 and GAPC2 interacted with cytosolic thiol peroxidases *in vivo* as demonstrated by FRET-measurements, and GAPC2 activity was protected against oxidation by 100 μM H<sub>2</sub>O<sub>2</sub> in the presence of the network. The obtained results advance our functional understanding of the cytosolic redox network and provide deeper insights into the redox interactions within the cytosol. The innovative network reconstitution opens new possibilities to address the regulation of novel targets in a controlled environment, mimicking the cytosol.



P 080

Enhanced Plant Growth and Stress Tolerance via miR156a Overexpression from chickpea:  
Insights into miR156-SPL Regulatory Network in Arabidopsis

G. Kalwan<sup>1</sup>

<sup>1</sup>National Institute for Plant Biotechnology, Molecular Biology and Biotechnology, Delhi, India

The interaction between miR156 and the SPL gene family is pivotal in plant biology, particularly in developmental processes and growth regulation. miR156, a microRNA in plants, targets mRNA transcripts of the SPL gene family, which includes transcription factors governing critical aspects of plant development like phase transitions, leaf initiation, flowering, and fruit ripening. miR156 binds to SPL gene transcripts, reducing their expression either through degradation or translational inhibition. In this work, we generated overexpressed transgenic lines of chickpea mir156 (CamiR156a) in Arabidopsis. CamiR156a at higher levels and we observed the huge differences in transgenic and wild plants changes at various stages. As we know mir156 is also well known for its role in different stresses, hence we also studied these transgenic lines under drought, salinity. Under these stress conditions transgenic plants were showing tolerance in comparison to wild type Arabidopsis thaliana (Col0). Under drought and salinity stress, the transgenic Arabidopsis seedlings developed longer main roots than the natural type. Furthermore, under these stresses, the Arabidopsis lines overexpressing CamiR156a showed superior growth compared to the wild type plants confirmed by biochemical and molecular analysis. They also showed unique developmental traits such dwarfism, prolonged vegetative growth period, increased shoot branching, and shorter siliques, in addition to producing more seeds. Importantly it is well known various SPL genes were found to involved in various roles in plant development, hence we also validated the CamiR156 targeted SPL genes and most of the SPL gene were down regulate in transgenic lines as compare to wild, which may result in significant phenotypic differences. The miR156-SPL module is evolutionarily conserved and has significant agricultural implications, offering potential for crop improvement by manipulating flowering time, yield, and stress tolerance in chickpea. Understanding this regulatory network is key to advancing plant biology and agricultural practices.

P 081

Characterization of the plastidial acetyltransferase GNAT3 in *Arabidopsis thaliana*

M. C. Stenkamp<sup>1</sup>, F. Bösing<sup>1</sup>, A. Brünje<sup>1</sup>, J. Mühlenbeck<sup>1</sup>, I. Finkemeier<sup>1</sup>

<sup>1</sup>University of Münster, Münster, Germany

Chloroplast acetyltransferases in *Arabidopsis thaliana* are a part of the *General control nonrepressible 5-related N-Acetyltransferase superfamily* (GNAT), which is characterized by a high structural conservation. Among the acetylated chloroplast proteins, those involved in photosynthesis make up a large proportion, indicating that the GNATs may be important regulators of photosynthesis (Hartl et al. 2017). All chloroplast GNATs show dual N-terminal as well as lysine acetylation activity to a different extent. When expressed heterologously, 2 of the 8 plastidial acetyltransferases show more relaxed acetylation activities compared to the remaining six, pointing to a putative redundancy in their function or to the dependency of these proteins on complex partners (Bienvenut et al. 2020). Previous research already highlighted the tendency of GNAT proteins to form complexes critical for their function in diverse organelles (Liszcak et al. 2013). Our research focusses on the plastidial acetyltransferase GNAT3, which showed reduced substrate specificity when expressed heterologously. GNAT3 exists in seven different splice forms and the goal of our research is to shed light on the specific functions of these by investigating whether these splice forms show altered localisation or acetylation activities. Furthermore, we want to examine the physiological role of the acetyltransferase and identify its putative interaction partners *in vivo*.

References

- Bienvenut, Willy V.; Brünje, Annika; Boyer, Jean-Baptiste; Mühlenbeck, Jens S.; Bernal, Gautier; Lassowskat, Ines et al. (2020): Dual lysine and N-terminal acetyltransferases reveal the complexity underpinning protein acetylation. In: *Molecular systems biology* 16 (7), e9464. DOI: 10.15252/msb.20209464.
- Hartl, Markus; Füßl, Magdalena; Boersema, Paul J.; Jost, Jan-Oliver; Kramer, Katharina; Bakirbas, Ahmet et al. (2017): Lysine acetylation profiling uncovers novel histone deacetylase substrate proteins in Arabidopsis. In: *Molecular systems biology* 13 (10), S. 949. DOI: 10.15252/msb.20177819.
- Liszcak, Glen; Goldberg, Jacob M.; Foy, Håvard; Petersson, E. James; Arnesen, Thomas; Marmorstein, Ronen (2013): Molecular basis for N-terminal acetylation by the heterodimeric NatA complex. In: *Nature structural & molecular biology* 20 (9), S. 1098–1105. DOI: 10.1038/nsmb.2636.

P 082

Sugar-dependent regulation of the flavonoid biosynthesis during high light acclimation: Interplay between SnRK1 and PAP1

J. Dieckmann<sup>1</sup>, R. Stracke<sup>2</sup>, A. S. Richter<sup>1</sup>

<sup>1</sup>Universität Rostock, Pflanzliche Stoffwechselphysiologie, Rostock, Germany

<sup>2</sup>Universität Bielefeld, Genetik und Genomik der Pflanzen, Bielefeld, Germany

Flavonoid biosynthesis produces a variety of specialized plant metabolites with diverse functions. One class of end products, the anthocyanins, play a role in attracting pollinators, defending against pathogens and herbivores, reducing free radicals and shielding against UV and visible light. Their carbon-intensive biosynthesis is regulated by abiotic factors and depends on the stadium of development. The genes for flavonoid biosynthesis are grouped into the early (EBG) and late biosynthetic genes (LBG), the latter of which are essential for the biosynthesis of anthocyanins. The expression of LBG is controlled by the MYB-bHLH-TTG1 transcription factor complex (MYB-bHLH-WD40).

We previously found that the SNF1-RELATED PROTEIN KINASE 1 (SnRK1), which integrates stress and energy signals, acts as a repressor of LBG and inactivation of SnRK1 is essential to permit high light-induced anthocyanin biosynthesis <sup>1</sup>.

However, it remained open how SnRK1 participates in the regulation of MBW complex components and the LBG. Here, we show that overexpression of *PAP1*, a dominant MYB component of the MBW complex, rescues low anthocyanin accumulation of *Arabidopsis thaliana* mutants overexpressing the catalytic SnRK1 subunit (*KIN10*). Parallel overexpression of *KIN10* and *PAP1* results in the derepression of LBG and higher anthocyanin contents compared to *KIN10ox* alone and wild type in high light. Our results provide genetic evidence and suggest that SnRK1 regulates the anthocyanin biosynthesis upstream of *PAP1* during high light acclimation.

A time-resolved expression analysis showed that *MYB LIKE 2* (*MYBL2*), a repressor of anthocyanin biosynthesis, is constitutively repressed during high light exposure which requires inactivation of SnRK1. Previously, it was shown that *MYBL2* is a direct or indirect transcriptional target of SnRK1 <sup>2</sup> and we propose that, in addition to MBW components, *MYBL2* functions downstream of SnRK1 to inactivate anthocyanin biosynthesis when not needed.

References

<sup>1</sup> Zirngibl, M. E., et al., 2023 (Plant Communications, 4: 100423)

<sup>2</sup> Broucke, E., et al., 2023 (The Plant Journal, 115: 1193-1213)

P 083

Characterizing the role of the transamidosome complex to understand the biological relevance of the indirect Glu-tRNA<sup>Gln</sup> synthesis pathway in plants

S. Schwartz<sup>1</sup>, B. Brandt<sup>1</sup>, F. Hagn<sup>2</sup>, R. Janowski<sup>3</sup>, H. H. Kunz<sup>1</sup>

<sup>1</sup>LMU Munich, Biology I, Planegg-Martinsried, Germany

<sup>2</sup>Technical University Munich, Department of Bioscience, München, Germany

<sup>3</sup>Helmholtz-University Group, Institute of Structural Biology, München, Germany

Protein translation is a pillar of cellular life. Hence, amino acid incorporation into the nascent peptide chain must proceed with high a degree of accuracy. Despite multiple translational control mechanisms, misincorporation of amino acids can occur due to tRNA mis-acylation and mis-decoding. Generally, mis-acylation is avoided by specific aminoacyl-tRNA synthetases (RS) which precisely load tRNAs with their cognate substrate amino acids. However, glutamine (Gln) presents an exception to this rule. Chloroplasts, mitochondria, and many prokaryotes lack a Gln-specific tRNA synthetase. Here, Gln-tRNA<sup>Gln</sup> is synthesised by an indirect pathway: First Glutamate is attached to the tRNA<sup>Gln</sup>. Subsequently, the resulting Glu-tRNA<sup>Gln</sup> is trans-amidated to Gln. This amidation reaction is catalysed by the AdT complex. In prokaryotes, this indirect pathway is predominantly taking place in a large oligomeric complex containing GluRS, tRNA and AdT, the so-called trans-amidosome [1]. However, the importance of this indirect Gln-tRNA<sup>Gln</sup> synthesis pathway and biochemical properties of the protein complex in plant cells is unknown.

Our findings indicate that the loss of any proteins composing the transamidosome complex is lethal while their impaired function increases mistranslation rates in the chloroplast. Furthermore, plant plastids exhibit a high tolerance for proteome plasticity, compensating for mistranslated and malfunctioning proteins unlike yeast and animal mitochondria [2]. Moreover, preliminary data suggest that a stable transamidosome complex does not form in chloroplasts, instead proteins comprising this complex interact separately with the tRNA (Gln). The importance of this pathway and its plant-specific nuances are unclear at this point but may have had significant implications during the second endosymbiotic event which gave rise to the chloroplast.

References

1. Bailly, M., et al., *The Transamidosome: A Dynamic Ribonucleoprotein Particle Dedicated to Prokaryotic tRNA-Dependent Asparagine Biosynthesis*. Molecular Cell, 2007. 28(2): p. 228-239.

2. Echevarría, L., et al., *Glutamyl-tRNA<sup>Gln</sup> amidotransferase is essential for mammalian mitochondrial translation in vivo*. Biochemical Journal, 2014. 460(1): p. 91-101.

P 084

Transcriptomic dissection of the H<sub>2</sub>O<sub>2</sub>-Ca<sup>2+</sup> crosstalk in the leaves and roots of barley  
 S. Bhattacharyya<sup>1</sup>, B. Meier<sup>2</sup>, C. Bleker<sup>3</sup>, K. Gruden<sup>3</sup>, E. Peiter<sup>2</sup>, U. Vothknecht<sup>1</sup>, F. Chigri<sup>1</sup>

<sup>1</sup>Institute for Cellular and Molecular Botany (IZMB), Plant Cell Biology, Bonn, Germany

<sup>2</sup>Institute for Agricultural and Nutritional Sciences, Halle a. d. Saale, Germany

<sup>3</sup>National Institute of Biology, Department of Biotechnology and Systems Biology, Ljubljana, Slovenia

ROS and Ca<sup>2+</sup> are important components of signal transduction cascades involved in plant acclimation to changing environmental conditions. H<sub>2</sub>O<sub>2</sub> is a ROS generated in the cell in response to various stimuli and has also been shown to induce characteristic Ca<sup>2+</sup> signatures in plants. However, the molecular connection between Ca<sup>2+</sup> signaling and H<sub>2</sub>O<sub>2</sub> responses in all its facets is not well understood, especially in grain crops like barley. To identify Ca<sup>2+</sup>-dependent H<sub>2</sub>O<sub>2</sub>-responsive genes in leaves and roots of barley we analyzed the transcriptional responses to H<sub>2</sub>O<sub>2</sub> treatment under conditions that inhibited the formation of cytosolic Ca<sup>2+</sup> transients using the plasma membrane Ca<sup>2+</sup> channel blocker LaCl<sub>3</sub>. Application of H<sub>2</sub>O<sub>2</sub> alone resulted in a total of 4246 differentially expressed genes (DEGs). By comparing the expression of these genes between H<sub>2</sub>O<sub>2</sub> and LaCl<sub>3</sub>+H<sub>2</sub>O<sub>2</sub> treatment, followed by data mining, we obtained in total 331 Ca<sup>2+</sup>-dependent H<sub>2</sub>O<sub>2</sub>-responsive genes in leaves and 1321 in roots, which could be further separated into five and four clusters, respectively. Furthermore, using the compiled Stress Knowledge Network ([www.skm.nib.si](http://www.skm.nib.si)), we were able to identify a small number of transcription factors as the potential nodal points of this important crosstalk between Ca<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> in leaves and roots of barley.

P 085

Computational analysis of protein interactions during the photosystem II assembly

M. Lange<sup>1</sup>, U. Armbruster<sup>1</sup>, T. Rudack<sup>2</sup>

<sup>1</sup>Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

<sup>2</sup>Ruhr-Universität Bochum, Biophysik, Bochum, Germany

Photosynthesis supports life on Earth through the fixation of carbon dioxide into carbohydrates and release of molecular oxygen. The photosynthetic process is initiated at Photosystem II (PSII), a large enzyme complex consisting of proteins, lipids and pigments. This complex uses light energy to split water and start the photosynthetic electron transport chain by reducing plastoquinone. The assembly of PSII into the thylakoid membrane requires several proteins and assembly factors. The first key intermediate is the Reaction Center (RC) complex consisting mainly of chlorophyll-binding proteins D1 and D2. In *Arabidopsis thaliana*, it was shown that the two proteins PAM68 (Photosynthesis Affected Mutant 68) and DEAP2 (Decreased Electron Transport At PSII) are required to accelerate the transition from the RC to the next assembly intermediate RC47 (RC + CP47). If plants lack both PAM68 and DEAP2, they are unable to make a functional PSII. How PAM68 and DEAP2 facilitate the assembly of the RC47 is not yet fully understood. To discover the molecular mechanism of these two assembly factors, we predicted structural models of RC and RC47 together with PAM68 and DEAP2 using AlphaFold. We refined the obtained structural models with molecular dynamics simulations, and analyzed the dynamic inter subunit contact interaction pattern. The results suggest that both proteins bind the CP47 protein at different sites. While the CP47 interaction with DEAP2 remains in the RC47 intermediate, the PAM68 interaction is disrupted, suggesting that PAM68 gets displaced during this assembly step.

P 086

Natural variation in gene regulatory networks

A. Korte<sup>1</sup>

<sup>1</sup>JMU Würzburg, Würzburg, Germany

Understanding the causal relationship between genotype and phenotype is a primary goal in biology. Statistical methods such as Genome-Wide Association Studies (GWAS) enable the connection of genetic variations within a population to distinct phenotypes. The results of these efforts indicate that numerous physiological traits are controlled by intricate gene regulatory networks. These networks possess noticeable genetic heterogeneity. This is particularly evident in gene regulatory networks associated with stress-related traits, which manifest differently in plants adapted to various environments. This data underscores the prevalence of local adaptation in *Arabidopsis thaliana*. Furthermore, by using gene expression as a molecular phenotype, we demonstrate that some genes are globally influenced by shared variants, whereas others are affected by variants specific to sub-populations. We can identify gene regulatory networks that have evolved differently in various natural accessions, attempt to link them to their corresponding phenotypes, and thereby highlight the extent of adaptive evolution within *Arabidopsis thaliana*. Finally, I will emphasize that even within the same plant, gene regulatory networks may vary across different cell types.

P 087

**Cryo-EM structure of photosystem II supercomplex from *Chlorella ohadii*, a green microalga with extreme phototolerance**

R. Arshad<sup>1,2</sup>, I. Skalidis<sup>1</sup>, D. Kopečný<sup>2</sup>, S. Brabencová<sup>3</sup>, M. Opatiková<sup>2</sup>, P. Ilík<sup>2</sup>, P. Pospíšil<sup>2</sup>, S. Č. Zeljković<sup>2</sup>, P. Roudnický<sup>3</sup>  
D. Lazár<sup>2</sup>, E. Elias<sup>4</sup>, R. Croce<sup>4</sup>, P. Kastritis<sup>1</sup>, R. Kouřil<sup>2</sup>

<sup>1</sup>Martin-Luther University Halle, Halle a. d. Saale, Germany

<sup>2</sup>Palacky University Olomouc, Olomouc, Czech Republic

<sup>3</sup>Central European Institute of Technology, Masaryk University, Brno, Czech Republic

<sup>4</sup>University of Amsterdam, Amsterdam, Netherlands

Photosystem II (PSII) is essential for energy conversion during oxygenic photosynthesis in plants and algae<sup>1</sup>. Adaptation to harsh environments has led photosynthetic organisms to develop various photoprotective mechanisms. *Chlorella ohadii*, a green microalga adapted to the extreme conditions of desert sand crusts<sup>2</sup>, lacks the conventional photoprotective mechanisms of PSII typically mediated by LhcSR/PsbS proteins or protein phosphorylation<sup>3</sup>, raising the need for detailed structural research. Here we report a cryo-electron microscopy structure of *C. ohadii* PSII supercomplex at 2.9 Å resolution. This structure reveals additional subunits, PsbR and PsbY, along with a novel isoform of PsbO, which collectively enhance the stability of the core complex through extensive interactions. Our findings also show that the attachment of trimeric light-harvesting complexes (LHCII) to the PSII core is regulated by specific light-harvesting proteins, whose expression is adjusted under high-light conditions, leading to a strategic reduction in the number of associated LHCII trimers. These findings point to key properties that maintain stability and regulate photoprotection of PSII under extreme environmental conditions. In addition, however, it is important to consider other elements that could contribute to the unusually high resistance of the photosynthetic apparatus of *C. ohadii*. For example, our observed accumulation of polyamines under high light conditions could fundamentally regulate electron transport in the thylakoid membrane, which is crucial for maintaining photochemical activity even under the challenging conditions that *C. ohadii* faces in its natural environment.

References

1. Blankenship, R. E. *Molecular Mechanisms of Photosynthesis*. 2nd ed. Wiley-Blackwell, Chichester (2014).
2. Treves, H. *et al.* A newly isolated *Chlorella* sp. from desert sand crusts exhibits a unique resistance to excess light intensity. *FEMS Microbiol. Ecol.* **86**, 373–380 (2013).
3. Niyogi, K. K., Li, X. P., Rosenberg, V. & Jung, H. S. Is PsbS the site of non-photochemical quenching in photosynthesis? *J. Exp. Bot.* **56**, 375–382 (2005)

P 088

**Cold acclimation of RuBisCo expression**

F. Rösch<sup>1</sup>, J. Legen<sup>1</sup>, B. Lenzen<sup>1</sup>, C. Schmitz-Linneweber<sup>1</sup>

<sup>1</sup>Humboldt University Berlin, Berlin, Germany

The chloroplast genome encodes key components of the photosynthetic light reaction machinery as well as the large subunit of the enzyme central for carbon fixation, RuBisCo. Its expression is predominantly regulated post-transcriptionally, with nuclear-encoded RNA binding proteins (RBPs) playing a key role. Mutants of chloroplast gene expression factors often exhibit impaired chloroplast biogenesis, especially in cold conditions. Low temperatures pose a challenge for plants as this leads to electron imbalances and oxidative damage. A well-known response of plants to this problem is to increase the production of RuBisCo and other Calvin Cycle enzymes in the cold, but how this is achieved is unclear. The chloroplast RBP CP29A has been shown to be essential for cold resistance in growing leaf tissue of *Arabidopsis thaliana*. Here, we examined CP29A-RNA interaction sites at nucleotide resolution. We discovered that CP29A preferentially binds to the 5'-UTR of *rbcL*, downstream of the binding site of the pentatricopeptide repeat (PPR) protein MRL1. MRL1 is an RBP known to be necessary for the accumulation of *rbcL*. In *Arabidopsis* mutants lacking CP29A, we were unable to observe significant effects on *rbcL*, possibly due to CP29A's restricted role in a limited number of cells at the base of leaves. In contrast, CRISPR/Cas9-induced mutants of tobacco NtCP29A exhibit cold-dependent photosynthetic deficiencies throughout the entire leaf blade. This is associated with a parallel reduction in *rbcL* mRNA and RbcL protein accumulation. Our work reveals a novel molecular player behind cold acclimation of the photosynthetic dark reaction.

P 089

Establishment of BioID-based proximity labelling for in-depth proteome analyses of Arabidopsis peroxisomes

E. Ntiriakwa<sup>1</sup>, A. Parasyri<sup>1</sup>, I. Dinler<sup>1</sup>, T. Mair<sup>2</sup>, H. Schlüter<sup>2</sup>, S. Reumann<sup>1</sup>

<sup>1</sup>University of Hamburg, Plant Biochemistry and Infection Biology, Hamburg, Germany

<sup>2</sup>Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

Plant peroxisomes are involved in major metabolic functions, including lipid metabolism, photorespiration, hormone biosynthesis, and abiotic and biotic stress responses. Even though a great number of peroxisomal proteins have been unravelled by proteomics and forward and reverse genetic studies, many still remain unidentified, including low-abundance, stress-inducible, and membrane proteins. To increase the sensitivity in identifying novel peroxisomal matrix proteins, we employed the method of proximity-dependent biotin identification (BioID, Khan et al., 2018), where a prokaryotic biotin ligase has been mutated to unspecifically biotinylate proteins in close proximity at surface-exposed lysine residues. To direct the biotin ligase specifically to the peroxisomal matrix, we extended the enzyme by a C-terminal peroxisomal targeting signal type 1 (PTS1). After creating stable Arabidopsis overexpressor lines, mature leaves were infiltrated with biotin. Within a short period of time, the biotin ligase indeed rapidly biotinylated numerous matrix proteins. After optimization of several parameters, the biotinylated proteins were purified with and analysed by mass spectrometry. To increase the biotinylation efficiency and reduce the number of unspecifically bound non-peroxisomal proteins that were co-purified by the streptavidin-coated magnetic beads, various steps of the protocol had to be optimized, including the biotin supplementation and its removal, as well as the temperature and incubation time for optimal biotin ligase activity. Mass spectrometry analysis of affinity-purified biotinylated proteins allowed the identification of a large number of known matrix proteins as well as several novel candidates, confirming the suitability of the BioID method. Subcellular targeting of these novel candidate proteins is presently investigated *in vivo*. This paves the way for further method applications to identify novel stress-inducible and membrane proteins of Arabidopsis peroxisomes.

P 090

Occupancy of lysine acetylation in *Arabidopsis* proteome via chemical labelling and mass spectrometry measurements

J. Mussenbrock<sup>1</sup>, J. Eirich<sup>1</sup>, I. Finkemeier<sup>1</sup>

<sup>1</sup>Universität Münster, Institut für Biologie und Biotechnologie der Pflanzen, Münster, Germany

In course of the day, the environmental conditions are changing, so that the plants have different needs of proteins and their functions. As a result, the proteome needs to change constantly and in a dynamic way. Post-translational modifications (PTM's) change the properties of present proteins and have a massive impact on their functions, structures and activities. Such a modified protein has a changing mass, that is detectible by mass spectrometry. We are looking at the acetylation of lysine residues over the whole proteome of *Arabidopsis* (e.g. adult leaves). Using a method for chemical labelling of lysine with heavy (D6-)acetic anhydride (Baeza et al. 2020), we want to define the occupancy from lysine residues, that were naturally acetylated in *Arabidopsis*, through to different stages of the day/night. Therefore, we use various proteases to generate lots of peptides, with different cleaving sites, measure them by mass spectrometry and determine the occupancy of lysine acetylation. For a better understanding which impact the site-specific acetylation of lysine has in plants and how big the changes are during different stages and environmental conditions.

P 091

Evolutionary conservation of a lipid-droplet anchoring protein-protein interaction in angiosperms

P. Prüsener<sup>1</sup>, T. Ischebeck<sup>1</sup>, J. Dabisch<sup>1</sup>

<sup>1</sup>Universität Münster, Institut für Biologie und Biotechnologie der Pflanzen, Münster, Germany

Lipid Droplets (LDs) are generally accepted as an energy source in form of neutral lipids with high importance in seedling establishment. Recently an increasing number of LD functions is being discussed, for example as being a sink and source for membrane lipids. Key to the functionality of LDs are their associated proteins. Two recently described proteins are SEED LIPID DROPLET PROTEIN (SLDP) 1 and 2 in *Arabidopsis thaliana*. It tethers LDs to the plasma membrane through its interaction with the LIPID DROPLET PLASMA MEMBRANE ADAPTOR (LIPA) (Ischebeck *et al.* 2020, Krawczyk *et al.* 2022). While the physiological role of this tethering is still unknown, investigating protein homologs and degree of evolutionary conservation of LIPA and SLDP and their interaction in different plant species could help to find an answer.

This study combines evolutionary and cell biological aspects and detects the LIPA-SLDP interaction and the resulting LD tethering to the plasma membrane in several angiosperm plant species to investigate the evolutionary conservation of this mechanism.

Interactions are assayed by confocal laser scanning microscopy of tobacco pollen transformed with fluorophore-tagged SLDP and LIPA. Additionally, a confirmation via yeast two-hybrid is performed. We identified potentially relevant residues in the binding site of the SLDP and LIPA based on AlphaFold and PyMOL predictions. Mutagenesis experiments could identify specific amino acids involved. Furthermore, we use proteomics analyses to uncover the LD proteome in the seeds of crop species, including lipid isolation and LC-MS/MS analysis.

We have shown that LIPA localizes to the plasma membrane not only in *A. thaliana* but in *Helianthus annuus*, *Glycine max* and other species as well. Additionally, LIPA from several species is colocalizing with the *A. thaliana* SLDP at the plasma membrane, indicating a co-interaction across species. Earlier experiments by Krawczyk *et al.* (2022) identified the interaction site of LIPA with SLDP in *A. thaliana* what allows us to specify this region promising a specific prevention of this interaction.

References

Ischebeck, T., Krawczyk, H. E., Mullen, R. T., Dyer, J. M., & Chapman, K. D. (2020, December). Lipid droplets in plants and algae: Distribution, formation, turnover and function.

Krawczyk, H. E., Sun, S., Doner, N. M., Yan, Q., Lim, M., Scholz, P., ... & Ischebeck, T. (2022). SLDP and LIPA mediate lipid droplet-plasma membrane tethering in *Arabidopsis thaliana*.

P 092

Prioritization of abiotic and biotic plant stress responses by a phosphatase and calcium-dependent protein kinase switch

H. Seybold<sup>1</sup>, J. Bortlik<sup>1</sup>, X. Jiang<sup>2</sup>, A. Liese<sup>2</sup>, B. Conrads<sup>1</sup>, W. Hoehenwarter<sup>2</sup>, S. Matschi<sup>2</sup>, T. Romeis<sup>2,1,3</sup>

<sup>1</sup>Freie Universität Berlin, Department of Plant Biochemistry, Dahlem Centre of Plant Sciences, Institute for Biology, Berlin, Germany

<sup>2</sup>Leibniz-Institute of Plant Biochemistry Halle, Biochemistry of Plant Interactions, Halle a. d. Saale, Germany

<sup>3</sup>MLU Halle-Wittenberg, Institute of Biology, Halle a. d. Saale, Germany

In nature, plants are constantly challenged by simultaneous abiotic and biotic stresses, and under conflicting stress scenarios prioritization of stress responses is required for plant survival. Calcium-dependent protein kinase CPK5 is a central hub in local and distal immune signaling, required for salicylic acid (SA)-dependent immunity and pathogen resistance. Here we show that CPK5-dependent immune responses and pathogen resistance are inhibited upon abscisic acid (ABA) treatment or in genetic mutant backgrounds lacking PP2C phosphatase activities including *abi1-2*, whereas immune responses are enhanced by co-expression of active ABA INSENSITIVE 1 (ABI1) phosphatase variants. Biochemical studies and mass spectrometry-based phospho-site analysis reveal a direct ABI1 phosphatase-catalyzed de-phosphorylation of CPK5 auto-phosphorylation site T98 *in vitro*. Mimicking continuous de-phosphorylation in CPK5T98A *in planta* leads to enhanced ROS production and more resistant plants, whereas mimic of the auto-phosphorylated status in CPK5T98D reduces CPK5-mediated immune responses. Further mechanistic insight implicates that differential phosphorylation at T98 in the N-terminal domain of CPK5 regulates the interaction between the kinase and its substrate protein RESPIRATORY BURST OXIDASE HOMOLOGUE D (RBOHD). In identifying the ABI1 phosphatase and CPK5 kinase hub our work uncovers a mechanism for stress response prioritization in plants.

P 093

Functional analysis of TatA and TatB subunits of the Tat machinery in chloroplasts

M. Zhou<sup>1</sup>, M. Jakob<sup>1</sup>, R. B. Klösger<sup>1</sup>

<sup>1</sup>Institut für Biologie, Martin Luther University Halle-Wittenberg, Pflanzenphysiologie, Halle a. d. Saale, Germany

The Twin-arginine translocation (Tat) mechanism is featured by mediating the translocation of fully folded proteins across cellular membranes. It operates at the cytoplasmic membranes of bacteria and archaea, at the inner membrane of plant mitochondria, as well as at the thylakoid membrane of chloroplasts. Tat machinery consists of three subunits: TatA, TatB and TatC. Subunits TatB and TatC constitute the hetero-oligomeric TatBC-complex which serves as a receptor for the Tat substrates and which in chloroplasts is located in the thylakoid membrane. In contrast, TatA, which contributes to the actual membrane translocation step, is found in both thylakoid membrane and stroma. The exact mechanism and interplay between TatA, TatB and TatC in the protein translocation process is still not fully understood. In this study, we developed an *in thylakoido* reconstitution assay where the intrinsic TatA or TatB activity is suppressed by specific antibodies. Subsequently, the assays are supplemented with soluble TatA or TatB obtained from either *in vitro* translation or heterologous overexpression. With this approach we could show that Tat transport activity of such antibody-blocked thylakoids can be restored by supplementing external TatA or TatB proteins. Interestingly, excessive amounts of externally added Tat proteins seemed to have a negative impact on Tat transport activity and to destabilize the TatBC-complex to some extent. Thus, our study provides insight also into the interplay between the Tat components in the protein trans-membrane process.

P 094

Revealing the Regulatory Network of KEA3 in Photosynthesis: Focus on the interactions of C-terminal Domain with pH and nucleotides

S. Käbe<sup>1,2</sup>, M. Uflewski<sup>3</sup>, T. Rindfleisch<sup>3,4</sup>, U. Armbruster<sup>1,2,4</sup>

<sup>1</sup>Heinrich Heine University, Molecular Photosynthesis, Düsseldorf, Germany

<sup>2</sup>CEPLAS - Cluster of Excellence on Plant Sciences, Heinrich Heine University, Düsseldorf, Germany

<sup>3</sup>Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

<sup>4</sup>University of Bergen, Computational Biology Unit, Department of Chemistry, Bergen, Norway

Plants dynamically regulate photosynthesis to optimize growth under fluctuating environmental conditions, particularly light. The thylakoid K<sup>+</sup>/H<sup>+</sup> antiporter KEA3 is involved in the regulation of photosynthesis in response to different light conditions and thus seems to be a key player in the dynamics of photosynthesis.

However, the complex regulatory network of KEA3 remains elusive. It was shown that the KEA3 activity is strongly linked to the pH in chloroplasts. Furthermore, binding of ATP and other nucleotides was experimentally demonstrated. Here, the C-terminal domain of KEA3 was described as crucial for regulation of KEA3. We hypothesize that KEA3 integrates light and carbon fixation signals through a complex network involving the C-terminal domain.

The previous computational models which suggested regulation of KEA3 activity via conformational changes of the C-terminus have not yet been experimentally proven. Therefore, this study aims to investigate the regulatory mechanisms of KEA3 by focusing on the C-terminal domain. Using *in vitro* approaches, we want to characterize the structure of this domain and its interaction with key nucleotides, i.e. ATP and NADPH. By investigation of these interactions, we aim to understand how KEA3 integrates with other regulatory pathways and optimizes photosynthesis under varying light conditions.

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P 095

### Functional characterization of the coiled-coil domain of the viral silencing suppressor (P15) of the peanut clump virus in dimerization and siRNA binding

L. M. Halscheid<sup>1</sup>, S. Wirling<sup>1</sup>, M. Bolz<sup>1</sup>, S. Reumann<sup>1</sup><sup>1</sup>University of Hamburg, Institute of Plant science and microbiology, Plant biochemistry and infection biology, Hamburg, Germany

Many plant viruses encode a small suppressor of RNA silencing that combats the plant defence system of RNA interference (RNAi). The P15 protein of the peanut clump virus was shown to inhibit RNAi upon binding of siRNAs and their subsequent sequestration into plant peroxisomes (Incarbone et al., 2017). The 15-kDa protein consists of an N-terminal cysteine-rich  $\alpha/\beta$ -domain and a long C-terminal  $\alpha$ -helix. In this Master's thesis study, P15 was biophysically and functionally characterized with a focus on its C-terminal predicted coiled-coil domain (CCD). An in-depth *in silico* analysis allowed the identification of a typical hydrophobic stripe formed by five so-called heptad repeats. For *in vitro* analyses, P15 was produced with an N-terminal His6-MBP-tag and purified by Ni-NTA chromatography to very high purity. As deduced from size-exclusion chromatography and supplemented by multi-angle light scattering, P15 forms stable dimers and tetramers, even in the absence of siRNAs. Several point mutations were introduced into the CCD to determine their influence on the oligomeric state. The hydrophobic residues of the first four of in total five heptad repeats were shown to be necessary for dimer formation as the abundance of oligomers was drastically reduced in the corresponding mutated constructs. Hence, the hydrophobic stripe is essential for P15 dimerization, consistent with the typical function of CCDs in mediating protein-protein interactions. In contrast, the C-terminal decapeptide including the fifth heptad is not involved in oligomerization and instead interacts with the peroxin PEX5, the cytosolic receptor of peroxisome targeting signal type 1 (PTS1)-containing proteins, as shown by yeast-two hybrid assays and subcellular localization experiments. When investigating the effect of the point mutations in P15 on siRNA binding by microscale thermophoresis, we detected a strong reduction or a complete abolishment of siRNA binding for mainly monomeric constructs. From this major finding we concluded that dimerization of P15 is required to enable efficient binding of siRNAs. The protomer orientation in the P15 dimer (i.e., parallel or antiparallel CCDs) is currently under investigation using small-angle X-ray scattering and X-ray crystallography.

#### Reference

Incarbone M, Zimmermann A, Hammann P, Erhardt M, Michel F, Dunoyer P. Neutralization of mobile antiviral small RNA through peroxisomal import. *Nat Plants*. 2017 Jun 19;3:17094. doi: 10.1038/nplants.2017.94.

P 096

### Squalene Cyclases in non-seed plants

F. Heumesser<sup>1</sup>, M. Lim Sheng Satha<sup>1</sup>, J. de Vries<sup>2</sup>, T. Ischebeck<sup>1</sup><sup>1</sup>University of Münster, Institute of Plant Biology and Biotechnology, Münster, Germany<sup>2</sup>Georg-August-Universität Göttingen, Institut für Mikrobiologie und Genetik, Göttingen, Germany

Metabolic diversification is a crucial step for the adaptation to new environments. Triterpenes are metabolites with a 30-carbon backbone that can contain up to six rings. They have multiple functions in plants – as pathogen and pest repellents, membrane components, or intercellular signaling agents. In seed plants, squalene as the precursor of all triterpenes is converted to 2,3-oxido-squalene before cyclization by oxidosqualene cyclases, while in bacteria direct cyclization of squalene by squalene cyclases occurs. However, squalene cyclases are also found in bryophytes and ferns. So far, only little is known about the origin, physiological role, and catalytic scope of squalene cyclases from non-seed plants. Their investigation could contribute to the understanding of the evolution of early land plants. Combining these findings on novel triterpene synthases with our current understanding of lipid metabolism could also improve the use of lipid droplets as subcellular factories for specialized metabolites. In this project, transient overexpression in *N. benthamiana* and subsequent product analysis as well as microscopy and phylogenetic analysis are used to characterize putative squalene cyclases from *M. polymorpha*, *P. patens* and *A. filiculoides* and pave the way for future biotechnological applications.



P 097

How to decode cellular signals: Investigating the biochemical mechanism-of-action of calcium-dependent protein kinases

K. Maguemoun<sup>1</sup>, M. Bredow<sup>2</sup>, J. Monaghan<sup>1</sup>

<sup>1</sup>Queen's University, Biology, Kingston, Canada

<sup>2</sup>Iowa State University, Plant Pathology and Microbiology, Iowa, IA, United States

Calcium (Ca<sup>2+</sup>) is a crucial signalling molecule involved in growth and stress responses across eukaryotes (1). Our research aims to elucidate the biochemical mechanism-of-action of a group of Ca<sup>2+</sup>-binding proteins in plants called Ca<sup>2+</sup>-dependent protein kinases (CDPKs or CPKs). These proteins are capable of decoding and processing various Ca<sup>2+</sup> signals received by cells in a highly regulated manner. Some CDPKs can process multiple and different signals, resulting in various cellular responses. Recent research from our laboratory suggests that site- and context-specific phosphorylation may play a role in the cellular response of CDPKs (2). However, the mechanisms by which CDPKs decode distinct Ca<sup>2+</sup> signals are yet to be identified. Our laboratory has recently found that autophosphorylation of CPK28, a CDPK in the model plant *Arabidopsis thaliana*, at position Ser318, primes the kinase domain for Ca<sup>2+</sup> activation *in vitro* and is required for CPK28 to function in a subset of pathways *in vivo* (3,4). Moreover, results from another recent study on CPK23 suggest that phosphorylation at Ser362 promotes the shift from a low Ca<sup>2+</sup>-sensitivity to a Ca<sup>2+</sup>-insensitive kinase activity (5). This indicates that phosphorylation could be involved in Ca<sup>2+</sup> signature decoding. Structural analysis has revealed that CPK28-Ser318 and CPK23-Ser362 are situated in or close to a regulatory region known as the autoinhibitory junction (AIJ), which is a flexible segment enabling mobility between active and inactive conformations of CDPKs. Comparative analysis of Ca<sup>2+</sup>/calmodulin-dependent protein kinases and CDPKs across eukaryotes has further shown that this region contains several phosphorylated or phosphorylatable residues (Ser, Thr, Tyr), some of which are associated with activation (2). Based on these findings, we hypothesize that phosphorylation in this region allows CDPKs to decode distinct Ca<sup>2+</sup> signatures. In this poster, I will present our ongoing work to test this hypothesis.

References

1. Luan S, Wang C. *Annu Rev Cell Dev Biol.* 2021 Oct 6;37(1):311–40.
2. Bredow M, Monaghan J. *Current Opinion in Plant Biology.* 2022 Aug;68:102251.
3. Bender KW et al. *Journal of Biological Chemistry.* 2017 Mar;292(10):3988–4002.
4. Bredow M et al. *Proc Natl Acad Sci USA.* 2021 May 11;118(19):e2024272118.
5. Liese A, Eichstädt B, Lederer S, Schulz P, Oehlschläger J, Matschi S, et al. *The Plant Cell.* 2023 Jul 11; koad196.

P 098

The enigma of respiratory supercomplexes in plant mitochondria

H. Röhrich<sup>1</sup>, E. H. Meyer<sup>1</sup>

<sup>1</sup>MLU Halle-Wittenberg/Institute of Biology, Cellphysiology, Halle a. d. Saale, Germany

Oxidative phosphorylation (OXPHOS) as final step of cellular respiration is one of the most important energy converting processes of life. Located in the inner mitochondrial membrane, the OXPHOS system is mainly comprised of five transmembrane protein complexes (I-V). Individual OXPHOS complexes were found to assemble into higher-order homo and/or hetero-oligomeric protein complexes of defined stoichiometry. These so called respiratory supercomplexes were observed in all organisms investigated so far. Some supercomplexes such as the complex V dimers were shown to play an important structural role but the biological function of complex I containing supercomplexes has not yet been elucidated.

We isolated two viable *Arabidopsis thaliana* mutant lines with the complete set of individual OXPHOS complexes but undetectable levels of complex I containing supercomplexes. The growth of the *A. thaliana* lines was challenged with various abiotic stresses (e.g. cold, heat, high light, darkness) and respiratory inhibitors (e.g. rotenone, antimycin A) but the phenotype of the supercomplex mutants was undistinguishable from the wildtype. However, when seeds are germinated on water, without the addition of any buffer or chemicals, the mutants show a transient germination delay which was increased in aged seeds.

Nonetheless, so far, the biological relevance of these supercomplexes still remains an enigma.

P 099

### Impact of Natural LPR1 Proteoforms on Fe-dependent Phosphate Sensing

N. Leutert<sup>1</sup>, C. Naumann<sup>1</sup>, S. Abel<sup>1</sup>, M. T. Stubbs<sup>2</sup>

<sup>1</sup>Leibniz-Institut für Pflanzenbiochemie (IPB), Halle a. d. Saale, Germany

<sup>2</sup>Martin-Luther-Universität Halle-Wittenberg, Institut für Biochemie und Biotechnologie, Halle a. d. Saale, Germany

Phosphorus is an essential plant nutrient, which is taken up in form of anorganic phosphate (Pi) from the soil. This process can pose a challenge due to interactions of Pi with positively charged metal ions, especially in deeper soil layers. Pi starvation, often caused by antagonistic Pi-Fe interactions, is sensed at the root tip in a Fe-dependent manner and results in an adjustment of root system architecture (RSA), including primary root growth inhibition and increased formation of lateral roots and root hair (topsoil foraging). In *Arabidopsis*, the cell wall-localized multicopper oxidase LOW PHOSPHATE ROOT 1 (LPR1) is a key determinant of Fe-dependent Pi sensing by root tips. LPR1 originally was identified by a comparative QTL (Quantitative Trait Locus) analysis to unravel accession specific, Pi-dependent root growth inhibition in *Arabidopsis* traits. Further biochemical and phylogenomic analysis revealed that LPR1 confines a novel class of bacterial-type specific ferroxidases, which was obtained by land plant progenitors from terrabacteria to facilitate plant terrestrialization and Pi acquisition. To date, analysis of LPR1 function *in planta* has been limited to null alleles and site-directed mutagenesis. However, there is allelic *LPR1* diversity in various *Arabidopsis* accessions, leading to different LPR1 proteins (proteoforms) which may account for habitat specific soil adaptations. Within this project, we will biochemically analyze LPR1 proteoform specific enzymatic parameters including substrate specificity and Michaelis-Menten kinetics. Additionally, to understand the impact of natural protein variation towards soil adaptation we will perform phenotypic and genetic analysis of select *Arabidopsis* accessions accounting for the most abundant LPR1 proteoforms with focusing on protein secretion and transcriptional regulation.

P 100

### Unraveling the role of subunit e of the plant mitochondria ATP synthase

J. Xavier de Brito Silva<sup>1</sup>, T. Kiesel<sup>1</sup>, E. H. Meyer<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle-Wittenberg, Plant Physiology, Halle a. d. Saale, Germany

Subunit e is a small subunit found in the structure of the mitochondrial ATP synthase (CV). Together with subunit g, subunit e has been shown in yeast and mammals to be important for the dimerization of CV. Recently, subunits e and g were found to be the only subunits not to be depleted in the mitochondria of an *Arabidopsis* mutant with reduced levels of ATP synthase monomers, suggesting that they may have an additional function in plants. To unravel the function of subunit e in plants, we designed a reverse genetics approach. In *Arabidopsis*, subunit e is encoded by two isoforms *At3g01130 (ATPe1)* and *At5g15320 (ATPe2)*. Two double mutants (*dm1* and *dm2*) were obtained. Both lines contain very low *ATPe* transcript levels, although *dm1* shows higher residual levels than *dm2*. Under standard growth conditions, the double mutants display no growth phenotype. However, some mitochondria in *dm1* were observed to be enlarged and shaped like a cup by electron microscopy. In both double mutants, enlarged mitochondria were observed using confocal microscopy. Surprisingly, the abundance of CV monomers and dimers is not significantly altered in *dm1* and *dm2*. The analysis of the CV dimer band from *dm1* by proteomics indicates that *dm1* represents a knock-down line of subunit e. In addition, low levels of the three isoforms of subunit g were also detected. This suggests that those two subunits have a common function but, in opposition to their role in yeast, they do not seem to be involved in the formation of CV dimers. The link between reduced levels of subunits e and g and the observed enlarged mitochondria requires further experimental investigation.

Keywords: mitochondria ATP synthase, subunit e, oligomers.

P 101

### Investigating the function of Arabidopsis HISTONE DEACETYLASE 14 in chloroplasts

C. Markiton<sup>1</sup>, F. Kotnik<sup>1</sup>, J. Eirich<sup>1</sup>, I. Finkemeier<sup>1</sup>

<sup>1</sup>Universität Münster, Institut für Biologie und Biotechnologie der Pflanzen, Münster, Germany

Lysine acetylation is a crucial post-translational modification involved in plant development and responses to environmental stimuli. While much attention has been focused on the role of HDACs in histone acetylation, their involvement in deacetylating non-histone proteins remains less explored. Among the 18 HDACs in *Arabidopsis*, HDA14 stands out for its dual localization in plastids and mitochondria. To investigate the role of HDA14, we used quantitative mass spectrometry and identified 1509 acetylation sites on 881 protein groups, with 56 sites deregulated in a *hda14* mutant compared to WT. Most of the upregulated acetylation sites are associated with chloroplast proteins. Based on these results, proteins known for their substantial position in regulating organellar metabolic processes were chosen to perform interaction studies via enzymatic activity assays, BIFC and phenotyping. Moreover, HDA14 activity was further characterized upon changes in the amino acid code. The findings underline the importance of HDA14 in modulating lysine acetylation dynamics in *Arabidopsis*, revealing not only its influence on other proteins but also that it is modified itself by N-terminal acetylation through GNAT2, which is one of the N-acetyltransferases in chloroplasts. The function of the N-terminal acetylation of HDA14 is still unknown and requires further investigation.

P 102

Kinetic analysis of Arabidopsis PI4K $\beta$ 1 and effects of phosphorylation by mitogen-activated protein kinases

A. L. Meza Lopez<sup>1</sup>, M. Heilmann<sup>1</sup>, I. Heilmann<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle-Wittenberg, Institute of Biochemistry and Biotechnology, Halle a. d. Saale, Germany

Phosphatidylinositol 4-kinase  $\beta$ 1 (PI4K $\beta$ 1) is a key enzyme of plant phosphoinositide biosynthesis at endosomal membranes, where it mediates the conversion of phosphatidylinositol (PtdIns) to PtdIns 4-phosphate (PtdIns4P). Here we explore the biochemical properties of purified recombinant PI4K $\beta$ 1. PI4K $\beta$ 1 was heterologously expressed in *E. coli* as a fusion to an N-terminal maltose-binding protein (MBP)-tag and enriched by affinity purification. In *in vitro* lipid phosphorylation assays in the presence of  $\gamma$ [32P]ATP, MBP-PI4K $\beta$ 1 converted PtdIns to PtdIns4P in a concentration-dependent manner. Conversion rates with increasing PtdIns concentrations (but not with ATP) displayed pronounced sigmoidal kinetics, indicating a cooperative effect of allosteric binding of either PtdIns or the reaction product, PtdIns4P. The data are consistent with an effect of previously reported binding of PI4K $\beta$ 1 to PtdIns4P through its Plant PI4K Charged (PPC) domain. The kinetic analyses enabled further characterization of PI4K $\beta$ 1 regulation. PI4K $\beta$ 1 is a substrate for phosphorylation by upstream protein kinases, including mitogen-activated protein kinases (MAPKs). Based on our platform for kinetic *in vitro* analysis, we explored how phosphorylation by MPK4 or MPK6 impacts on the catalytic function of PI4K $\beta$ 1. Purified recombinant MBP-PI4K $\beta$ 1 protein was phosphorylated *in vitro* by constitutively-active variants of MPK4 or MPK6, consistent with previous reports. Phosphosites in PI4K $\beta$ 1 reported based on phosphoproteomics analyses include S186 and S454. The degree of *in vitro* phosphorylation of MBP-PI4K $\beta$ 1 substitution variants S186A or S454A was significantly reduced by ~53% and ~63%, respectively. Kinetic analysis of MBP-PI4K $\beta$ 1 substitution variants showed substantially altered kinetics with both PtdIns or the co-substrate, ATP. In particular, an MBP-PI4K $\beta$ 1 S186D variant, possibly mimicking a phosphorylated state of the enzyme, displayed increased catalytic activity compared to wild type MBP-PI4K $\beta$ 1 and also showed increased affinities for both PtdIns ( $K_m$  0.45 mM compared to 0.62 mM) and ATP ( $K_m$  200  $\mu$ M compared to 300  $\mu$ M). The data suggest an activation of PI4K $\beta$ 1 upon MAPK-mediated phosphorylation in position S186. Based on our kinetic and biochemical characterization of recombinant MBP-PI4K $\beta$ 1, we propose allosteric regulation of the enzyme by PtdIns4P and additionally by MAPK-mediated phosphorylation. The described mode of regulation of PI4K $\beta$ 1 might be relevant in the context of cytokinesis.

P 103

Molecular basis of the structural changes acquired by the C<sub>4</sub> photosynthetic NADP-malic enzyme from its housekeeping ancestor

J. M. Böhm<sup>1</sup>, S. Willms<sup>1</sup>, M. B. Arango<sup>1</sup>, O. Ferrao<sup>1</sup>, M. Hüdig<sup>1</sup>, C. Alvarez<sup>2</sup>, V. G. Maurino-Larcher<sup>1</sup>

<sup>1</sup>University of Bonn, IZMB, Institut für Zelluläre & Molekulare Botanik, Bonn, Germany

<sup>2</sup>University of Rosario, Centro de Estudios Fotosintéticos y Bioquímicos, Rosario, Argentina

NADP-malic enzyme (NADP-ME) catalyzes the oxidative decarboxylation of malate to produce pyruvate, CO<sub>2</sub> and NADPH. NADP-ME isoforms are present in the cytosol and the plastids and are involved in various metabolic pathways. In C<sub>4</sub> plants, two plastidic isoforms are of particular interest: C<sub>4</sub>-NADP-ME and nonC<sub>4</sub>-NADP-ME. C<sub>4</sub>-NADP-ME plays a vital role in the C<sub>4</sub> photosynthetic pathway, where it releases CO<sub>2</sub> in the chloroplasts of bundle sheath cells. This carbon concentrating mechanism reduces the oxygenase function of RuBisCO, thereby increasing plant productivity. C<sub>4</sub>-NADP-ME evolved from the housekeeping nonC<sub>4</sub>-NADP-ME by gene duplication and was subsequently co-opted for the C<sub>4</sub> photosynthetic pathway. The plastidic isoforms from *Zea mays* (maize) differ in their kinetic and structural properties. Compared with nonC<sub>4</sub>-NADP-ME, C<sub>4</sub>-NADP-ME acquired a higher affinity for malate and NADP and an overall two- to fivefold higher catalytic efficiency. In addition, C<sub>4</sub>-NADP-ME organizes as a tetramer, whereas the oligomeric organization of nonC<sub>4</sub>-NADP-ME is less clear. To understand the evolution of C<sub>4</sub>-NADP-ME from its nonC<sub>4</sub> ancestor, we performed several structural and biochemical analyses. Analytical ultracentrifugation suggests that nonC<sub>4</sub>-NADP-ME exists in a dynamic dimer-tetramer equilibrium, with the dimeric form being predominant when analysed by native PAGE. Size Exclusion Chromatography-Small Angle X-ray Scattering (SEC-SAXS) and protein crystallization showed that nonC<sub>4</sub>-NADP-ME forms tetramers similar to those of C<sub>4</sub>-NADP-ME. In conclusion, C<sub>4</sub>-NADP-ME has evolved into a stable tetramer, while the nonC<sub>4</sub> isoform is less stable and probably exists in an equilibrium of dimers and tetramers *in vivo*. An important question is which changes in the amino acid sequence are involved in changes of protein stability and thus in protein function. We identified 20 differentially conserved amino acids that may be important for the evolution of C<sub>4</sub>-NADP-ME from nonC<sub>4</sub>-NADP-ME. Through structural and biochemical analysis of various mutants, we show that an N-terminal region and four specific amino acids are involved in the oligomeric organization of the enzymes.

P 104

## The development of untargeted peptide and FTIR approaches for identifying the geographic origins of pomelos

W. Sriprapat<sup>1</sup>, S. Kittisenachai<sup>2</sup>, W. Ruankaew<sup>3</sup>, S. Roytrakul<sup>2</sup><sup>1</sup>Department of Agriculture, Thailand, Biotechnology Research and Development Office, Pathumthani, Thailand<sup>2</sup>National Center for Genetic Engineering and Biotechnology, Pathumthani, Germany<sup>3</sup>Office of Agricultural Research and Development Region 5, Chai Nat, Thailand

There has been an increasing emphasis on ensuring the safety and quality of agricultural products, particularly in verifying the authenticity and origin of food items. Consequently, it is evident that modern analytical techniques are required to address these authenticity issues. This study utilized advanced analytical methods to examine the peptide and chemical signatures of pomelo, employing MALDI-TOF MS and Fourier transform infrared (FT-IR) spectroscopy. Additionally, LC-MS was employed to assess the types and quantities of peptides and proteins. The results demonstrate that MALDI-TOF MS and FT-IR not only enable the straightforward discrimination of pomelo varieties but also the clear classification of pomelo fruit from three distinct growing locations. Peptide fingerprints obtained from the juice vesicle and peel of pomelos of the same variety but grown in different locations revealed a peptide mass distribution ranging from 2,000 to 10,000 Daltons. The average mass of the samples exhibited distinct peptide mass fingerprints for each group. The peptides identified, such as NADH dehydrogenase I subunit H, ATP synthase subunit alpha, and tyrosine decarboxylase, vary in quantity depending on the area, which can be used as biomarkers for the production source. The spectral signatures observed from FT-IR between 3500 and 2500 cm<sup>-1</sup> and 1700 and 1600 cm<sup>-1</sup> correspond to stretching vibrations of hydroxyl (OH) and carbonyl (C=O) functional groups found in plant phenolic compounds, while wavenumbers between 1300 and 800 cm<sup>-1</sup> indicate stretching vibrations of functional groups such as C-O, C-C, C-O-H, and the glycosidic bond of C-O-C in plants. Differentiation among the three locations was achieved within this range. Principal Component Analysis (PCA) of peptide mass and FT-IR spectra can distinguish pomelo growing locations into three distinct regions. Consequently, the results indicate that combining MALDI-TOF MS and FT-IR spectroscopy with PCA analysis is an effective preprocessing method for accurately predicting authenticity issues in pomelo samples.

P 105

## Meiotic temperature resilience in wild barley

Y. Gao<sup>1</sup>, S. Dreissig<sup>1</sup><sup>1</sup>Martin-Luther Universität, Institut für Argrar- & Ernährungswissenschaft, Halle a. d. Saale, Germany

Meiosis is an important cell division occurring in the maturation of germ cells in sexually reproducing organisms. While meiosis plays a significant role in generating genetic diversity, the faithful segregation of chromosomes is also essential for fertility, and in case of cereal crops, grain yield. It has been demonstrated that certain environmental stresses directly affect the meiotic machinery. When environmental stresses exceed the organism's tolerance, such as in the case of temperatures that are either too high or too low, the meiotic process is unable to complete successfully. This is due to the disruption of the spindle or the synaptonemal complex (SC), which ultimately results in sterility. Male meiosis is more susceptible to temperature fluctuations than female meiosis. Improving meiotic temperature resilience might ensure grain yield under future climate conditions by maintaining fertility.

The aim of this study is to examine the impact of short-term heat stress on meiotic chromosome structure and crossover frequency, and to explore natural variation in meiotic temperature resilience in wild barley (*Hordeum vulgare* ssp. *spontaneum*). To address this question, we characterised meiotic progression in genetically diverse wild barley accessions subjected to short-term heat stress during meiotic prophase (30°C for 24h). We found that different barley accessions exhibited varying degrees of temperature resilience during meiosis and exhibited distinct responses to high temperatures. The majority of chromosomal aberrations observed following high-temperature treatment were univalents, with only one accessions showing mostly multivalents upon heat stress. A significant reduction in the number of chiasmata under high temperature stress compared to normal temperature (22°C) was found in almost all accessions.

These data will be used in the future to identify genes that influence meiotic temperature resilience via RNA-seq-based differential gene expression analysis, and to improve our understanding of the underlying mechanisms.

P 106

Barley panproteome: analyzing its nuances through *in silico* structural biology

V. H. Rabesquine Nogueira<sup>1</sup>, A. Souza Câmara<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genebank Department, Seeland/OT Gatersleben, Germany

Proteins play pivotal roles in connecting the genetic information stored in the genome to diverse phenotypical traits. In the German Federal Ex situ Gene Bank, located at the IPK, the phenotypical diversity of plants is stored as extensive collections of accessions, many of which can be genotyped thanks to advancements in sequencing technologies. Concurrent recent progress in artificial intelligence and AlphaFold's breakthroughs [1, 2] allow us to predict, from the genome, reliable structural models for all the proteins present in a species – its proteome. We present here the first outcomes of a *Hordeum vulgare* (barley) panproteome. Our aim is to identify distinctive protein structural features that are conserved or have diverged among different barley variants and assess the extent of their structural variability. The latest version of the barley pangenome [3] contains information regarding 76 different genotypes, whose genes translate to more than 850,000 unique amino acid sequences, of which 75.6% are functionally annotated, forming 10,404 annotation clusters. So far, we have predicted about 20% of unique structures in the proteome, excluding proteins annotated as unknown or as transposable elements. These proteins were then clustered according to their structural domain features [4], revealing common and variable aspects that can help to elucidate some phenotypic differences between barley variants. Our results are constantly being incorporated into a data exploration portal for easy consultation and use. Further steps will include structural predictions and analyses of all the remaining accessions in the pangenome of barley, as well as a comparison between homologous proteins of different species.

References

- [1] Jumper, J *et al.* Highly accurate protein structure prediction with AlphaFold. Nature (2021).
- [2] Varadi, M *et al.* AlphaFold Protein Structure Database in 2024: providing structure coverage for over 214 million protein sequences. Nucleic Acids Research (2024).
- [3] Jayakodi, M. *et al.* Adaptive diversification through structural variation in barley. bioRxiv (2024).
- [4] Barrio-Hernandez, I. *et al.* Clustering predicted structures at the scale of the known protein universe. Nature (2023).

P 107

Calcium transporters in the endoplasmic reticulum determine plant development, fertility, and nutritional responses

O. Mariani<sup>1</sup>, M. Grenzi<sup>2</sup>, C. Naumann<sup>3</sup>, A. Janssen<sup>1</sup>, S. Abel<sup>3</sup>, A. Costa<sup>2</sup>, B. Meier<sup>1</sup>, E. Paiter<sup>1</sup>

<sup>1</sup>Institute of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Plant Nutrition Laboratory, Halle a. d. Saale, Germany

<sup>2</sup>University of Milan, Department of Biosciences, Milan, Italy

<sup>3</sup>Leibniz Institute of Plant Biochemistry, Department of Molecular Signal Processing, Halle a. d. Saale, Germany

Upon sensing environmental and developmental stimuli, temporally and spatially defined changes in free Ca<sup>2+</sup> concentrations ([Ca<sup>2+</sup>]) appear in the cytosol and within organelles. Calcium signalling has been intensively studied in the cytosol but is more poorly understood in organellar compartments, such as the endoplasmic reticulum (ER), the role of which is mostly described as Ca<sup>2+</sup> store or buffer for cytosolic Ca<sup>2+</sup> homeostasis. Recent studies showed that increases in [Ca<sup>2+</sup>]<sub>ER</sub> follow [Ca<sup>2+</sup>]<sub>cyt</sub> rises to restore basal [Ca<sup>2+</sup>]<sub>cyt</sub>. Indeed, ER Ca<sup>2+</sup> homeostasis is important for the proper folding of secreted proteins.

We have identified ion transporters resident in the ER that are predicted to load Ca<sup>2+</sup> into this compartment. Mutants lacking these transporters showed an altered plant development, a defect in fertilization, and an aberrant response to phosphate deficiency, which may all be linked to a misregulated [Ca<sup>2+</sup>]<sub>ER</sub> homeostasis. We therefore aim to uncover the mechanistic impact of these transporters on Ca<sup>2+</sup>-related processes in the ER. To allow visualization of Ca<sup>2+</sup> dynamics within the ER in response to various stimuli, we are currently employing the ER-GCaMP6-210 reporter located into the ER of plants devoid of the transporters.

P 108

**Structural Phloem Proteins: Exploring the Reaction Mechanism of the Plant Defense System**L. Wrobel<sup>1</sup>, D. Prüfer<sup>1</sup>, G. Noll<sup>1</sup><sup>1</sup>University Münster, Institute for Biology and Biotechnology of Plants, Münster, Germany

The phloem does much more than distribute sugars, amino acids, and proteins. It also plays a crucial role in long-distance signaling by transporting phytohormones and secondary metabolites. This vital network of sieve elements (SEs) connects every part of the plant, but it also brings with it the risk of spreading harmful substances and pathogens. In the event of wounding, phloem-specific proteins (P-proteins), encoded by the SEO-gene family, leap into action and form a protein plug on the downstream sieve plate (SP). The rapid plug formation of these highly conserved structural proteins raises intriguing questions about the underlying mechanisms, triggers and function.

Currently, most studied P-proteins are the spindle-shaped forisomes, which are exclusively found in the Fabaceae plant family. In response to Ca<sup>2+</sup>, they reversibly form plugs on the SPs independent of any energy source. We could show that the structure of the protein complexes contains negatively charged regions that allow the interaction with Ca<sup>2+</sup> and provide stability through cysteines, ensuring reversibility (Rose et al., 2022). Forisomes can be isolated or artificially produced, with incredible potential applications as smart biomaterials in engineering, such as valves or as bioactive coatings (Noll et al., 2022, Becker et al., 2023).

Investigations into forisomes have spotlighted the conventional P-proteins, which are highly conserved across dicotyledonous plants and form a filamentous network within SEs. The exact function of these P-protein plugs remains unclear, though they are believed to minimize phloem sap loss and/or prevent pathogens from entering the phloem network.

Using confocal laser scanning microscopy (CLSM), we observed the P-protein response *in vivo* in *Arabidopsis thaliana* roots, utilizing mutant lines with fluorescently labeled P-proteins. This research also highlighted the role of long-distance calcium signaling in plug formation, detected via an SE-specific calcium sensor. Blocking calcium channels confirmed the link between calcium signaling and P-protein response, revealing a complex and highly organized defense mechanism in the phloem. For the future, the understanding of plant defense mechanisms against pathogens and other stresses has a great potential to advancements in crop development and resilience.

P 109

**Biosynthesis and cell biology of piperine accumulation in black pepper, *Piper nigrum***W. Kouas<sup>1</sup><sup>1</sup>Leibniz-Institut für Pflanzenbiochemie, Cell and Metabolic Biology, Halle a. d. Saale, Germany

Black pepper (*Piper nigrum* L.) is the world's most popular spice and is also used as an ingredient in traditional medicine. Its pungent perception is due to the interaction of its major alkaloid, piperine (1-piperoyl-piperidine) (piperine) with ion-channels, specifically the human vanilloid receptor, TRPV-1. Based on a differential RNA-Seq approach combined with synthesis of substrates we established three specific and consecutive enzymatic steps of piperine formation including the critical amide formation catalyzed by piperine synthase (PS) in developing black pepper fruits (Figure 1). We showed that piperine accumulation take place in highly specialized cells of the fruit endosperm where we also localized PS and related enzyme, termed piperamide synthase (PAS) with a more promiscuous substrate specificity (Figure 2). We also identified piperine and piperamide containing idioblasts in the root cortex.

We will now investigate the accumulation of piperine and piperamide biosynthetic enzymes of the fruit perisperm and the root cortex at the cellular level by (targeted) metabolomics, proteomics, and transcriptomics. This will promote ideas how these specialized cells work. Isolation of individual cells will be achieved by laser capture microdissection and in parallel, by isolation of protoplasts combined with cell sorting by FACS(sc) that may be required for single cell (sc) RNAseq analysis. Differential metabolomics in combination with proteomics should indicate which specialized metabolic pathways will take place in the specialized cell factories of the perisperm and which pathway are localized in pericarp cells. With respect to biosynthesis of piperine we continue to investigate the enigmatic C-2-chain elongation of the presumed phenylpropanoid precursor feruloyl CoA that can in principle be catalyzed by either chalcone synthase (CHS) or ketoacyl synthase (KAS) like polyketide synthases (PKS) for functional expression of the complete pathway in different hosts.

P 110

**Chloroplast Envelope Metabolite Channels in Lipid Remodelling and Photosynthetic Efficiency Under Cold Stress**

W. T. Lo<sup>1</sup>, C. Tullberg<sup>1,2</sup>, F. Hagn<sup>3</sup>, T. Kleine<sup>1</sup>, M. Lehmann<sup>1</sup>, D. Leister<sup>1</sup>, S. Schwenkert<sup>1</sup>

<sup>1</sup>Ludwig Maximilian University of Munich, Faculty of Biology, München, Germany

<sup>2</sup>Lund University, Department of Chemistry, Lund, Sweden

<sup>3</sup>Technical University of Munich, School of Natural Sciences, München, Germany

In plants, the chloroplast serves as the cellular site of photosynthesis and harbours a host of essential and interwoven metabolic pathways. Therefore, acclimation to challenging environmental conditions is driven by the movement of metabolites across the chloroplast envelopes, which are equipped with channel and transport proteins that support metabolic crosstalk. Originally identified as a chloroplast outer envelope protein, OEP23 has been suggested to act as a metabolite channel. However, the physiological function of OEP23 remains unclear. Using CRISPR technology *oep23* knockouts were generated and characterised under challenging environmental conditions. Interestingly, *oep23* showed a strong response during cold acclimation. While *oep23* mutants presented a wildtype-like phenotype under standard growth conditions, they accumulated less anthocyanins and suffered severe reductions in photosynthetic efficiency after cold treatment. Changes in the proteome and lipidome in *oep23* mutants after cold treatment suggested that OEP23 plays a role in lipid remodelling. More detailed investigation of its topology also revealed its localisation to the inner rather than the outer chloroplast envelope. In summary, OEP23 is a novel[SS1] inner envelope protein implicated in lipid remodelling under low temperature stress.

P 111

**Plant cells can form and retain protrusions - are plant and animal cells more alike than we think?**

J. E. M. Dickmann<sup>1</sup>, M. Martin<sup>1</sup>, C. Lionnet<sup>1</sup>, O. Hamant<sup>1</sup>

<sup>1</sup>Laboratoire Reproduction et Développement des Plantes, ENS de Lyon, INRAE, CNRS, UCBL1, Lyon, France

Plant cells can be caricatured as passive bags of membrane, pressed against a rigid cell wall by turgor pressure. In contrast, animal cells are shaped by a cytoskeletal cell cortex that can actively deform the membrane, e.g. forming protrusions.

In fact, plant cells can also form protrusions, as exemplified during exposure to a high osmotic environment. In such hyperosmotic environment, the protoplast retracts from the cell wall in a process called plasmolysis. During plasmolysis, the plasma membrane stays connected to the cell wall at discrete points, the Hechtian attachment sites, from which membranous tubes, the Hechtian strands, emerge, that are continuous with the plasma membrane of the retracting protoplast.

We found that in isolated protoplasts, obtained by digesting the cell wall, 16±7 % of the protoplasts show similar membranous protrusions. Both the plasma membrane and the cytoplasm of these protrusions are continuous with the protoplast. The protrusions can contain cytoskeleton as well as ER. Together, these observations show that forming and retaining protrusions is not exclusive to animal cells but indeed readily observed in plant cells, too. The protrusions observed in plant cells echo focal adhesions in animals and hence are attractive candidates as mechanosensing hubs: the Hechtian attachment sites might bundle mechanical forces at discrete points on the plasma membrane; the protoplast protrusions might probe the environment for potential attachment sites. In this way, plant cells may be more similar to animal cells than currently appreciated.

P 112

**Cooperative reaction kinetics of Arabidopsis phosphatidylinositol 4-phosphate 5-kinase 6 (PIP5K6) mediated by allosteric PtdIns4P-binding**

J. C. Nordmeier<sup>1</sup>, R. Golbik<sup>1</sup>, A. Schutkowski<sup>1</sup>, I. Heilmann<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle-Wittenberg, Pflanzenbiochemie, Halle a. d. Saale, Germany

The lipid composition of cellular membranes contributes substantially to the control of various physiological processes in plants. The phosphatidylinositol 4-phosphate 5-kinase (PI4P 5-kinase) PIP5K6 is a key enzyme of phosphoinositide metabolism in *Arabidopsis thaliana* and involved in essential plasma membrane-associated processes, including the control of polar tip-growth and pathogen defense. PIP5K6 converts the membrane lipid phosphatidylinositol 4-phosphate (PtdIns4P) to phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P<sub>2</sub>), creating an important positional signal for the membrane recruitment of relevant proteins. The biochemical features of plant PI4P 5-kinases are not well understood, and the enzymology of these important enzymes has not received much attention. Here we analyze the kinetic behavior of PIP5K6 and its regulation in a membrane environment containing PtdIns4P substrate lipids. Kinetic analysis of purified recombinant PIP5K6 protein revealed pronounced sigmoid reaction kinetics in the presence of increasing concentrations of PtdIns4P. The data are consistent with a cooperative effect of allosteric PtdIns4P binding. No sigmoid kinetics were observed with the cosubstrate ATP. As a member of the plant-specific PI4P 5-kinase subfamily B, PIP5K6 differs in domain architecture from mammalian or yeast counterparts by containing plant-specific N-terminal domains with proposed regulatory function. Allosteric PtdIns4P-binding occurred in the N-terminal regulatory domains of PIP5K6, as determined by microscale thermophoresis, and a truncated PIP5K6 variant representing only the dimerization and catalytic domains displayed altered kinetic features in vitro. The kinetic data reveal that N-terminal domains can contribute to the control of PIP5K6 catalytic activity in a membrane environment highly enriched in PtdIns4P. Potential effects of the kinetic properties of PIP5K6 on asymmetric membrane lipid nano-distribution are discussed. This research was supported by a PhD-student stipend from the Landesgraduiertenförderung Sachsen-Anhalt.

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Evaluating the physiological role of plastid nucleus interaction: plastid to nuclear H<sub>2</sub>O<sub>2</sub> transportC. Guizani<sup>1</sup>, M. Schattat<sup>1</sup>, V. Karsten<sup>1</sup><sup>1</sup>MLU, biologicum, Halle a. d. Saale, Germany

Retrograde control has evolved to coordinate the expression of nuclear genes encoding plastid localized proteins, ensuring the delivery of needed proteins to plastids depending on their metabolic and developmental state. Plastids employ multiple retrograde signals to orchestrate major changes in nuclear gene expression [1]. Despite the notable progress in understanding the nature of retrograde signals and the changes they induce in the nucleus, it is still unclear how the signals are effectively transferred from the plastid to the nucleus and whether the physical interaction between chloroplasts and the nucleus may serve as a direct route for signaling. It has been proposed that clustering of plastids at the nucleus leads to the establishment of physical interactions between plastids and nuclei, which in consequence supports retrograde signaling [2]. Interestingly, these interactions become more frequent when cells are exposed to conditions that induce retrograde signals, such as stimuli triggering the innate immune response [2,3]. Despite these observations, the quantitative experimental evidence, which would support the idea of plastid clusters supporting signal transfer is missing.

To test the hypothesis, we will measure how fast a chosen retrograde signal accumulates in the nucleus after triggering its release from plastids. Comparing the accumulation dynamics obtained from wild-type plants with the accumulation dynamics obtained from plants with altered plastid-nucleus association. we choose the retrograde signal to follow by critical evaluation of the available literature and available techniques to track different known signals. We decided, to use H<sub>2</sub>O<sub>2</sub> as a metabolite signal. H<sub>2</sub>O<sub>2</sub> will be visualized by the genetically encoded fluorescence-based sensor Hyper7, which has been shown to be currently the fastest and the most sensitive variant of the Hyper protein family. The composition of the plastid-nucleus complex will be manipulated using mutants with an increased number of nucleus-associated plastids and mutants with decreased number of nucleus-associated plastids.

## References

[1] Fernandez, A.P. and Strand, .. (2008) *Curr. Opin. Plant Biol.* 11(5), 509– 513.[2] Caplan JL et al. (2015) *Dev Cell* 34: 45-57;[3] Prautsch J et al. (2022) *Plant Physiol* 191:161-176

P 115

## The mechanism of PEP7 maturation in SIRK1 signaling pathway

H. Kuhn<sup>1</sup>, L. Xi<sup>1</sup>, X. Wu<sup>1</sup>, W. Schulze<sup>1</sup><sup>1</sup>University of Hohenheim, Plant Systems Biology, Stuttgart, Germany

In recent years, research on plant peptides has shown the importance of these small signaling molecules for cell-to-cell communication. They not only coordinate various cellular functions and regulate plant development, but also enable the plant to adapt to various biotic and abiotic stresses. Previously, we identified plasma membrane-intrinsic sucrose-induced receptor kinase 1 (SIRK1) and Qian Shou kinase 1 (QSK1) as receptor/co-receptor pair involved in phosphorylation of aquaporins in response to osmotic conditions induced by sucrose. Recently, we identified a member of the elicitor peptide (PEP) family, namely PEP7, as a ligand glue that binds to the extracellular domain of SIRK1 and stabilizes the SIRK1-QSK1 signaling complex. However, the maturation process of bioactive PEP7 in response to external sucrose, is still unclear.

The common mechanism of peptide maturation indicates the PEP7 derives from the inactive precursor PROPEP7. However, PROPEP7 stands out insofar as it is a non-post-translationally modified precursor and does not carry the usual N-terminal secretion signal. Recently, it was discovered that metacaspase 4 (MC4) cleaves the PEP family in a Ca<sup>2+</sup>-dependent manner. The water influx in the *mc4* knockout mutant was response to sucrose similar to that in *sirk1* or *pep7* mutants, suggesting that the cleavage of PROPEP7 by MC4 was strongly associated with the sucrose-induced SIRK1 signaling pathway. In order to reveal the relationship between *in vivo* sucrose status and PEP7 secretion, our preliminary results showed that the abundance of PEP7 in the apoplasm responds to external sucrose changes. Additionally, three phosphorylation sites on PROPEP7 were found to be associated with internal sucrose status in Arabidopsis. We hypothesize that the regulation mechanism of PROPEP7 phosphorylation plays a key role in the sucrose-regulated MC4-PEP7-SIRK1 signalling pathway. In this study, we aim to examine the function of phosphorylation sites during PEP7 processing at the end of the day and the end of the night, and to identify the putative kinase(s) involved in the phosphorylation of PROPEP7. Our research will expand the understanding of the role of phosphorylation in signaling peptide processing.



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Modulating membrane lipid composition influences cellulose deposition in *Arabidopsis thaliana*

I. Stenzel<sup>1</sup>, G. Hause<sup>2</sup>, S. Krüger<sup>2</sup>, I. Heilmann<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle Wittenberg, Institut für Biochemie und Biotechnologie Abteilung Pflanzenbiochemie, Halle a. d. Saale, Germany

<sup>2</sup>Martin-Luther-Universität, Halle a. d. Saale, Germany

The half life and abundance of intrinsic membrane proteins are determined in part by the balanced rates of their membrane insertion and endocytotic recycling. Both secretion and endocytosis involve membrane lipids to facilitate transitional membrane hemi-states or to recruit proteins of the secretory or endocytotic machineries. Therefore, membrane lipid composition can influence the abundance of intrinsic membrane proteins. Cellulose synthase complexes (CSCs) are intrinsic membrane proteins with profound impact on plant cell wall deposition. Through the directional deposition of cellulose fibers, CSC also represent an important morphogenic factor in plants. So far, it is not well understood how membrane lipid composition influences CSCs or cellulose deposition.

Based on the observation that an *Arabidopsis pip5k1 pip5k2* double mutant displayed thicker cell walls and contained significantly higher cellulose levels compared with wild type controls, we tested whether modulated membrane lipid composition would result in altered patterns of cell wall deposition. Modulation of membrane lipid metabolism was assessed in *Arabidopsis pip5k* mutants; upon developmentally induced RNAi knock-down of *PIP5K*-genes; or upon developmentally induced overexpression of *PIP5K* genes. Cellulose levels were monitored according to biochemical cell wall analysis, and cell wall thickness was assessed by transmission electron microscopy. Interestingly, changes in PI4P 5-kinase activity or in PI(4,5)P<sub>2</sub> levels arising from the genetic modulation appeared unexpectedly complex. For instance, we observed compensatory activation of PI4P 5-kinase genes in *pip5k1 pip5k2* mutants resulting in overproduction of PI(4,5)P<sub>2</sub> at later stages of development, whereas young seedlings of the same mutants displayed reduced levels of the lipid. Our data suggest that enhanced degrees of cell wall deposition were a consequence of PI(4,5)P<sub>2</sub> overproduction rather than reduced levels of the lipid, even if thicker cell walls were first observed in a *pip5k1 pip5k2* double mutant. We conclude that plant cell wall composition can be influenced by modulating membrane lipids, and that membrane lipid metabolism is subject to complex intrinsic control by compensatory transcriptional regulation of iso-genes.

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Decoding the Role of Plastid Ca<sup>2+</sup> Signaling in Plant Defense

S. Mühlbauer<sup>1</sup>, I. Nunes<sup>1</sup>, L. Holzner<sup>1</sup>, C. Völkner<sup>1</sup>, B. Brandt<sup>1</sup>, D. Jaslan<sup>2</sup>, C. Grimm<sup>2</sup>, C. Tisserant<sup>1</sup>, S. Robatzek<sup>1</sup>, B. Hause<sup>3</sup>

S. Zenker<sup>4</sup>, A. Bräutigam<sup>4</sup>, H. H. Kunz<sup>1</sup>

<sup>1</sup>LMU Munich, Biology, Planegg-Martinsried, Germany

<sup>2</sup>LMU Munich, Medicine, München, Germany

<sup>3</sup>Leibniz Institute of Plant Biochemistry, Halle a. d. Saale, Germany

<sup>4</sup>Universität Bielefeld, Biology, Bielefeld, Germany

Most plants are sessile and cannot escape abiotic and biotic stressors. Consequently, plants have evolved a slew of molecular mechanisms to respond to these stimuli, facilitating adaptation, and minimizing damage. Over the last years, the chloroplast, which is capable to generate its own Ca<sup>2+</sup> signature in response to stress, has emerged as an important hub to coordinate these cellular acclimation responses. Recently, we have identified two members of a novel plastid-localized ion channel family, which we designated plastid envelope ion channels (PEC1/2) [1]. The loss of these proteins attenuates stress-induced stromal Ca<sub>2+</sub> transients, which in turn results in the trapping of Ca<sup>2+</sup> in the cytosol. However, the exact physiological relevance of stress-induced plastid Ca<sup>2+</sup> signals has remained enigmatic. Through patch-clamping isolated *Arabidopsis* chloroplasts, we obtained preliminary evidence suggesting that PEC1/2 function as the fast-activating chloroplast cation (FACC) channels previously characterized in pea chloroplasts [2], and thus exhibit permeability for K<sup>+</sup> and Ca<sup>2+</sup>. Our research further reveals that the expression of PEC1/2 is highly regulated by the canonical Jasmonic acid (JA) signaling pathway, which is activated during plant defense against herbivores and necrotrophic pathogens. Consequently, in *pec1pec2* loss-of-function mutants the expression of plant defense-related genes is negatively impacted.

In summary, our findings indicate that through the regulation of PEC1/2 expression, JA can enhance plastid Ca<sup>2+</sup> transients, which are necessary to elicit the complete defense response. Concomitantly, overexpression of PEC1 leads to constitutively elevated Ca<sup>2+</sup> transients and increased resistance against *Botrytis cinerea*. Our research suggests that the stress-induced expression of PECs modulates stromal Ca<sup>2+</sup> release to attenuate or exacerbate JA signaling. We hypothesize that this two-component stress signaling mechanism primes the plant for sustained long-term defense. Considering that the JA derivative methyl jasmonate is volatile, PEC1/2 activity may also participate long-distance plant-to-plant signaling.

References

- Völkner, C., et al., *Two plastid POLLUX ion channel-like proteins are required for stress-triggered stromal Ca<sup>2+</sup> release*. Plant Physiol, 2021. 187(4): p. 2110-2125.
- Pottosin, II, J. Muniz, and S. Shabala, *Fast-activating channel controls cation fluxes across the native chloroplast envelope*. Journal of Membrane Biology, 2005. 204(3): p. 145-156.

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The role of regulatory lipids during the initiation of endocytosis at the plant plasma membrane

J. Uhlenberg<sup>1</sup>, M. Heilmann<sup>1</sup>, I. Heilmann<sup>1</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg (MLU), Plant Biochemistry, Halle a. d. Saale, Germany

The plasma membrane (PM) acts as the outermost barrier of each cell, serving as a critical interface between the cell and its environment. The ability to dynamically regulate PM composition is crucial for physiological functions and therefore cell survival. The maintenance of PM protein composition relies on the two complementary processes, exocytosis and endocytosis, with clathrin mediated endocytosis (CME) being the main pathway for internalizing PM-located cargo proteins. Exocytosis and endocytosis at the PM are highly dynamic and strictly regulated in time and space. While it is known that anionic phospholipids, such as phosphoinositides (PIs), can contribute to the regulation of CME, and that endocytic cycling of target proteins is impaired in plant cells with perturbed PI metabolism, the molecular mechanisms behind the regulation of CME by membrane lipids are not fully understood. One potential link between PIs and the regulation of early endocytosis stages are adaptor protein complexes, including the adaptor protein complex-2 (AP-2), which are key factors for initiating CME at the PM. Here we show specific binding of AP-2 subunits from Arabidopsis to PIs and to lipid kinases involved in PI metabolism *in vitro*. The lipid binding capability of AP-2 subunits has an impact on the function of AP-2 complexes, as indicated by endocytosis assays using membrane dyes or fluorescence-tagged cargo proteins. As membrane lipids, PIs - as well as sphingolipids or sterols - are not uniformly distributed throughout the PM but are enriched in specific areas, forming dynamic membrane nanodomains. We hypothesize that PI binding of AP-2 subunits is required also for their dynamic PM distribution. Changes in PM lipid composition do not only lead to a perturbed endocytosis but also to changes in the degree of overall membrane liquid phase order, as we observe by applying a phase sensitive membrane dye to Arabidopsis mutants with different defects in PM lipid biosynthesis. We further hypothesize that any modulation of PM lipid composition has complex consequences and may impact various lipids through a complex network of interconnected pathways for the biosynthesis of different PM lipid classes. This work is supported by the German Research Foundation (DFG, grant He3424/6-2).

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Actin-plasma membrane-contacts mediated by the class VIII myosin ATM2 enable a self-reinforcing loop of binding and promoting PtdIns(4,5)P<sub>2</sub>, controlling actin dynamics in pollen tubes

V. Wagner<sup>1</sup>, M. Fratini<sup>1</sup>, C. Kastner<sup>1</sup>, A. Schutkowski<sup>1</sup>, M. Heilmann<sup>1</sup>, I. Heilmann<sup>1</sup>

<sup>1</sup>Martin-Luther University Halle-Wittenberg, Plant Biochemistry, Halle a. d. Saale, Germany

The intersection of actin filaments with the plasma membrane of plant cells involves lipid-protein interactions by the Arabidopsis class VIII-myosin, ATM2, that are currently not understood. Using pollen tube cells as a model, we describe how ATM2 modulates membrane-lipid nano-organization and at the same time locally stabilizes actin-plasma membrane contacts. ATM2 in its active state binds both to actin and to anionic plasma membrane lipids, which mediate ATM2-plasma membrane-attachment. Overexpression of ATM2 stabilized actin dynamics and altered pollen tube morphology, implicating ATM2 in the control of actin dynamics. Actin dynamics were not altered upon overexpression of ATM2 variants defective in lipid binding, despite enhanced association with actin filaments. ATM2 effects on actin dynamics required not only membrane association but were specifically dependent on the regulatory phosphoinositide, phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P<sub>2</sub>), as artificial depletion of PtdIns(4,5)P<sub>2</sub> abolished effects of full length ATM2 on actin dynamics. ATM2 colocalized and interacted with the PI4P 5-kinase, PIP5K2, which resides at actin-plasma membrane-contacts and forms PtdIns(4,5)P<sub>2</sub>-nanodomains that as GDFs facilitate ROP-dependent actin-stabilization. Upon ATM2 overexpression, a coexpressed fluorescent PtdIns(4,5)P<sub>2</sub>-biosensor displayed expanded plasma membrane distribution *in vivo*, indicating a promoting effect of ATM2 on PtdIns(4,5)P<sub>2</sub>-distribution in pollen tube cells. *In vitro*, catalytic PI4P 5-kinase-activity and PtdIns(4,5)P<sub>2</sub>-formation were enhanced by coinubation of recombinant PIP5K2 protein with a purified C-terminal ATM2 fragment. We conclude that ATM2 activates PIP5K2 at actin-plasma membrane-contacts. Stimulation of PtdIns(4,5)P<sub>2</sub>-formation creates a self-reinforcing loop promoting recruitment of ATM2 as well as ROP-activation, thereby mediating the stabilization of membrane-proximal actin-filaments. This work was supported by the German Research Foundation (DFG) through grant He3424/6-2 and the DFG Research Training Group RTG 2498 "Communication and Dynamics of Plant Cell Compartments" (DFG grant 400681449/ GRK2498 TP01).

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Using plant pathogen effectors to identify genes regulating stress driven plastid morphology adaptation

T. Staps<sup>1</sup>, M. Schattat<sup>1</sup>, C. Lampe<sup>1</sup>, J. Marx<sup>2</sup>, S. Ortmann<sup>1</sup>, J. Erickson<sup>2</sup>

<sup>1</sup>Martin-Luther-Universität Halle-Wittenberg, Biology, Halle a. d. Saale, Germany

<sup>2</sup>Leibniz-Institut für Pflanzenbiochemie, Halle a. d. Saale, Germany

One aspect of the morphological plasticity of plastids is the formation of plastid envelope out-folds, called stromules. These structures have been implied to play a role in the control of organelle constellations and interactions in response to stress. Although stromules appear to be evolutionarily conserved, and their formation tightly regulated, our current knowledge about their precise role for the plastids in plant cells or the underlying mechanisms of stromule formation and regulation are not known. Intriguingly, stromules are formed in response not only to one specific kind of stress but to a number of stresses, suggesting that stromules are connected to so far unknown fundamental stress-related cellular processes. Because stromules provide an easy visual read out they are the ideal model system to study organelle shape change and at the same time to uncover and study the underlying fundamental stress responses. Thus, the central aim of the project is to utilize stromules to uncover the molecular mechanisms of dynamic stress driven organelle shape adaptation and to uncover the fundamental stress related cellular processes inducing this shape change. To achieve these goals, we have utilized plant microbe-encoded effector proteins that interfere with core cellular processes in plants to screen for stromule phenotypes. This led to the identification of a set of stromule-inducing effectors. Transcription analysis using those effectors enabled us to identify a set of transcription factors, which are sufficient to positively regulate stromules and connect stromules to fundamental stress responses. In future, analysis of genes regulated by these transcription factors and their activity in cells will help to understand in which way plastid stromules support stress responses.

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Elucidating the role of actin-depolymerizing factors (ADFs) in regulating *Nicotiana tabacum* pollen tube polarity

M. Fradini<sup>1</sup>, G. Hamm<sup>1</sup>, M. Heilmann<sup>1</sup>, I. Heilmann<sup>1</sup>

<sup>1</sup>Martin-Luther-University Halle-Wittenberg, Plant Biochemistry, Halle a. d. Saale, Germany

Polarized tip growth of pollen tubes requires the coordinated action of vesicle trafficking at the tip of the cell and a motile actin cytoskeleton. Although pollen tube tip growth is intensively studied, it remains unclear, how these processes are coordinated. One influence is the contribution of regulatory membrane phosphoinositides, low-abundant lipids which specifically interact with proteins. Phosphatidylinositol 4,5-bisphosphate (PIP2) is a lipid essential for polar tip growth of pollen tubes. In the subapical plasma membrane, PIP2 localizes in nanodomains, which influence the function of actin regulating proteins, such as ROPs. To elucidate further factors controlling actin dynamics - and thus pollen tube growth - in a PIP2-dependent manner, we investigate candidate proteins, including actin-depolymerizing factors (ADFs). ADFs (in mammals also known as cofilins) control actin dynamics by favoring actin depolymerization. Importantly, ADFs bind to PIP2 *in vitro*, mediating recruitment and inactivation of ADFs. Here we use confocal live imaging to probe localization of fluorescence-tagged tobacco ADF2 and its interplay with regulatory factors influencing actin dynamics in tobacco pollen tubes *in vivo*. ADF2 displays fast dynamics of plasma membrane association and localizes in proximity to nanodomains decorated by fluorescent biosensors for PIP2 or by fluorescently tagged PI4P5 kinase 2 (PIP5K2). Moreover, ADF2 interacts with PIP5K2 *in vitro*. PIP5K2, when overexpressed, induces pollen tube tip swelling and disrupts actin dynamics, but co-overexpression with ADF2 maintains ADF2's fast dynamics despite the swelling. Furthermore, overexpression of the pollen tube-specific Myosin Class VIII ATM2 results in ADF2 becoming cytosolic, losing its binding to F-actin. Finally, systematic analysis of pollen tube morphologies following ADF2 overexpression shows that PIP2 overproduction does not affect ADF activity, challenging previous *in vitro* findings that suggested PIP2 binding reduces ADF activity. This discrepancy can be attributed to the complex interactions within the native membrane environment, involving lipid nanodomains, the actin cytoskeleton, and membrane-associated protein kinases. Overall, this study, is the first of its kind that examines ADF2 localization and its relationship with PI4P5 kinase 2 *in vivo* at the pollen tube tip.

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- Quantitative 3D Modelling of Organelle Ultrastructure in Native Guard Cells Using Cryo-FIB-SEM**  
 X. Zhang<sup>1</sup>, B. Franzisky<sup>2</sup>, C. J. Burkhardt<sup>3</sup>, E. Majorovits<sup>4</sup>, E. Hummel<sup>4</sup>, A. Schertel<sup>4</sup>, C. M. Geilfus<sup>2</sup>, C. Zörb<sup>1</sup>  
<sup>1</sup>University of Hohenheim, Institute of Crop Science, Quality of Plant Products, Stuttgart, Germany  
<sup>2</sup>Hochschule Geisenheim University, Department Soil Science and Plant Nutrition, Geisenheim, Germany  
<sup>3</sup>University of Tübingen, NMI Natural and Medical Sciences Institute, Tübingen, Germany  
<sup>4</sup>Carl Zeiss Microscopy GmbH, Oberkochen, Germany

The stomata serve as crucial gatekeepers responsible for the exchange of atmospheric CO<sub>2</sub> and water vapor, with the dynamic aperture and ultrastructure of guard cells exhibiting high sensitivity to environmental stimuli. The Faba bean serves as an excellent model for studying stomata due to its responsiveness to external factors and easy epidermis isolation. Traditional sample preparation methods for electron microscopy typically involve chemical fixation, heavy metal staining, and resin embedding, all of which pose a risk of distorting cell morphology and compromising membrane integrity. To address these concerns, plunge-freezing in liquid ethane has been employed to rapidly transition cells to a vitreous state. This approach preserves cell structure in a fully hydrated, near-native state, facilitating subsequent electron microscopy observation under cryogenic conditions. Cryo-Focused Ion Beam- Scanning Electron Microscopy (cryo-FIB-SEM) volume imaging was utilized to visualize the subcellular ultrastructure of Faba bean guard cells. By processing cryo- FIB-SEM data, 3D models of guard cell organelles such as chloroplasts, stromules, mitochondria, and vacuoles are reconstructed, allowing quantification of their surface area and volume. This near-native volume imaging technique enables investigation into how environmental factors such as drought or salinity influence stomatal behavior and the morphology of guard cells and organelles.

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- Exploring manganese function and biology with genetically encoded Mn<sup>2+</sup>-Biosensor**  
 N. Zhou<sup>1</sup>, L. Wallrad<sup>1</sup>, L. Schmitt<sup>2</sup>, J. Kudla<sup>1</sup>  
<sup>1</sup>University of Münster, Institute of Plant Biology and Biotechnology, Münster, Germany  
<sup>2</sup>Institute of Biochemistry, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

Manganese (Mn) is an essential metal ion in all living organisms that acts as an important cofactor in many enzymes, and Mn homeostasis is necessary for plant growth and development. Recently two different sensors are reported to monitor Mn in bacteria and mammalian cells respectively, but currently little is known about the actual concentration of Mn in plant cell due to the absence of Mn sensors for plant. Here we report the genetically encoded fluorescent sensor engineered from Yellow Cameleon 3.6 called Yellow Mangaleon (YM) for manganese. Our data indicate that this sensor has high affinity and selectivity for Mn<sup>2+</sup> over Ca<sup>2+</sup> and it reports the Mn<sup>2+</sup> uptake and dynamics not only in plant root cell but also in HEK293T cells. Therefore, our studies establish a new sensor as a versatile tool for Mn detection in plant cells and mammalian cells.

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- A quantitative study of the mitochondrial transcriptome in the model plant *Arabidopsis thaliana***  
 M. Marofke<sup>1</sup>, K. Kühn<sup>1</sup>  
<sup>1</sup>MLU Halle-Wittenberg, Institute for Biology/Plant Physiology, Halle a. d. Saale, Germany

As organelles of endosymbiotic origin, plant mitochondria contain a residual genome and have their own gene expression system. Although mitochondrial gene expression and oxidative phosphorylation (OXPHOS) depend on a large number of nuclear encoded and imported proteins, all products of the mitochondrial genome are necessary to maintain mitochondrial function.

Investigations of mitochondrial gene expression usually focus on large populations of mitochondria, but each individual mitochondrion functions in the living cell. Therefore, the aim of this study has been the absolute quantification of the mitochondrial transcriptome in *Arabidopsis thaliana*. Based on the strategy of an earlier proteomic analysis, which had estimated absolute numbers of individual proteins in a single mitochondrion, we assessed absolute copy numbers of mitochondrial transcripts in a single, average Arabidopsis mitochondrion using quantitative Northern Blots and RT-qPCR. As a reference, RNA spike-ins were added during the extraction of RNA from a defined amount of isolated mitochondria.

The mRNA copy numbers calculated per mitochondrion were unexpectedly low. Even abundant transcripts, i.e. those encoding OXPHOS complex subunits, were represented by less than ten copies per mitochondrion. We also observed mRNAs with less than one copy per mitochondrion, coding for ribosomal proteins and proteins of the cytochrome *c* maturation system.

The determined transcript copy numbers per single mitochondrion provide new insight into the composition of this organelle. Considering that not every mitochondrion contains a genome, these results enable new hypotheses on how do plant mitochondria maintain their gene expression and balance OXPHOS biogenesis based on only a few mRNA molecules per mitochondrion.

P 126

The Ca<sup>2+</sup>-signaling associated protein kinases CIPK23 and CPK21 have an opposite impact on stomatal movements

S. Huang<sup>1</sup>, T. Arjus<sup>2</sup>, H. Kollist<sup>2</sup>, D. Geiger<sup>1</sup>, R. Roelfsema<sup>1</sup>

<sup>1</sup>University of Würzburg, Molecular Plant Physiology and Biophysics, Würzburg, Germany

<sup>2</sup>University of Tartu, Plant Signal Research Group, Tartu, Estonia

Stomatal movements are controlled by a variety of protein kinases, including members of the CBL-interacting protein kinase (CIPK) and calcium-dependent protein kinase (CPK) families. Studies with loss-of-function mutants revealed that protein kinases in both families have important functions in guard cells, but due to redundancy is difficult to assign specific roles for individual proteins. Here we present an approach, in which the expression of a single protein kinase is specifically induced in guard cells, after treatment with estrogen. Estrogen-induced expression of an overactive CIPK23 stimulated stomatal opening, which was linked to reduction in activity of S-type anion channels in guard cells. In contrast, the expression of an overactive CPK21 lead to stomatal closure and strongly impaired light-induced stomatal opening. Our data thus reveal that CIPK23 and CPK21 have opposite roles in regulation of stomatal movement, despite of the reports that both protein kinases are activated by elevated cytosolic Ca<sup>2+</sup> concentrations and can activate S-type anion channels. Future studies will need to reveal, if the contradictory functions of CIPK23 and CPK21 are an exception, or if they represent general roles of protein kinases in these families.

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Investigation of novel membrane contact sites in plants

V. Valencia<sup>1</sup>, A. Weber<sup>1</sup>

<sup>1</sup>Heinrich Heine University, Plant biochemistry, Düsseldorf, Germany

Eukaryotic cells consist of different functional units, called organelles. Cell homeostasis critically relies on the communication between these organelles via the exchange of substrates as signals, lipids and metabolites. Known transport mechanisms are vesicular trafficking and transport protein, but more and more data points towards an additional pathway – the exchange via membrane contact sites. In recent years, there has been increasing research on membrane contact sites, which are characterized as direct and physical interaction sites between two membranes. It is assumed that these contact sites exist at all membranous compartments. However, there is a disparity in knowledge of membrane contact sites in yeast and mammalian cells versus plants. In most studies, a homology-based method was used, which led to the discovery of specific interaction sites between ER and plasma membrane. Plants also contain chloroplasts as an additional compartment, which house the photosynthetic apparatus embedded within one of the largest membrane systems seen in nature. This implies that membrane contact sites in plants are likely to be unique and more complex.

This project aims to uncover novel membrane contact sites in plants. It uses a combined approach for visualization and proteome characterization. Initially, potential contact sites are visualized in a proximity-dependent approach using a split fluorescent protein (FP). Therefore, different membrane proteins of ER, chloroplasts and mitochondria are selected as anchors for the split FP. Subsequently, taking advantage of visualized contact sites, a proximity labeling approach using TurboID is applied in order to investigate their putative interaction network. This comprehensive approach promises to uncover new insights into the organization and function of membrane contact sites in plants.

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Altered Ca<sup>2+</sup> signature promotes side branch formation via actin cytoskeleton modification in the moss *Physcomitrium patens*

J. Knab<sup>1</sup>, B. Kost<sup>1</sup>

<sup>1</sup>Friedrich-Alexander Universität Erlangen-Nürnberg, Cell Biology, Erlangen, Germany

Apical protonemal cells of the moss *Physcomitrium patens* elongate by tip-growth, a highly polarized process, which depends on an oscillating tip-focused cytoplasmic Ca<sup>2+</sup> gradient. An F-actin focal point near the cell apex, which is required for tip growth, was proposed to disassemble at peak Ca<sup>2+</sup> concentrations and to reassemble afterwards. Ca<sup>2+</sup>/actin interplay was also suggested to be important for the determination of lateral growth sites during protonemal branching.

Plant CNGCs (cyclic nucleotide gated channels) were reported to control spatio-temporal changes in cytosolic Ca<sup>2+</sup> concentrations with important functions in the regulation of many biological processes. In *Physcomitrium patens* the CNGC family consists of eight members, which based on sequence homology can be assigned to two different groups. CNGC quadruple knock-out (*cngc4xKO*) mutants lacking all members of one of these two groups were found to display disturbed signatures of tip-focused Ca<sup>2+</sup> oscillations. This observation demonstrates, that the CNGCs missing in these mutants are involved in modulating cytoplasmic calcium concentrations at the tip of apical protonemal cells. Phenotypic analysis of *cngc4xKO* mutants revealed developmental defects including excess branching and the formation of multiple apical cells, which indicates polarity defects. These defects appear to be caused by enhanced F-actin formation resulting from aberrant calcium oscillations. Treatment with Latrunculin B, which destabilizes F-actin, rescued the mutant phenotype. In contrast, jasplakinolid treatment, which stabilizes F-actin, resulted in increased branching of WT moss colonies. Furthermore, based on low-speed sedimentation assays *cngc4xKO* mutants were determined to contain increased F-actin levels as compared to WT plants.

Interestingly, *cngc4xKO* mutants were also found to contain reduced levels of ROS (reactive oxygen species) produced by RBOH (respiratory burst oxidase homolog) proteins, and were observed to be partially phenocopied by *rboh* mutants missing multiple RBOH isoforms. These results suggest that positive feedback regulation of cytoplasmic Ca<sup>2+</sup> concentrations and ROS levels may play an essential role in control of protonemal development.

P 129

mTERF21 – an essential protein for RNA processing in Arabidopsis mitochondria

T. Schoeller<sup>1</sup>, C. Goldhardt<sup>1</sup>, L. Brings<sup>1</sup>, M. Kim<sup>1</sup>, M. Schattat<sup>1</sup>, E. H. Meyer<sup>1</sup>, E. Vierling<sup>1</sup>, K. Kühn<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle-Wittenberg, Plant Physiology, Halle a. d. Saale, Germany

Mitochondria play pivotal roles in cellular energy metabolism and are essential to cell survival. The complex post-transcriptional processes involved in mitochondrial gene expression, which largely depend on factors that are encoded in the nucleus and imported into mitochondria, are still not fully understood at the molecular level.

In mammals as well as in plants, members of the mitochondrial transcription termination factor (mTERF) family play major roles in organellar gene expression. Belonging to the superfamily of solenoid-shaped helical-repeat proteins, mTERFs have been shown to possess nucleic acid binding properties. For the 35 mTERF proteins identified in *Arabidopsis thaliana*, only a few different mTERF mutant alleles have been recovered and studied so far.

One of the essential proteins identified in *A. thaliana* is the mitochondrially localized mTERF21. *mterf21* T-DNA insertion mutants are mostly unable to develop viable embryos. Studies performed with hypomorphic *mterf21* mutants revealed defects in two complexes of the oxidative phosphorylation system in Arabidopsis mitochondria. RT-qPCR experiments investigating the abundance of spliced and unspliced mitochondrial transcripts in *mterf21* mutants indicated defective splicing for 19 out of the 23 mitochondrial introns, which is unique for an mTERF protein.

Due to the high number of affected introns, we suspected an interaction of mTERF21 with other proteins. To test this hypothesis, we rescued an *mterf21* loss-of-function mutant with a *pmTERF21::mTERF21-GFP* construct and performed co-immunoprecipitation experiments on mitochondrial extracts. Surprisingly, we were not able to identify any protein-protein interaction. However, we pulled down a high number of RNA-binding proteins in non-RNase treated samples, indicating an interaction with nucleic acids. Subsequent electrophoretic mobility shift assays detected a non-specific interaction of mTERF21 with ssRNA, but not with dsRNA or DNA. In order to identify transcripts associated with mTERF21 *in vivo*, we are currently performing RNA-immunoprecipitation experiments.

Altogether, mTERF21 is an ssRNA-binding protein that is essential for the accumulation of mature mRNAs from most intron-containing mitochondrial genes in *A. thaliana*.

P 130

Development of a high-throughput split-nanoLUC screen for novel protein interaction partners in Arabidopsis protoplasts

M. Reimers<sup>1</sup>, C. Delker<sup>1</sup>, J. Stuttmann<sup>2</sup>, M. Quint<sup>1</sup>, L. Eschen-Lippold<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle-Wittenberg, Institut für Agrar- und Ernährungswissenschaften, Halle a. d. Saale, Germany

<sup>2</sup>Institute of Biosciences and Biotechnologies of Aix-Marseille, Aix en Provence, France

Signal transduction processes within a given cell generally require formation of specific functional protein complexes. Thus, identification of physiologically relevant protein-protein interactions is the key to elucidation of signal transduction pathways. We are developing an *in vivo* screening method using split-nanoLuciferase (nLUC) technology to identify novel interaction partners of any protein of choice expressed in Arabidopsis mesophyll protoplasts. Potential interaction partners are co-expressed from stimulus- or tissue-specific subtracted cDNA libraries to enrich for clones relevant to the studied signal transduction process. The potential partners carry corresponding split-nLUC-tags to reconstitute functional nLUC in case of interaction, resulting in quantifiable light emission. Since nLUC displays up 100 times brighter luminescence than standard firefly or Renilla luciferases, we aim at screening complex cDNA libraries in high-throughput format by transforming sub-pools of clones in 96-well plates. Pools containing putative interactors will then be further analyzed in detail. Results of test experiments conducted during the setup of the screening system will be presented, possible difficulties and pitfalls are discussed.

P 131

Monitoring the Activity of a Dually-targeted Rna Polymerase *in Vivo*

T. Kiesel<sup>1</sup>, S. Dwiani<sup>1</sup>, K. Kühn<sup>1</sup>

<sup>1</sup>Martin-Luther-University, Cellphysiology, Halle a. d. Saale, Germany

In *Arabidopsis thaliana* there are three different nucleus-encoded organellar phage-type RNA polymerases: RPOTp in plastids, RPOTm in mitochondria, and RPOTmp which is localized in plastids and mitochondria. RPOTmp performs transcription of genes in both organelles. This project aims to further unravel the roles of RPOTmp and RPOTm in mitochondria by evaluating contributions of these enzymes to transcriptional processes. Previous work done in our group has used differential RNA-Seq (dRNA-Seq) to determine RPOTmp- and RPOTm-dependent genes and promoters in mitochondria. While this work has given important insight into the roles of RPOTmp and RPOTm, it also showed that a nascent transcript 5' end sequencing approach (5'-GRO-Seq) is needed to capture promoters that give rise to fast-processed transcripts and to quantitatively evaluate the contributions of both enzymes to transcriptional processes. For this, protocols developed for analysing nuclear nascent transcripts have been adapted to Arabidopsis mitochondria. This approach is currently being optimized and combined with the previously established mitochondrial dRNA-Seq strategy performed on mitochondrial total RNA. Using a formerly developed algorithm to determine transcription start sites (TSS), 64 TSS were identified from a first 5'-GRO-Seq dataset. 51 TSS were newly discovered, while 13 of the TSS had already been identified in previous dRNA-Seq experiments. The data from the combined 5'-GRO-Seq and dRNA-Seq experiments provided us with preliminary information on contributions of individual mitochondrial RNA polymerases to the steady-state transcriptome. Comparing coverages of TSS between the nascent and steady-state datasets showed that coverages at 4 TSS were significantly enriched in the steady-state RNA pool. Thus, the corresponding transcript 5' ends seem to be post-transcriptionally stabilized. Interestingly the data also revealed that in both the steady-state and nascent RNA pools, the highest read coverages are observed for RPOTm-dependent TSS. This indicates an important contribution of transcriptional processes to the mitochondrial steady-state RNA pool.

P 132

The PPR protein PPR596 is important for splicing of *nad2* and assembly of Complex I  
in mitochondria of *Arabidopsis thaliana*

L. Peters<sup>1</sup>, K. Kühn<sup>1</sup>

<sup>1</sup>Martin Luther Universität Halle-Wittenberg, Zellphysiologie, Halle a. d. Saale, Germany

Pentatricopeptide repeat (PPR) proteins have been shown to be involved in every stage of organellar gene expression. Typically targeted to either mitochondria or chloroplasts, proteins that belong to this family are capable of binding to RNA and subsequently influence RNA metabolism, e.g. by contributing to RNA stabilization, editing or splicing. Two different PPR subclasses (P- and PLS-subclass) have been defined, differing in the types of PPR motifs they are composed of. PPR596, an *Arabidopsis thaliana* P-subclass PPR protein, has previously been reported to be involved in C-to-U RNA editing as well as splicing. This study aims to show that the severe growth phenotype displayed by homozygous *ppr596* mutant plants is primarily a result of a splicing defect for *nad2* intron 3. In order to prove this, mitochondrial transcript analyses by northern blots and RT-qPCR as well as various protein analysis methods were used. NAD2 is a core subunit of the Complex I (CI) of the OXPHOS system in mitochondria. Integrated into the membrane arm of the complex at an early stage, NAD2 is essential for proper assembly of CI. Defective splicing of *nad2* results in a reduced assembly of complex I. Due to this, plants lacking PPR596 display lower CI abundance and activity, thus impairing respiration and plant development.

As the specific mechanisms involving PPR596 and its functions in splicing and RNA editing remain elusive, additional studies involving potential interaction partners of PPR596 could provide further knowledge.

#### References

Barkan, Alice; Small, Ian (2014): Pentatricopeptide repeat proteins in plants. In *Annual review of plant biology* 65, pp. 415–442. DOI: 10.1146/annurev-arplant-050213-040159.

Doniwa, Yoko; Ueda, Minoru; Ueta, Masami; Wada, Akira; Kadowaki, Koh-ichi; Tsutsumi, Nobuhiro (2010): The involvement of a PPR protein of the P subfamily in partial RNA editing of an *Arabidopsis* mitochondrial transcript. In *Gene* 454 (1-2), pp. 39–46. DOI: 10.1016/j.gene.2010.01.008.

Sayyed, Aqib; Chen, Baoyin; Wang, Yong; Cao, Shi-Kai; Tan, Bao-Cai (2024): PPR596 Is Required for *nad2* Intron Splicing and Complex I Biogenesis in *Arabidopsis*. In *International journal of molecular sciences* 25 (6). DOI: 10.3390/ijms25063542.

P 133

Plastid morphology impacts jasmonate biosynthesis

R. Baral<sup>1</sup>, H. Stellmach<sup>1</sup>, B. Hause<sup>1</sup>

<sup>1</sup>Leibniz institute of plant biochemistry, Halle a. d. Saale, Germany

Plastids are double-membrane organelles, highly dynamic and exist in various interconvertible forms in response to developmental or environmental cues. They are involved in different metabolic processes including photosynthesis, amino acid synthesis, lipid metabolism and hormone biosynthesis, including biosynthesis of 12-*cis*-oxo-phytodienoic acid (OPDA), the main precursor of jasmonic acid (JA). JA is one of the vital phytohormones that regulate multifarious aspects of development as well as stress responses. With the utilization of genetic, molecular, and analytical approaches, we aimed to elucidate the impact of plastid morphology on JA biosynthesis. Our study shows the development of a microscopy-based method to characterize plastid morphology, especially chloroplasts in true leaves of *Arabidopsis thaliana*. Mutation of *ARC* genes (*ACCUMULATION AND REPLICATION OF CHLOROPLAST*; *ARC3* and *ARC5*) leads to mesophyll cells with altered numbers of chloroplasts showing an altered morphology. Wounding of leaves of *arc3* and *arc5* mutants resulted in OPDA levels that were different from those of wild type (*Landsberg erecta*). These changes also include altered expression of genes encoding JA biosynthetic enzymes located in the plastids. The study paves the way for better morphological characterization of organelles as well as depicts the impact of plastids on OPDA/JA biosynthesis.

P 134

Towards the role of dual or even triple organellar localization of Whirly proteins in plant cells

N. Kistner<sup>1</sup>, R. B. Klösigen<sup>1</sup>, B. Bennewitz<sup>1</sup>

<sup>1</sup>MLU Halle-Wittenberg, Halle a. d. Saale, Germany

Whirly proteins are a class of highly conserved DNA-binding proteins that are found in a wide range of plant species. They are encoded by a small family of nuclear genes and synthesized in the cytoplasm before being targeted to chloroplasts, mitochondria, or both endosymbiotic organelles. Whirly proteins play crucial roles in maintaining organellar function, e.g. as stress and redox sensors. Since Why-1 has furthermore been found also in the nucleus, it was suggested that the Whirly proteins are involved in retrograde signalling orchestrating, for example, the coordinated expression of nuclear and plastidial photosynthetic genes. Yet, the exact mechanisms by which the Whirly proteins are targeted to the different subcellular compartments are still being explored. In particular, the pathway into the nucleus (directly from the cytosol or by export or release from the endosymbiotic organelles?) remains enigmatic. Understanding these processes will not only shed light on the complex biology of Whirly proteins but also provide insights into the regulation and integrative cooperation of plant organelles.



P 135

Anion Uptake by Guard Cells

N. Ahmad<sup>1</sup>, R. Roelfsema<sup>1</sup>

<sup>1</sup>Julius-Maximilians-Universität of Würzburg (JMU), Pflanzenphysiologie und Biophysik, Würzburg, Germany

Guard cells control stomatal movements by increasing and decreasing their cell volume, through the uptake and release of ions from their cell walls. The influx of ions causes an osmotic gradient that drives water uptake through osmosis, resulting in the expansion of guard cells and subsequent stomatal opening. With respect to cations, guard cells mainly transport potassium ions (K<sup>+</sup>), to change their osmotic value, while the SLAC1/SLAH3 channel are responsible for release of nitrate (NO<sub>3</sub><sup>-</sup>) and chloride (Cl<sup>-</sup>). However, the mechanisms facilitating anion uptake during stomatal opening is hardly explored. To close this gap, we employ the Scanning Ion Selective Electrode (SISE) technique to measure hydrogen (H<sup>+</sup>) fluxes across the plasma membrane of guard cells in *Vicia faba*. Since anion uptake is facilitated by co-transport with H<sup>+</sup>, this approach can be used to monitor the uptake of specific anions i.e. NO<sub>3</sub><sup>-</sup> or Cl<sup>-</sup>. Our aim is to clarify if the nutrient availability of *V. faba* plants affects the selectivity of guard cells for uptake of NO<sub>3</sub><sup>-</sup> versus Cl<sup>-</sup>. Such a change in anion preference may have consequences for the control of stomatal movements and thus affect the water usage efficiency of this crop plant.

P 136

Nuclear/cytoplasmic distribution and posttranslational regulation of the Arabidopsis PI4P 5-kinase PIP5K1

M. Lebescond<sup>1</sup>, L. Schwalgun<sup>1</sup>, M. Heilmann<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle, Plant Biochemistry, Halle a. d. Saale, Germany

Phosphoinositide (PI) signaling has been implicated in the regulation of multiple cellular processes. Studies have shown that in addition to already known plasma membrane-localized PI-regulated processes, PIs and PI-forming lipid kinases very probably also exist in the cell nucleus and studies in all eukaryotic systems show that they may have an influence on the cell cycle, DNA repair, mRNA export and gene transcription in the cell nucleus. In Arabidopsis Phosphatidylinositol 4-phosphate 5-kinases (PIP5Ks), enzymes producing the regulatory PI phosphatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub>), are present in a variety of subcellular locations, including the plasma membrane and the nucleus. Recent results from our group show that the PIP5K1 isoform contains a functional nuclear localization signal sequence (NLS) and interacts with importin alpha isoforms, indicating that nuclear localization of PIP5Ks is controlled by the active nuclear import machinery. However, the nuclear localization of PIP5Ks in plants appears to be either dynamic and/or tightly regulated, as the nuclear localization of PIP5Ks is not equally concise in all developmental stages and all cell types. Moreover, the distribution between the plasma membrane and the nucleus suggests that, in addition to functional nuclear localization signals, further regulation is required so that function is not restricted. We hypothesize that nuclear localization of PIP5Ks is dynamically regulated by NLS that might be masked or active, depending on posttranslational modifications, such as phosphorylation. We have already demonstrated *in vitro* phosphorylation of PIP5K1 by the protein kinase casein kinase 2 (CK2) from *Arabidopsis thaliana* and identified three serine residues as CK2 phosphorylation sites in the PIP5K1 amino acid sequence by mass spectrometry. Phosphomimicry and phosphoablation variants of PIP5K1 were designed and the initial results indicate that CK2 phosphorylation leads to a reduced *in vitro* activity and an increased nuclear localization of PIP5K1 in Arabidopsis protoplasts, indicating, that CK2-dependent PIP5K1 phosphorylation is both a spatial and a temporal mechanism for the regulation of PI dynamics in Arabidopsis. These results will be confirmed by further *in vivo* and *in planta* studies in the future, which will hopefully also provide information on the physiological relevance.

P 137

Calcium and CDPK in the longevity of plants

O. Nagel<sup>1</sup>, S. Lederer<sup>1</sup>, A. Liese<sup>1</sup>, T. Romeis<sup>1</sup>, F. P. Sylvester<sup>1</sup>, P. Schulz<sup>1</sup>

<sup>1</sup>Leibniz-Institute for Plant Biochemistry, Halle a. d. Saale, Germany

During their lifetime plants undergo diverse physiological changes based on a complex regulatory network. The period of senescence is defined as the last one. Beside of hormones, reactive oxygen species and other regulatory mechanisms, calcium is discussed as a positive regulator in the process of senescence. The underlying molecular mechanism is unknown. Calcium-dependent protein kinases (CDPKs) are regulators in signal transduction pathways, which can sense calcium signals and translate them into specific downstream target phosphorylation. We identified Arabidopsis *CPKSEN*, which functions as a positive regulator of the longevity. The *in vitro* biochemical analysis with recombinant, purified enzyme reveals a clear calcium dependency in kinase assay, displaying a low K<sub>50</sub> for calcium. This indicates a high responsiveness at subtle intracellular calcium changes for *in vivo* function, and single EF hand motifs differ in their contribution to this calcium sensitivity. *CPKSEN* overexpression lines reveal increased longevity correlating with higher chlorophyll content in dark-induced senescence compared to the wildtype, whereas *cpkSEN* mutant lines are characterized by an early senescence phenotype. Our data implicate *CPKSEN* function at the link between developmental calcium signalling and senescence

P 138

### Molecular Characterization of the Activation Mechanism of the Small GTPase, Arabidopsis Immune-Associated Nucleotide-Binding Protein 12 (AtIAN12), and the Regulation of its Targeting to the Membrane of ER Bodies

S. Pokhrel<sup>1,2</sup>, C. Falter<sup>1</sup>, S. Seemann<sup>1</sup>, F. Mousavimehr<sup>1</sup>, S. Reumann<sup>1</sup><sup>1</sup>Universität Hamburg, Institute for Plant Science and Microbiology, Hamburg, Germany<sup>2</sup>University of California, Institute of Neurodegenerative Diseases, San Francisco, CA, United States

Small GTPases are versatile molecular switches that regulate important cellular processes. Members of the small GTPase family of immune-associated nucleotide-binding (IAN) proteins mediate immune responses in mammals and plants. To date fundamental properties such as GTPase classification, activation mechanism, and structure remain unknown even for the 13 members of Arabidopsis IAN GTPases. We selected AtIAN12 as a representative model protein, which consists of a conserved G-domain and a C-terminal coiled-coil domain terminating with a prenylation site, to determine central features of the family members. Recombinant His6-tagged AtIAN12 could be produced in *E. coli* at high homogeneity and indeed showed high GTPase activity. Yeast two-hybrid analyses revealed that AtIAN12 homodimerizes via the G-domain, and this interaction was dependent on one or two acidic amino acid residues in the G4 motif (E175 and D176), as confirmed *in planta* by rBiFC analyses. While D176 was essential for AtIAN12 homodimerization, E175 was not. The importance of D176 for GTP binding and thus AtIAN12 homodimerization is consistent with the AlphaFold 3 model. Besides, the invariant R144 of the AIG1 family-specific conserved box within the G-domain was identified as a catalytic residue that, analogously to human GIMAP7, most likely serves as an Arg finger in *trans* complementing the active site of the opposing protomer. These properties allowed the assignment of AtIAN12 as the first family member of plant IAN proteins to the unconventional group of small GTPases that are activated by dimerization (GADs). *In vivo* subcellular targeting analyses in Arabidopsis cotyledons showed that solely the EYFP-tagged constitutively active mutant (R144L) of AtIAN12 is directed specifically to the membrane of ER bodies, which are ER-derived organelles of Brassicaceae important for the plant immune system. We conclude that AtIAN12 is a membrane-anchored small GTPase of the GAD family and is directed in its activated ON state to the membrane of ER bodies to carry out its yet unknown role in pathogen defence. Present work aims at performing structural analyses of different mutated AtIAN12 versions via Small-angle X-ray scattering and crystallisation in the presence of non-hydrolyzable GTP analoga.

P 139

### Characterization of a copper deficiency-sensitive natural accession in barley

L. Yang<sup>1</sup>, N. von Wirén<sup>1</sup><sup>1</sup>Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Department Physiology and Cell Biology, Seeland, Germany

Copper is one of the eight plant micronutrients and takes over essential functions in photosynthesis, respiration, antioxidant defense, and lignin synthesis. To date, only very few genes related to copper homeostasis have been identified in crops, including barley. Here, we analyzed the phenotypes of a 6-row spring barley (*Hordeum vulgare* L.) panel and found that one accession exhibited distinct copper deficiency symptoms, expressing as leaf chlorosis and leaf tip necrosis. ICP-MS analysis showed that copper concentrations in the symptomatic leaf tips were around 5 µg/g, which is close to critical Cu deficiency levels, while copper concentrations in other leaves were about twofold higher. Spray application trials showed that these symptoms were alleviated by foliar supply of copper chelates. In further hydroponic experiments, leaf symptoms in this accession were clearly alleviated by increased copper supply. In addition, moderate increases in copper supply promoted root elongation of this accession, while its roots were less sensitive to copper toxicity. We conclude that phenotypes of the identified accession may result from a genetic defect in copper uptake or translocation. As we believe that studying this accession will help to uncover mechanism of copper homeostasis in barley, we are currently crossing this line to generate a biparental population for subsequent QTL mapping of the corresponding locus. Keywords: Barley, copper deficiency, leaf chlorosis, root sensitivity

P 140

Conquering new grounds: Plant organellar C-to-U RNA editing factors can be functional in the plant cytosol

M. Thielen<sup>1</sup>, B. Gärtner<sup>1</sup>, V. Knoop<sup>1</sup>, M. Schallenberg-Rüdinger<sup>1</sup>, E. Lesch<sup>2,1</sup>

<sup>1</sup>University of Bonn, Biology - IZMB, Bonn, Germany

<sup>2</sup>University of Münster, Biology - IBBP, Münster, Germany

Up to thousands of cytidines are specifically converted into uridines in plant organellar RNAs, mainly to correct genetic information. Key players are the PLS-type pentatricopeptide repeat (PPR) proteins. They consist of an N-terminal PPR array that binds to their respective target RNA and a C-terminal DYW-type cytidine deaminase domain. The model moss *Physcomitrium patens* features a simple and likely ancestral editing machinery with nine editing factors that target 13 organellar editing sites, whereas RNA binding and cytidine deamination functionalities may be split into two proteins that have to reassemble in higher plants.

Although translated in the cytosol, PPR editing factors only modify organellar RNAs. To explore, whether cytosolic editing can be induced artificially, we aimed to overexpress three mitochondrial editing factors of *Physcomitrium* – PPR56, PPR65 and PPR78 – together with their respective native target sites encoded in the 3'-UTRs. Strong constitutive overexpression, however, failed and no cytosolic editing could be detected, although mitochondrial editing defects were complemented in the respective knockout lines. Intriguingly, using an inducible promoter finally enabled the strong overexpression of editing factors that elicited editing of the co-transcribed cytosolic targets, even upon retention of the organellar targeting peptides.

RNA sequencing uncovered that RNA editing activities are not restricted to the co-transcribed targets in close proximity to the nascent protein, but extend to further nucleo-cytosolic transcripts. Off-target profiling revealed specific interactions of the three editing factors with the targeted transcripts that widely follow the PPR-RNA binding code. Additionally, nucleotide preferences beyond the “core code” were noted, and the amounts of off-targets and level of specificity strongly differed between the three tested proteins.

Our study finally proved that (i) PPR-type editing factors are not *per se* incompatible with the plant cytosol, (ii) their un- and re-folding upon organellar import, likely including the cleavage of the organellar targeting peptides, is not a prerequisite for their functionality, and (iii) plants must restrict their activities to the organelles by limiting their expression, because they lack specificity in the larger nucleo-cytosolic transcriptome. Moreover, we offer a new platform to study the specificities of native, chimeric and synthetic PPR editing factors in the future.

P 141

Characterization of evolutionary conserved PFA-DSP-type phosphohydrolases in *Arabidopsis thaliana*

K. Lami<sup>1</sup>, P. Gaugler<sup>1</sup>, R. Schneider<sup>1</sup>, G. Schaaf<sup>1</sup>, V. Gaugler<sup>1</sup>, G. Liu<sup>2</sup>, D. Qiu<sup>2</sup>, H. J. Jessen<sup>2</sup>, D. Laha<sup>3</sup>

<sup>1</sup>Bonn University, Plant Nutrition, Bonn, Germany

<sup>2</sup>Freiburg University, Institut für Organische Chemie, Freiburg i. Br., Germany

<sup>3</sup>Indian Institute of Science, Bengaluru, India

Inositol pyrophosphates (PP-InsPs), such as InsP7 and InsP8, are molecules derived from myo-inositol (Ins) esterified with unique patterns of monophosphates (P) and diphosphates (PP), and have been described as versatile messengers in yeast, amoeba, and animal cells. With recent discoveries that PP-InsPs regulate nutrient sensing, hormone signalling and plant immunity, these molecules have become a novel focus of research in plant physiology. However, despite the rapid hydrolysis in plant extracts, very little is known about the molecular identity of the phosphohydrolases that convert these messengers back to their inositol polyphosphate precursors. Work in baker's yeast revealed the presence of a PP-InsP phosphohydrolase, named Siw14, with a high specificity for the  $\beta$ -phosphate at position C5 of 5-InsP7. This enzyme is a member of the Plant and Fungi Atypical Dual Specificity Phosphatases (PFA-DSPs) that belong to a large family of protein tyrosine phosphatases. Blast search analyses revealed that the *Arabidopsis thaliana* genome encodes five PFA-DSPs, which adopts an  $\alpha/\beta$  fold typical for cysteine phosphatases, with the predicted catalytic cysteine residing at the bottom of a positively-charged pocket. Here, we investigate whether *Arabidopsis* PFA-DSP1-5 catalyze inositol pyrophosphate phosphohydrolase activity. We will provide evidence that all five homologs display *in vitro* and *in vivo* phosphohydrolase activity with a high specificity for the 5- $\beta$ -phosphate of inositol pyrophosphates. We further show that heterologous expression of *Arabidopsis* PFA-DSP1-5 rescues wortmannin-sensitivity and deranged inositol pyrophosphate homeostasis caused by the deficiency of the PFA-DSP-type inositol pyrophosphate phosphohydrolase Siw14 in yeast. We also outline strategies to generate and employ higher order mutants to clarify the role of these enzymes in phosphate signaling and plant immunity. Our findings lay the biochemical basis and provide the genetic tools to uncover the roles of inositol pyrophosphates in plant physiology and plant development.

P 142

**Regulation of calcium homeostasis in the Golgi apparatus of *Arabidopsis thaliana***

N. Rössner<sup>1</sup>, S. Morghen<sup>2</sup>, F. Daamen<sup>1</sup>, J. He<sup>3</sup>, O. Mariani<sup>1</sup>, B. Meier<sup>1</sup>, E. Peiter<sup>1</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg, Plant Nutrition, Halle a. d. Saale, Germany

<sup>2</sup>Norwegian University of Science and Technology, Biology, Trondheim, Norway

<sup>3</sup>Yangzhou University, College of Bioscience and Biotechnology, Yangzhou, China

Our knowledge on mechanisms and impact of Ca<sup>2+</sup> homeostasis in the cellular compartments of the plant's secretory pathway is still scant. The Golgi apparatus may serve as Ca<sup>2+</sup> store or buffer in plants, and functions of this compartment are likely regulated by Ca<sup>2+</sup>. Differences in function between early and late Golgi compartments suggest that Ca<sup>2+</sup> regulation within the Golgi may be cisternae-specific. In this study, we investigated the dynamics and regulation of [Ca<sup>2+</sup>]<sub>Golgi</sub> by employing aequorin targeted specifically to *cis*- and *trans*-Golgi cisternae. Both showed a temporary rise in [Ca<sup>2+</sup>] in response to NaCl, whereby the response of the *trans*-Golgi was less pronounced. Two proteins were likely candidates to mediate this NaCl-induced Ca<sup>2+</sup> elevation - ECA3, a Golgi-localised P2A-type Ca<sup>2+</sup>/Mn<sup>2+</sup>-ATPase, and BICAT3, a Ca<sup>2+</sup>/Mn<sup>2+</sup> transporter related to GDT1 in yeast. The latter did not contribute markedly to the [Ca<sup>2+</sup>]<sub>cis-Golgi</sub> increase, which corresponded with its specific localisation in the *trans*-Golgi. In contrast, this response was strongly depressed in mutants devoid of ECA3. Intriguingly, these mutants were severely hypersensitive to salinity, which was due to the ionic effect of Na<sup>+</sup>, rather than Cl<sup>-</sup> or osmotic stress. The results indicate a cisternae-specific Ca<sup>2+</sup> handling in the Golgi that impacts stress tolerance in plants.

P 143

**CIPK25 as a direct or indirect regulator of the NRT2.1**

R. John<sup>1</sup>, T. Straub<sup>1</sup>, W. Schulze<sup>1</sup>

<sup>1</sup>University Hohenheim, Plant Systems Biology, Stuttgart, Germany

Nitrogen is an important plant nutrient involved in important process ranging from its role in nucleic acids to protein synthesis. Plants take up nitrogen in two major forms, nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) via specific transporters in the plasma membrane. The NRT2.1 is a high affinity transporter (HATS) in *Arabidopsis*, responsible for 75% of HATS activity. Post-translational modifications governing the abundance and activity of the NRT2.1 are still being elucidated. In the NRT2.1, five phosphorylation sites in the N and C-terminus were discovered. However, the understanding of signalling modules and the kinases involved in NRT2.1 regulation is still evolving.

Through a screening approach involving phosphoproteome profiling under different nitrogen regimes and by using mutants in transport and/or calcium signaling, we identified several kinases as putative candidate regulators of NRT2.1.

Here, we present Calcineurin B-Like Interacting Kinase (CIPK) 25 as a putative kinase involved in the NRT2.1 pathway. Studies with Calcineurin B-Like (CBL) quintuple mutants (*cb1/4/5/8/9*) revealed a positive correlation of phosphorylation at T440 at the C-terminal regulatory domain of CIPK25 and of NRT2.1 at the S11 inactivating phosphorylation site. Known roles of CIPK25 include the regulation of auxin transport, cell elongation as well as involvement in ROS regulation and formation related to root differentiation in primary roots.

In combination with receptor kinase H<sub>2</sub>O<sub>2</sub>-Induced Ca<sup>2+</sup> Increases Like 1 (HPCAL1), which was also identified as a putative regulator of NRT2.1, we propose an indirect signaling pathway of CIPK25 regulating NRT2.1 or a direct CIPK25-NRT2.1 pathway.

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**Regulation of plant immunity through (sub)compartmentalization of the RNA granule protein TZF9**

A. Karkhanis<sup>1</sup>, S. Matschi<sup>1</sup>, J. Lee<sup>1</sup>

<sup>1</sup>Leibniz-Institut für Pflanzenbiochemie, Biochemistry of Plant Interaction, Halle a. d. Saale, Germany

How Mitogen-Activated Protein Kinases (MAPKs) act in multiple plant processes without erroneous crosstalk is still poorly understood. We propose phospho-coding and sub-compartmentalization of MAPK substrates in biomolecular condensates are part of the mechanism for signaling specificity maintenance. Biocondensates are considered as membraneless organelles which are formed through liquid-liquid phase separation. They are known to be involved in compartmentalizing RNAs and proteins which may be involved in post transcription regulation. The Tandem Zinc Finger 9 (TZF9) protein is a phospho-substrate of the MAPKs, MPK3/6. This RNA binding protein forms cytoplasmic granules and the immune-induced disappearance of the granules may reflect the release from translational arrest mediated by TZF9 (Tabassum et al. 2020). TZF9's ability to interact with specific Poly-A Binding Proteins (PABPs) and induce PABP condensate formation may interfere with recruitment of translational initiation complex components, thereby facilitating translational arrest. Alternatively, PABPs binding to a so-called R-motif in the 5'UTR regions of some defense-related mRNAs has been reported to mediate selective translation of such mRNAs (Wang et al. 2022). However, it is unclear if/how TZF9-PABP sub-compartmentalization into biomolecular condensates, is involved in regulating selective translation during plant immunity. To address this: (1) R-motif containing translational reporter assays will be used to study the relevance of TZF9-PABPs in selective translation of mRNAs during plant immunity; (2) Proximity labelling techniques will be employed to identify the composition TZF9 or PABP biocondensates.

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Exploring Uncharted Territories: New Genes for Sulfur Starvation Responses in Plants

S. Basu<sup>1</sup>, S. Kopriva<sup>1</sup>, D. Ristova<sup>1</sup>

<sup>1</sup>University of Cologne, Köln, Germany

Achieving sustainable agricultural [MOU1] practices relies on enhancing the efficiency with which crops utilize essential nutrients. Plants have evolved various adaptive mechanisms to cope with nutrient deficiency stress. These adaptations occur at the morphological, biochemical, and rhizosphere levels to enhance nutrient acquisition and utilization efficiency. Optimizing nutrient use efficiency (NUE) is crucial for the primary macronutrients – nitrogen, phosphorus, potassium, and sulfur. Sulfur, in particular, plays a pivotal role in plant growth, development, metabolism, and the ability to withstand environmental stresses. As an essential macronutrient, sulfur is indispensable not only for plant health but also for human nutritional needs. However, the molecular machinery controlling sulfur starvation responses in plants is poorly understood. Improving the NUE of sulfur, alongside other key nutrients, is a vital step towards engineering plants that utilize nutrients more effectively. Despite the importance of sulfur for crop yield and quality, little is known about molecular machinery responsible for regulating sulfur starvation responses. *ETHYLENE-INSENSITIVE3-LIKE3 (EIL3)* or *SULFUR LIMITATION1 (SLIM1)*, emerged as the first transcriptional to orchestrate multiple gene expressions under sulfur deficiency. It is involved in upregulating sulfate acquisition while concomitantly inducing glucosinolate degradation, enabling sulfur recycling. However, the mechanistic details of *SLIM1*'s regulation of sulfur starvation responses remain incompletely elucidated. These investigations into sulfur metabolism and regulation have predominantly centered on *Arabidopsis*. Consequently, gaining a more comprehensive understanding of sulfur-related processes in major crop plants is imperative. To contribute to these endeavours, in this work, we have performed RNA-seq analysis in rice and setaria plants under sulfur-starved conditions and compared our dataset with other sulfur starvation RNA-seq datasets from *Arabidopsis* and tomato. The intersection between four species has resulted in 7 common DEGs. We have characterized 4 of the 7 intersect genes and dissected their involvement in mitigating sulfur starvation responses.

Keywords: Sulfur, crop, rice, nutrients, RNA-seq.

References:

Dietzen C, Koprivova A, Whitcomb SJ, et al. *Plant Physiology*. 2020;184(4):2120-36.

Ristova D, Kopriva S. *IScience*. 2022;25(5).

Zenzen I, Cassol D, Westhoff P, et al. *BMC Plant Biology*. 2024;24(1):257.

P 146

Possible involvement of a ribosomal protein (*uL13x*) in growth under low calcium conditions in *Arabidopsis thaliana*

A. Kumari<sup>1</sup>, H. Fukuda<sup>1</sup>, N. Sotta<sup>1</sup>, D. Ma<sup>1</sup>, T. Fujiwara<sup>1</sup>

<sup>1</sup>The University of Tokyo, Department of Applied Biological Chemistry, Bunkyo, Japan

In *Arabidopsis*, there are ~81 ribosome proteins (RPs) and each is encoded by a gene family that consists of two to seven paralogous genes. These paralogous genes could be redundant or may be involved in specific plant growth or developmental processes, and defence response to environmental conditions. However, nutrient-specific roles of RPs are scarcely reported. Therefore, the main purpose of this work is to identify ribosomal protein mutant(s) showing a specific phenotype to calcium (Ca) conditions. After the phenotypic screening of twenty-one T-DNA insertional mutants, *uL13x* was selected as a candidate gene, because a mutant of *uL13x* exhibited a decreased root length phenotype under low Ca condition (0.2 mM), while showed an increase in root length under normal Ca condition (2.0 mM). Next, the phenotype of paralogs viz., *uL13z-1*, *uL13y-1*, *uL13x-1*, and *uL13w-1* was observed and root phenotype was found specific to *uL13x-1* under low Ca conditions. Hence, to know whether the declined root length phenotype has some difference in the accumulation of Ca and other macro- and micro-nutrients, the ionic status of WT and *uL13x-1* and *uL13x-2* under Ca conditions was analyzed. To know the expression pattern of *uL13x* in tissues, we used GUS-promoter assay (*puL13x:GUS*), and found that this gene is expressed in cotyledon, leaves, primary root tips, and lateral roots. For the confirmation of the causal gene, two independent homozygous complementation lines were generated by introducing the *uL13x* gene in *uL13x-1* under the control of its own promoter and the recovery of WT phenotype is indicated in preliminary observations, however, to confirm statistically, analysis is ongoing. The allelic lines (*uL13x-1* and *uL13x-2*) were used to form crossing lines to confirm the root phenotype under low Ca conditions is also in-progress. Besides, there was a significant difference was found in the root hair density of mutants and WT under Ca conditions. So, we will show updates of our study at the conference.

P 147

**Genetic approach to acquire new insights into members of the *Tracheophyta*-specific HIPP protein family**

O. Barth<sup>1</sup>, A. Parasyri<sup>1,2</sup>, W. Zschiesche<sup>1</sup>, L. Rehneke<sup>1,3</sup>, K. Humbeck<sup>1</sup>

<sup>1</sup>Martin-Luther-Universität Halle-Wittenberg, Inst. f. Biologie, Bereich Pflanzenphysiologie, Halle a. d. Saale, Germany

<sup>2</sup>Universität Hamburg, Inst. f. Pflanzenwissenschaften und Mikrobiologie Pflanzenbiochemie und Infektionsbiologie, Hamburg, Germany

<sup>3</sup>Justus-Liebig-Universität Gießen, Inst. f. Phytopathologie/ AG Dr. R. Schäfer, Gießen, Germany

Plants were faced with an at least temporary limited water availability during colonization of land. Approx. 400 million years ago new plant species evolved, which were able to generate vessels to transport water and nutrients (Xylem) as well as assimilates (Phloem) across long distances. This contrivance permits as prerequisite to develop the advantageous organs root, stem and leaf, partially with amazing huge dimensions. Obviously, a lot of new proteins were necessary to facilitate that important evolutionary step. Members of one of these novel vascular plant specific protein families are specified as Hheavy metal binding, Iisoprenylated Plant Proteins (HIPPs) due to their conserved protein domain structure (Barth et al., 2009. *Plant Molecular Biology* 69, 213; de Abreu-Neto et al., 2013. *FEBS Journal* 280:1604–1616.). A HIPP protein contains one or two heavy metal associated domains (HMA domain, PF00403, around 60 amino acid residues) and a C-terminal isoprenylation motive (either a CaaX-, CxxX- or CxxxX-Box).

The presence of HMA domains within HIPP proteins suggests a molecular function as heavy metal chaperone. Therefore, HIPPs may be involved in transportation of essential heavy metal ions (Cu<sup>1+</sup> or Zn<sup>2+</sup>) from specific importers at the plasma membrane to target proteins. In addition, due to their isoprenylation motif, HIPPs might receive a hydrophobic anchor which could be important for proper cellular localization, e.g., membrane association or protein-protein interactions. Interestingly, many HIPPs contain additional Nuclear Localization Signals (NLS), arising interesting speculations about functions in the nucleus.

In our Lab *P35S::AtHIPP5* gain-of-function *Arabidopsis thaliana* plants were produced, which show an interesting *AtHIPP5* overexpression phenotype with retarded development including flowering and effects on physiological processes like photosynthesis. These overexpressors were used to perform a *P35S::AtHIPP5* suppressor mutant screening to identify factors, which are functionally directly linked to *AtHIPP5*. One of these putative interactors will be presented on the poster.

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**The ancestral UPF0016 proteins Hmx1 and Hmx2 enable efficient manganese uptake in cyanobacteria**

M. Reis<sup>1</sup>, F. Brandenburg<sup>2</sup>, M. Knopp<sup>3</sup>, S. B. Gould<sup>3</sup>, M. Eisenhut<sup>1</sup>

<sup>1</sup>Bielefeld University, Computational Biology, Bielefeld, Germany

<sup>2</sup>Helmholtz-Centre for Environmental Research - UFZ, Leipzig, Department of Solar Materials, Leipzig, Germany

<sup>3</sup>Heinrich-Heine University Duesseldorf, Institute for Molecular Evolution, Düsseldorf, Germany

Manganese (Mn) is an essential micronutrient with special importance for photosynthetic organisms due to its function in water oxidation. To avoid critical imbalances, Mn needs to be specifically and timely allocated to the place where it is needed, and sequestered in a safe storage place if accumulating in excess. That is, key to Mn homeostasis is the controlled uptake from the environment and appropriate intracellular distribution of the metal. We have identified the Unknown Protein Family (UPF) 0016 as a new group of Mn transporter. The founding member Manganese exporter (Mnx) in the model cyanobacterium *Synechocystis* sp. PCC6803 facilitates Mn transport from the cytoplasm into the thylakoid lumen and is involved in Mn delivery to Photosystem II as well as sequestration of excess Mn [1]. Mnx-type transporters were endosymbiotically conveyed to plants and are here constituting efficient Mn uptake systems at the chloroplast envelope and the thylakoid membrane [2]. Two additional UPF0016 members, Hemi manganese exchangers (Hmx) Hmx1 and Hmx2, are exclusively encoded in cyanobacterial genomes. In contrast to the Mnx-type transporters, Hmx proteins are only half-sized and likely functionally assemble as heteromers. According to our physiological analyses they function as the long sought constitutive Mn importer at the plasma membrane. Furthermore, our phylogenetic analysis indicates that Hmx1 and Hmx2 are the ancestral progenitors of eukaryote-type UPF0016 proteins and likely coevolved with the internalization of the water oxidation complex [3]. Here, results of the functional and phylogenetic characterization of Hmx1 and Hmx2 will be presented and discussed in terms of their function in Mn homeostasis.

References

[1] Brandenburg F, Schoffman H, Kurz S, Krämer U, Keren N, Weber APM, Eisenhut M (2017). *Plant Physiol* 173:1798-1810. doi.org/10.1104/pp.16.01895

[2] Eisenhut M, Hoecker N\*, Birkelund Schmidt S, Basgaran RM, Flachbart S, Jahns P, Eser T, Geimer S, Husted S, Weber APM, Leister D, Schneider A (2018). *Mol Plant* 11:955-969. doi.org/10.1016/j.molp.2018.04.008

[3] Reis M, Brandenburg F, Knopp M, Flachbart S, Bräutigam A, Metzger S, Gould SB, Eisenhut M (2023). bioRxiv 2023.02.16.528846. doi: <https://doi.org/10.1101/2023.02.16.528846>

P 149

Identification and Functional Analysis of Ion Transporters Providing Salinity Tolerance in Halophytic Barley Relatives

S. Isayenkov<sup>1</sup>, B. Meier<sup>1</sup>, E. Peiter<sup>1</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg, Institute of Agricultural and Nutritional Sciences, Halle a. d. Saale, Germany

Salinity and drought are significant threats to global agriculture, adversely affecting crop productivity. Over 1125 million hectares of arable land worldwide (nearly 20% of irrigated land) are salt-affected. Among wild crop relatives, the Triticeae tribe, which exhibits a halophytic phenotype, stands out due to its anatomical, physiological, and genomic similarities to major cereal crops. *H. marinum*, commonly found in coastal areas and salt marshes, can grow and reproduce at salinity levels exceeding 450 mM NaCl. This species is considered the primary source of salinity tolerance for wheat and other cereals. Our recent study showed elevated expression levels of genes encoding various channels and transporters, including homologs to SLAH Cl-/NO<sub>3</sub>- channels, NRT1/PTR NO<sub>3</sub>- transporters, the SKOR K<sup>+</sup> channel, the BOR4 borate transporter, and a CHX cation exchanger [1]. The *HmBOR4* and *HmCHX16* genes have been successfully cloned for further functional analysis. Transformation of yeast strains deficient in K<sup>+</sup> uptake with *HmCHX16* demonstrated partial restoration of wild-type phenotypes in the mutant lines. Additionally, ion content analysis of transformed yeast revealed higher K<sup>+</sup> accumulation within 4 hours of incubation in K<sup>+</sup>-deficient media, suggesting that the HvCHX16 transporter is involved in K<sup>+</sup> transport. Transient expression of *HmBOR4* and *HvCHX16 mCherry* fusions in barley protoplasts revealed a Golgi-specific pattern for both transport proteins. The creation of *HvCHX16* and *HmBOR4*-expressing *Arabidopsis* and *H. vulgare* lines, as well as CRISPR-Cas9-modified *H. marinum* knockout lines, is in progress.

Furthermore, we have selected another wild barley species, *H. intercedens*, for comparative analysis of adaptation strategies with the model barley halophyte *H. marinum*. *H. intercedens*, an increasingly rare annual species found in Californian saline beds and alkaline soils, will be studied for its physiology, ionome, and gene expression profiles. Our primary focus on transcript profiling of salt/sodicity-treated halophytic barley species aims to facilitate the discovery of novel stress tolerance genetic traits as well as the identification and isolation of stress-specific ion transporters.

References

1 Isayenkov S et al. (2020). Adaptation strategies of halophytic barley *Hordeum marinum* ssp. *marinum* to high salinity and osmotic stress. Int. J. Mol. Sci. 21, 9019

P 150

The calcium-nitrogen-interaction on barley root metabolism and exudation

I. Denjali<sup>1</sup>, R. Abdel-Basset<sup>2</sup>, M. Persicke<sup>3</sup>, T. Seidel<sup>1</sup>, V. Kumar<sup>1</sup>, K. J. Dietz<sup>1</sup>

<sup>1</sup>Bielefeld University, Faculty of Biology, Bielefeld, Germany

<sup>2</sup>Assiut University, Botany and Microbiology Department, Faculty of Science, Assiut, Egypt

<sup>3</sup>Bielefeld University, The Center for Biotechnology – CeBiTec, Bielefeld, Germany

Ca and N, being functional components of plant cells, are central to plant growth and development. Nitrogen is crucial for central and specialized metabolism. Optimal N-availability for plants often depends on microbial nitrogen fixation or artificial N-fertilization of agricultural soils. N-uptake and transport are highly regulated, including through role for Ca<sup>2+</sup> signaling elements like calcium dependent protein kinases (CDPKs), calcineurin B-like proteins (CBLs) or CBL-interacting protein kinases (CIPKs). These calcium signals trigger different developmental responses depending on external N-availability, putatively involving Ca-availability changes too. Considering natural diversity in N and Ca availability, plants need to adapt and respond accordingly. In a barley (*H. vulgare*) hydroculture system, we focussed on revealing this putative N, Ca-interdependence through a 4x4 concentration array starting from a maximum 3.5mM N/1mM Ca and moving towards complete starvation, imitating differential N and Ca-availability. A Ca concentration dependent gradient in terms of reduced plant biomass accumulation was evident at optimal and moderate N-availability, while at increased N-depletion, the Ca-effects disappeared. Interestingly, the Ca-effects were more prominent on shoot biomass accumulation. The data implies a relation between Ca-availability and N-utilization/assimilation. Although, total N-accumulation data does not reflect Ca-dependency, change in external Ca-availability leads to accumulation of cellular N-homeostasis-related metabolites like L-aspartate, glutamine, and β-alanine, highlighting Ca-interference. However, the complexity of this relation is highlighted by the sequentially reduced cellular Ca accumulation with progressing N-depletion. N-depletion also leads to change in plant water relations as perceived through reduced K accumulation in shoot and vice versa in roots. In both cases, at least at optimal and moderate N levels, K accumulation also responded to changes in external Ca. Potentially perturbed osmolarity and membrane characteristics of barley roots are further characterized through analysis of root exudation for electrical conductivity, ionic content as well as quantification of specific metabolites under different treatment conditions. Furthermore, the relative relevance of changes in Ca-availability on N-assimilation, homeostasis of other macro-nutrients and therefore plant survival is being worked out in a triple split root system

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### Metabolic responses to phosphate deficiency in barley (*Hordeum vulgare*)

M. Kuczkowska<sup>1</sup>, G. Schaaf<sup>2</sup>, P. Dörmann<sup>3</sup>

<sup>1</sup>Institute of Molecular Physiology and Biotechnology of Plants, University of Bonn, Bonn, Germany

<sup>2</sup>Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Bonn, Germany

<sup>3</sup>Institute of Molecular Physiology and Biotechnology of Plants (IMBIO), University of Bonn, Bonn, Germany

Phosphate (P) plays a vital role in energy metabolism, signaling, membrane lipid, and nucleic acid synthesis, making it essential for plant growth and development. P deficiency has significant impacts on plant growth and yield, affecting photosynthesis, metabolic fluxes, and storage compound allocation. To better understand these metabolic changes and develop more efficient and sustainable crop management strategies, we employed barley (*Hordeum vulgare*) for P deprivation experiments during cultivation under controlled hydroponic conditions. We found that after 9 days of P starvation, the anthocyanin content increased sharply in the oldest leaf parts, while the chlorophyll content decreased. Lipid metabolism in barley leaves was altered after 9 and 12 days of P deprivation, with decreased amounts of phospholipids and elevated production of glycolipids. We started to establish “smart plants” i.e. transgenic barley lines expressing the reporter gene RUBY under control of different P-dependent promoters, which can unambiguously report P deficiency by producing a red dye. In a proof of concept experiment, we introduced the RUBY gene under control of the maize U6 promoter in barley plants. Furthermore, using the CRISPR-Cas9 mutagenesis strategy, we targeted the two genes, VIH1 and VIH2, encoding diphosphoinositol pentakisphosphate kinases involved in inositol polyphosphate metabolism and P sensing. We obtained genome edited plants which are homozygous or heterozygous for one or both of the vih1 and vih2 mutations. We will isolate homozygous mutants for detailed physiological characterization, including metabolite measurements using targeted and non-targeted LC-MS analysis. In conclusion, this study will shed light on the metabolic changes that occur during P deprivation in barley and will highlight potential solutions for improving crop management. It will provide insights into the molecular and physiological responses of plants to P deficiency, which is crucial for ensuring global food security.

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### New insights into the pH-dependent reciprocity of NRTs and AMTs during N uptake balance in Arabidopsis

M. Rivero-Marcos<sup>1</sup>, N. von Wirén<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Physiology and Cell Biology, Gatersleben, Germany

Nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) are the two main forms of inorganic nitrogen (N) uptake by plants. While the uptake of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  through NRTs and AMTs, respectively, have opposing effects on the rhizospheric pH, NRT and AMT activity are also dependent on pH. Although  $\text{NO}_3^-$  and  $\text{NH}_4^+$  uptake through NRTs and AMTs are independent systems, there is evidence that these transporters may modulate each other to achieve a proper balance in cation:anion uptake ratio and in N acquisition. However, underlying mechanisms remain poorly understood. By employing mutant lines for NRT1.1 and AMT proteins in *Arabidopsis* for influx studies with  $^{15}\text{N}$ -labeled  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , together with gene expression analysis, we demonstrate that a higher external  $\text{H}^+$  availability is pivotal for high-affinity  $\text{NO}_3^-$  uptake, and  $\text{NO}_3^-$  influx is neither affected by co-provision of  $\text{NH}_4^+$  or  $\text{NH}_4^+$ -dependent  $\text{H}^+$  efflux. We also uncovered a putative interplay between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  transporters reflected by weaker up-regulation of the  $\text{NO}_3^-$  transceptor *NRT1.1* but increased high-affinity  $\text{NO}_3^-$  uptake in a quadruple *amt* mutant (*qko*) lacking the main high-affinity  $\text{NH}_4^+$  transporters. Conversely, at higher external pH an alternative mechanism independent of AMTs appears to facilitate  $\text{NH}_4^+$  uptake not only in *qko* but also in the *NRT1.1* loss-of-function mutant *chl1-5*. These findings provide new components for a better mechanistic understanding of the interplay of pH and reciprocal N uptake dynamics beyond the role of AMTs and NRT1.1 for individual substrate fluxes.

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### Nitrogen stress-induced alterations in the leaf proteome of rice varieties

S. Basri<sup>1</sup>, A. Ahmad<sup>1</sup>

<sup>1</sup>Aligarh Muslim University, Department of Botany, Aligarh, India

Nitrogen (N) is essential for proper plant growth and its application has proven to be critical for agricultural produce. However, for unavoidable economic and environmental problems associated with excessive use of N-fertilizers, it is an urgent demand to manage application of fertilizers. Improving the N-use efficiency (NUE) of crop plants to sustain productivity even at low N levels is the possible solution. In the present investigation, contrasting low-N sensitive (Aditya) and low-N tolerant (Vikramarya) genotypes were identified and used for comparative proteome-profiling of leaves under optimum and low N supply. The analysis of differential expression pattern of proteins was performed by 2-D gel electrophoresis. Significant variations in the expression of proteins were observed under low N, which were genotype specific. In the leaf proteome, 25 spots were influenced by N treatment and four spots were different between the two genotypes. Most of the proteins that were differentially accumulated in response to N level and were involved in photosynthesis and metabolism, affirming the relationship between N and carbon metabolism. In addition to this, greater intensity of some defense proteins in the low N tolerant genotype was found that may have a possible role in imparting it tolerance under N starvation conditions. The new insights generated on rice proteome in response to N-starvation and restoration would be useful toward improvement of NUE in rice.



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Biofortification with Vitamin B12 in Lettuce improves light use efficiency under high light

K. Huntenburg<sup>1</sup>, M. Vlaming<sup>1</sup>, C. Nikolopoulos<sup>1</sup>, L. Marcelis<sup>1</sup>

<sup>1</sup>Wageningen University, Plant Science Group, Wageningen, Netherlands

Around 10% of the population in European countries are on a vegetarian or vegan diet. Such a diet increases the chance to suffer from Vitamin B12 deficiency, because Vitamin B12 is naturally found in animal products. Plants do not produce Vitamin B12, but can take the water soluble Vitamin up with the nutrient solution. Lettuce (*Lactuca sativa* L.) is often grown hydroponically in controlled environment agriculture (CEA), where Vitamin B12 can easily be added, improving the nutritional value of the crop by incorporating it. However, the effect of B12 on the plant itself is unclear. The aim of the study was to understand the effect of Vitamin B12 fortification on plant physiology and growth as well as postharvest product quality. Experiments were carried out with different concentrations of Vitamin B12 in the nutrient solution and different light intensities in CEA. The addition of Vitamin B12 to the nutrient solution did not negatively affect plant growth or shelf life of the lettuce. In addition, Vitamin B12 improved photosynthetic efficiency and thereby biomass production under high light conditions (440  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). However, Vitamin B12 addition had no effect on ROS scavenging nor on oxidative processes. In conclusion, biofortification of lettuce with Vitamin B12 improves the nutritional value without decreasing product quality parameters and may improve plant resilience to abiotic stresses. Further research is needed to understand the mechanism by which Vitamin B12 improves light use under high light intensity.

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Chilling of germinating seeds accelerates flowering in *Aethionema arabicum* involving repression of *FLC*

R. Sharma<sup>1</sup>, L. Gramzow<sup>1</sup>, F. Rümpler<sup>1</sup>, G. Theißen<sup>1</sup>

<sup>1</sup>Friedrich Schiller University Jena, Matthias Schleiden Institute - Genetics, Jena, Germany

Many plants growing under temperate climate conditions require a prolonged period of wet cold to initiate flowering through the process of vernalization. The molecular basis of vernalization has been studied intensively in *Arabidopsis thaliana*. The MADS-domain transcription factor FLOWERING LOCUS C (*FLC*) represses flowering by binding in a complex together with SHORT VEGETATIVE PHASE (*SVP*) to regulatory DNA of floral promoters such as *FT* and *SOC1*. During vernalization, *FLC* expression is inhibited by a complex epigenetic mechanism [1]. *Aethionema arabicum* is an annual, diploid species of the crucifer family (Brassicaceae). It has been developed in recent years to a model system for studying the eco-evo-devo of fruit and seed dimorphism [2, 3]. *Ae. arabicum* grows in the Irano-Turanian region and parts of the Mediterranean. Not surprisingly, therefore, there is no obligate requirement of cold for germination and flowering initiation. Nevertheless, we found for the accession that we studied that flowering time is significantly reduced upon chilling of germinating seeds. In addition to a reduction of flowering time, we observed increased plant height and fewer branches and fruits in plants whose germinating seeds were chilled. As in *A. thaliana*, acceleration of flowering was correlated with a significant reduction of the expression of the *FLC* orthologue. *In vitro* studies revealed that the *FLC* and *SVP* orthologues of *Ae. arabicum* binds as dimeric or multimeric complexes to target genes that are orthologues of some *FLC* targets in *A. thaliana*. Taken together, our results unexpectedly show that a vernalization mechanism similar to that of some accessions of *A. thaliana* growing under temperate climate operates in the Mediterranean plant *Ae. arabicum*. Vernalization in *Ae. arabicum* appears to be facultative rather than obligate, however. In what way this vernalization mechanism affects the life history and fitness of *Ae. arabicum* under natural growth conditions needs to be determined and will be discussed.

References:

1. Berry and Dean (2015). *TPJ* 83(1), 133-48.
2. Lenser, T., et al. (2016). *Plant Physiol.* 172, 1691-1707.
3. Chandler, J.O., et al. (2024) *Plant Cell*, koae085.

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Cytochrome P450 enzyme CYP716A is a gatekeeper of bitter and hemolytic oleanolic acid biosynthesis in *Chenopodium quinoa*

P. Kundu<sup>1</sup>, G. Zinta<sup>1</sup>

<sup>1</sup>CSIR- Institute of Himalayan Bioresource Technology, Biotechnology, Palampur, India

*Chenopodium quinoa* is a nutritionally rich and climate-resilient pseudocereal gaining attention globally. Quinoa seeds are gluten-free and rich in protein and micronutrients. However, they taste bitter due to the presence of antinutritional oleanane-type triterpenoid saponins viz. oleanolic acid, hederagenin and ursolic acid. Oleanolic acid saponin is the major saponin found in *C. quinoa* seeds. Oleanolic acid containing saponins are bitter and hemolytic. Oleanolic acid is synthesized by the cyclization of 2,3-oxidosqualene by beta-amyrin synthase followed by the oxidation of beta-amyrin. Oxidation of beta-amyrin is catalyzed by the action of cytochrome 450 enzymes. Plant genomes contain cytochrome P450 (CYP) supergene family involved in the biosynthesis of saponin aglycones. Here, by performing homology-based sequence analysis we identified a CYP716A in *C. quinoa*, which converts beta-amyrin into oleanolic acid. The functional validation was carried out by homologous transient overexpression and virus-induced gene silencing (VIGS) of CYP716A through agro-infiltration in the leaves of *C. quinoa*, followed by UPLC-MS quantification of the metabolites. Furthermore, heterologous expression in tobacco and Arabidopsis demonstrated the biological functionality of CYP716A in growth and stress responses. In summary, we discovered a novel beta-amyrin 28-oxidase enzyme (CYP716A) that catalyzes the biosynthesis of oleanolic acid in *C. quinoa* and explored its role in growth and defense. These results provide a strong foundation for understanding triterpenoid saponin biosynthesis in *C. quinoa* and designing saponin-free varieties.

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Control of stomatal plasticity in fluctuating environmental conditions

S. Forlani<sup>1</sup>, K. Elhazzime<sup>1</sup>, A. Vaten<sup>1</sup>

<sup>1</sup>University of Helsinki, Organismal and Evolutionary Biology (OEB), Helsinki, Finland

Stomata are leaf epidermal pores that regulate carbon uptake, transpiration, inner organ temperature, and pathogen resistance. During leaf growth, stomata are produced asynchronously throughout epidermis. Stem cell-like cells called meristemoids are dispersed in the epidermis and undergo series of controlled asymmetric divisions until the final commitment to a guard mother cell (GMC). GMC will eventually divide symmetrically generating a pair of guard cells, which mature and form a functional stoma. At the same time, the underlying mesophyll layer forms in a coordinated way with the epidermal layer, resulting in a leaf structure with protective but porous outer surface combined with extensive internal air spaces, which allows efficient exchange of CO<sub>2</sub> and H<sub>2</sub>O between the plant and surrounding environment. Environmental conditions, such as water availability, CO<sub>2</sub> concentration, and salinity in turn feed into the stomatal developmental program, and thus, can modify stomatal numbers. The current view is that the bHLH transcription factor SPEECHLESS (SPCH), a master regulator of meristemoid divisions, is central for the regulation of stomatal numbers. Various environmental signals are known to regulate SPCH, and thus, stomatal numbers. What are the regulatory mechanisms involved in the control of plastic stomata production and further, how different signaling pathways are orchestrated to control coordinated environmental responses in each leaf layer and across layers are not fully understood.

To identify mechanisms controlling stomatal developmental plasticity, we combine both reverse and forward genetic approaches. First, we are performing a layer-enriched transcriptomic analysis, employing mutant with altered environmental sensing capacity. Second, we are using a genome-wide forward genetic approach, based on misexpression of environmental sensitive stomatal lineage specific reporter gene. Our goal is to identify what are the regulatory factors modulating developmental responses upon environmental fluctuations and to explore signal integration within and beyond stomatal lineage.

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Host-dependent physiological and genetic modulations in Vampire Weed (*Rhamphicarpa fistulosa*, Orobanchaceae):  
A Parasitic Generalist

O. Dayou<sup>1</sup>, G. Brun<sup>1</sup>, H. K. Kithinji<sup>1</sup>, L. Botton-Divet<sup>1</sup>, T. Stach<sup>1</sup>, S. Wicke<sup>1</sup>

<sup>1</sup>Institute for Biology, Humboldt University of Berlin, Plant Evolution and Biodiversity, Berlin, Germany

Facultative root parasite *Rhamphicarpa fistulosa* (Orobanchaceae), commonly referred to as vampire weed, poses a significant threat to lowland rice in sub-Saharan Africa, leading to devastating yield losses exceeding 70%. This notorious pest exhibits remarkable plasticity, infecting a diverse range of monocot and dicot hosts. However, despite this considerable economic impact and ecological significance, our understanding of the genetic mechanisms underpinning vampire weed's interactions with its hosts remains limited. Here, we elucidated the complex interplay between vampire weed and its multiple hosts at the molecular level, shedding light on the intricate adaptive strategies employed by this highly successful parasitic generalist. To this end, we sequenced *Rhamphicarpa*'s complete genome and obtained comparative transcriptomic data from the parasite growing on rice, pearl millet, cowpea, tomato, and as parasitizes itself. We demonstrate that the parasite displays at least 1.5-fold higher successful attachments on rice and tomato, coinciding with a 1.4-fold lower photosynthetic efficiency on these hosts compared with others. Imaging the host-parasite interface using microscopic sections and micro-computed tomography revealed that *Rhamphicarpa* invades the various hosts' vascular systems by establishing a xylem-xylem connection, signifying full compatibility with both monocot and dicot hosts. Unlike rice and tomato, where enzymatic degradation may have dominated, *Rhamphicarpa* breaks through the host epidermal and cortical cells by mechanical pressure in cowpea and pearl millet. Weighted gene co-expression network analysis (WGCNA) revealed an upregulation of pathways involved in phloem or xylem histogenesis and development, as well as water, nutrients, oligopeptide, and auxin transports, correlating with haustorium and photosynthesis phenotypes in *Rhamphicarpa*. Furthermore, WGCNA pinpointed essential *Rhamphicarpa* genes expressed host-specifically and differentially during infection, including galactan beta-1,4-galactosyltransferase (GALS1), auxin transporter-like protein 2 (LAX2), cysteine-rich repeat secretory protein 55 (CRR55) and No Transmitting Tract (NTT) genes, all launched for host tissue invasion. In conclusion, this study underscores the various host-dependent modulations of *Rhamphicarpa*'s genetic program and physiology during host-parasite interplay, providing a critical knowledge base to develop sustainable and durable management strategies

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Deciphering tomato photoperiodic flowering and its interactions with age and gibberellin pathways

M. H. Vicente<sup>1</sup>, G. Serrano-Bueno<sup>2</sup>, L. E. Pino<sup>1</sup>, F. Baile<sup>2</sup>, L. E. P. Peres<sup>1</sup>, M. Calonje<sup>2</sup>, F. Valverde<sup>2</sup>, F. Nogueira<sup>1</sup>

<sup>1</sup>Esalq/USP, Piracicaba, Brazil

<sup>2</sup>IBVF/CSIC, Seville, Spain

Several studies have shown that *CONSTANS* (*CO*) gene is a central hub in photoperiodic signaling, affecting distinct processes such as flowering, tuberization, fruit ripening, stress tolerance, and organ development. Although cultivated tomato (*Solanum lycopersicum*) shows day-neutral flowering behavior, its wild relative species show delay in flowering under long day (LD) conditions, which was associated with a high activity of the *SELF-PRUNING 5G* (*SP5G*) gene.

As *SP5G* mediates tomato photoperiodic response, we hypothesized that *SICO1*, a homolog of *CO*, could negatively affect tomato flowering by inducing *SP5G* expression, which was confirmed by our molecular and functional data. Plants overexpressing *SICO1* showed high levels of *SP5G*, and late flowering independently of photoperiodic conditions, especially in plants harboring the *SP5G* allele from *S. pennellii* (*SP5G<sub>pen</sub>*), a wild tomato species. We also verified an increased in the luciferase activity driven by the *SP5G* promoter in the presence of the *p35S:SICO1* effector construct. Furthermore, genetic experiments suggest that tomato photoperiodic flowering is negatively regulated by the photoreceptor *PHYTOCHROME B1* (*PHYB1*), since the mutant *phyB1* recovered the late flowering of *p35S:SICO1* and *SP5G<sub>pen</sub>* plants, showing an epistatic phenotype. Our results suggest that tomato photoperiodic flowering is regulated by *SP5G* activity in a mechanism that involves the central hub of photoperiodic signaling *SICO1* and the photoreceptor *PHYB1*.

In this work, we also explored how photoperiod and its interaction with gibberellin and age flowering pathways regulates flowering in tomato. For this, we generated double mutants/transgenics tomato plants in genes related to the different pathways, and evaluated flowering time in LD. Our results indicate that the photoperiodic, age, and GA pathways act synergistically to control floral induction in tomato. The phytohormone gibberellin consistently acts as a negative regulator of tomato flowering, and the *SP5G* represses the *SFT* (*SINGLE FLOWER TRUSS*, the tomato *FLOWERIG LOCUS T*) activation by miR156-targeted SBP13, probably through a protein-protein interaction. Understanding the interactions of these flowering pathways can help breeding programs for tomato and other crops.

Keywords: Photoperiod, *CONSTANS*, Flowering, Gibberellin, miR156/SBPs.

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From root to shoot: uptake, translocation, distribution and speciation of Eu(III) in hydroponically grown plants

M. Klotzsche<sup>1</sup>, R. Steudtner<sup>1</sup>, M. Vogel<sup>2</sup>, B. Drobot<sup>1</sup>, S. Schymura<sup>3</sup>, T. Stumpf<sup>1</sup>

<sup>1</sup>Helmholtz-Zentrum Dresden-Rossendorf e.V., Institute of Resource Ecology, Dresden, Germany

<sup>2</sup>VKTA – Strahlenschutz, Analytik & Entsorgung Rossendorf e. V., Dresden, Germany

<sup>3</sup>Wilhelm Ostwald Park, Grimma, Germany

Lanthanides (Ln) are essential for various industrial and scientific applications and serve as inactive analogs for trivalent actinides (An) such as curium and americium. Understanding the bioaccumulation behavior of non-essential metals like Ln and An is crucial for food safety and the development of effective phytoremediation strategies.

Our aim is to gain a molecular process understanding of the interaction between Ln/An and plants: from initial exposure, uptake and speciation in different parts of the root tissue, translocation via plant sap to deposition in shoots and leaves. Therefore, we used hydroponically grown *Avena strigosa*, a grass cultivated as animal feed in many countries. Using laser spectroscopy and chemical microscopy in combination with factor analysis for data deconvolution, liquid chromatography, autoradiography, biochemical methods and inductively coupled plasma mass spectrometry, we followed the path of Eu(III) through the plant. We found that the release of the root exudates malate, citrate and fumarate affected the speciation of Eu(III) in the medium. Further it was demonstrated that over 99.35% of the bioassociated Eu(III) accumulates in the root through a multistage process, where it can be found both apoplastically (54%) and intracellularly (15%). Chemical microscopy revealed the presence of various speciations in the root tissue. The metal enters the plant through root tips and root hairs, allowing for translocation into the green parts of the plant. Eu (III) is transported via the xylem sap, likely as a malate or citrate complex. The concentration of Eu(III) in the xylem sap is dependent on the duration of exposure of the plant roots to the Eu(III)-containing liquid medium. Furthermore, we visualized the distribution of the radionuclide in roots and leaves in experiments with Eu-152.

The present results allow us to better understand and predict the environmental behavior of Ln and radionuclides. The indicator plant *A. strigosa* is a potential candidate for the remediation of contaminated soils due to its Eu(III)-association behavior and high biomass production. Further experiments with Cm(III) are currently underway.

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Genome editing reveals that the transcription factor BRANCHED1 is not a primary determinant of dimorphic fruit ratio in *Aethionema arabicum*

J. Patzer<sup>1</sup>, F. Kleeberg<sup>1</sup>, S. S. Kathalingam<sup>1,2</sup>, L. Gauthier<sup>1,3</sup>, G. Theißen<sup>1</sup>

<sup>1</sup>Friedrich-Schiller-Universität, Matthias-Schleiden-Institut/Genetik, Jena, Germany

<sup>2</sup>Nanyang Technological University, School of Biological Sciences, Singapore, Singapore

<sup>3</sup>Friedrich Schiller University Jena, Institute for Applied Optics and Biophysics, Jena, Germany

In recent years *Aethionema arabicum* has been established as a valuable model organism to study developmental and evolutionary aspects of fruit and seed dimorphism [1, 2]. In *Ae. arabicum* two kinds of fruits develop on the same plant, dehiscent and indehiscent fruits with mucilaginous and nonmucilaginous seeds, respectively. The ratio of dehiscent to indehiscent fruits (fruit ratio) is strongly dependent on growth habit and environmental cues such as light conditions and growth temperature [1, 3]. Therefore, the fruit and seed dimorphism was hypothesized to represent a blend of developmental plasticity and a bet hedging strategy to cope with unpredictably varying environmental conditions [1]. Mechanic manipulation of plants suggested that the branching pattern is a primary determinant of the fruit ratio, with mainly dehiscent fruits developing on the main branch if side branches are removed [1, 3]. We hypothesized that the key factor in generating the precise dimorphism of *Ae. arabicum* fruits may be the high expression of the *AearBRC1* gene in indehiscent fruits [3]. *AearBRC1* is an ortholog of the branching repressor *BRANCHED1* from *Arabidopsis thaliana*, encoding a transcription factor of the TCP family. To test our hypothesis we knocked-out *AearBRC1* in *Ae. arabicum*, harnessing the CRISPR-Cas9 system. As expected, *AearBRC1* knock-out plants showed a considerable increase in the number of side branches. However, this did not change the fruit ratio on the different branches compared to wild-type plants, suggesting that the fruit ratio is controlled via an *AearBRC1* independent pathway. Implications of our findings for the molecular mechanism underlying fruit ratio plasticity and the evolution of fruit dimorphism will be discussed.

References:

1. Lenser, T., et al. (2016). *Plant Physiol.* 172, 1691-1707.
2. Chandler, J.O., et al. (2024) *Plant Cell*, koae085.
3. Lenser, T., et al. (2018). *Plant J.* 94, 352-371.

P 162

**A causal study of the stimulating effect of phosphite (PO<sub>3</sub><sup>3-</sup>) on plants**

W. Yiming<sup>1</sup>, Z. Zhou<sup>1</sup>, X. Sicheng<sup>1</sup>, K. Holger<sup>1</sup>, V. Joseph-Alexander<sup>1</sup>, C. Daguang<sup>1</sup>

<sup>1</sup>Institut für Phytopathologie, Department Molecular Phytopathology, Kiel, Germany

Phosphite (PO<sub>3</sub><sup>3-</sup>) has been used as a biostimulant for many years. It promotes plant root development, improves stress tolerance, and increases crop yields. Due to the restricting regulations on chemical fertilizers and pesticides, there is growing interest in using phosphite in practice. Thus, understanding the underlying mechanisms and the mode of action is urgently needed. Our transcriptomic analyses revealed that foliage application of phosphite affects the transcriptomic landscape of both *Arabidopsis* and oilseed rape. Three days after application (dpa), we found 3889/8638 genes upregulated, and 5380/7018 genes were downregulated in treated *Arabidopsis*/oilseed rape, respectively. The GO and KEGG analyses revealed that the major terms and signaling pathways enriched in treated plants were mostly related to plant growth, development, defense-related hormone signaling, and nitrogen metabolism. Notably, many genes involved in nitrogen metabolism and SA metabolism were significantly upregulated, whereas genes involved in water homeostasis regulation and ethylene metabolism were suppressed in both treated species. Moreover, we found that genes of nitrate transporters, *NRT1.1* and *NRT1.5*, nitrate reductase (NR), *NIA1* and *NIA2*, and nitrate signaling genes, *NID1* and *HY5*, were all highly upregulated at 3dpa, suggesting an enhanced nitrogen metabolism stimulated by phosphite. To confirm this, we measured the activity of NR, a key enzyme in nitrogen metabolism. We found that phosphite enhanced the transcription of *NIA1* and *NIA2* and maintained NR activity by preventing NR inactivation *via* 14-3-3 protein (a phosphorylated NR-binding inhibitor). These effects could be observed in *Arabidopsis nrt1.1* but were significantly impaired in *nia1* and *nia2* mutants. Taken together, these results provide the first molecular evidence for the stimulating effect of phosphite *via* enhancing NR activity and nitrogen metabolism in plants. A possible mode of action is currently under investigation.

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**Genome-wide association study reveals the molecular determinants of pre-anthesis tip degeneration in barley**

K. Tan<sup>1</sup>, Y. Huang<sup>1</sup>, R. Kamal<sup>1</sup>, T. Schnurbusch<sup>1,2</sup>

<sup>1</sup>The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Breeding, Gatersleben, Germany

<sup>2</sup>Martin Luther University Halle-Wittenberg, Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, Halle a. d. Saale, Germany

Barley (*Hordeum vulgare*), an indispensable global crop classified within the Triticeae tribe of the Poaceae family, holds the status of being the fourth most crucial cereal, following rice (*Oryza sativa*), maize (*Zea mays*), and wheat (*Triticum* spp.). Barley possesses an indeterminate spike-type inflorescence with single-flowered spikelets that ultimately develop into grains. Barley plants pass through three development phases: vegetative, reproductive, and grain-filling. The reproductive phase is further divided into early and late reproductive phases: early reproductive phase determines the number of initiated spikelet primordia, and the late reproductive phase determines the number of spikelets that develop into the fertile floret. After the main culm spike has reached its maximum yield potential stage, the inflorescence meristem dome ceases its growth and starts to degenerate, followed by the degeneration of the subjacent spikelet primordia. Due to apical spikelet primordia degeneration (henceforth named pre-anthesis tip degeneration; PTD), the full potential of a barley spike is not realized. Hence, uncovering, identifying and optimizing some key genes involved in PTD is promising to increase grain number. We utilized 416 six-rowed barley accessions representing genotypic diversity for genome-wide association studies (GWAS) analyses of the PTD phenotype. Results show that there are three separate peaks that were detected on chr1H, chr3H, and chr7H, respectively. The most interesting but also challenging peak is located at the beginning of chr1H, where a total of 11 tandemly repeated genes encoding pentatricopeptide repeat (PPR) proteins. Interestingly, all of those PPR genes are homologs of rice *restorer of fertility 4* whose copy number variation was reported to associate with spikelet fertility. Benefiting from the high-quality and comprehensive pan-genome assemblies of barley, we successfully identified a large deletion (~7 kb) that showed co-segregation with the significant SNP and contained two PPR genes with complete structure and mRNA expression. Thus, we believe that copy number variation of the PPR cluster may be a strong causal candidate factor. We identified two major haplotypes within the locus that showed different geographical preference (Eastern versus Western), suggesting that this locus may have undergone different natural or artificial selection during evolution.

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**Early nutrition strategies of parasitic *Cuscuta* spp.**

M. Hartenstein<sup>1</sup>, D. Kischka<sup>1</sup>, R. Stadler<sup>1</sup>, M. Albert<sup>1</sup>

<sup>1</sup>Friedrich-Alexander-Universität Erlangen-Nürnberg, Molecular Plant Physiology, Erlangen, Germany

Holoparasitic plants of the genus *Cuscuta* are unable to photosynthesize and penetrate shoots of host plants with haustoria. With a haustorium *Cuscuta* spp. build a connection to the host vasculature to exhaust water, solutes and carbohydrates. Such infections usually stay unrecognized by the host and lead to host plant damage. During the haustorium penetration phase, the cells of the ingrowing parasitic haustorium require nutrients, energy and sugars but do not yet possess a symplasmic connection to host plant cells. How are parasite cells supplied with nutrients and energy during the first week of the infection process? Addressing this question, we discovered and studied a set of membrane transport proteins, genes of which are sequentially expressed and that are involved in an apoplastic nutrition mechanism. Our findings demonstrate how the early parasitic prehaustorial cells are first provided with sugars from parasite's storages and how they later switch to acquire nutrients from host cells' apoplast as early as possible and before a connection to host vascular bundles. These findings shed light on the early nutrition strategies of *Cuscuta* spp. that are taking place during the days before a connection to host vasculature. Holoparasitic plants of the genus *Cuscuta* are unable to photosynthesize and penetrate shoots of host plants with haustoria. With a haustorium *Cuscuta* spp. build a connection to the host vasculature to exhaust water, solutes and carbohydrates. Such infections usually stay unrecognized by the host and lead to host plant damage. During the haustorium penetration phase, the cells of the ingrowing parasitic haustorium require nutrients, energy and sugars but do not yet possess a symplasmic connection to host plant cells. How are parasite cells supplied with nutrients and energy during the first week of the infection process? Addressing this question, we discovered and studied a set of membrane transport proteins, genes of which are sequentially expressed and that are involved in an apoplastic nutrition mechanism. Our findings demonstrate how the early parasitic prehaustorial cells are first provided with sugars from parasite's storages and how they later switch to acquire nutrients from host cells' apoplast as early as possible and before a connection to host vascular bundles. These findings shed light on the early nutrition strategies of *Cuscuta* spp. that are taking place during the days before a connection to host vasculature.

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**Glutathione affects plant growth indirectly by controlling the amount of nitric oxide**

M. T. Safi<sup>1</sup>, A. Meyer<sup>1</sup>, J. M. Ugalde<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Institute of Crop Science and Resource Conservation, Bonn, Germany

Glutathione (reduced form: GSH; oxidized form: GSSG) is the most abundant low molecular thiol in plants and fulfils various functions in detoxification of endogenously produced toxic metabolites, nitric oxide (NO<sup>•</sup>), xenobiotics and heavy metals, as well as detoxification of reactive oxygen species (ROS) along with maintenance of cellular redox homeostasis. The redox potential  $E_{\text{GSH}}$  of the GSH/GSSG redox pair equilibrates with the redox potential of protein thiols in reactions mediated by glutaredoxins (GRXs) and thus changes in  $E_{\text{GSH}}$  can cause alterations in function of the respective GRX target proteins. Arabidopsis growth is long known to be highly dependent on glutathione, which is most obvious from short root phenotypes of partially GSH-deficient mutants. This phenotype might be caused through direct or indirect effects on the cell cycle in meristematic cells, but the underlying processes remain poorly characterized. At this point, it is not known whether root growth and maintenance of the cell cycle depends on absolute concentrations of glutathione or the actual  $E_{\text{GSH}}$  in the cytosol or the nucleus. To distinguish possible effects of total GSH and  $E_{\text{GSH}}$  at subcellular level, we used roGFP2 as a genetically encoded biosensor for monitoring relative changes of  $E_{\text{GSH}}$  in Arabidopsis wild-type seedlings, mutants with defects in glutamate-cysteine ligase (GSH1) as the first enzyme of GSH biosynthesis and mutants with defects in GSSG reduction and GSH degradation. The obtained results strongly supporting a critical role for the absolute amount of GSH in root growth. Therefore, we further elucidated the role of GSH in nitric oxide (NO<sup>•</sup>) homeostasis because NO<sup>•</sup> is known as a plant growth regulator and GSH is known to be involved in metabolising NO<sup>•</sup> through formation of S-nitrosoglutathione (GSNO) and its subsequent reduction by GSNO reductase (GSNOR). Deficiency of GSNOR in *gsnor1-3* mutants resulted in increased NO<sup>•</sup> content, which correlated with partially inhibited plant growth. This is further corroborated by mutants with defects in NO<sup>•</sup> production. The results of our work suggest that the effects of GSH on plants growth to a significant part are indirect through the role of GSH in controlling the amount of NO<sup>•</sup> and thus protein nitrosylation.

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The Role of Endomembrane Potassium Transporters in Plant Abiotic Stress Tolerance: Combining Knowledge from Soybean and Arabidopsis

J. Hamacher<sup>1</sup>, B. Becker<sup>1</sup>, Y. Qu<sup>2</sup>, M. Gilliam<sup>2</sup>, S. Wege<sup>1</sup>

<sup>1</sup>University of Bonn, Plant Nutrition, Institute of Crop Science and Resource Conservation (INRES), Bonn, Germany

<sup>2</sup>ARC Centre of Excellence in Plants for Space, School of Agriculture, Food and Wine & Waite Research Institute, Glen Osmond, Australia

Soybean (*Glycine max*) is a globally important crop for food and edible oil production. Soybean plants are sensitive to salinity (NaCl), with significant yield losses reported under saline conditions. *GmSALT3* is a dominant gene underlying a major quantitative trait locus for salt tolerance in soybean and it encodes a potassium transporter of the cation/proton exchanger (CHX) protein family. Interestingly, the potassium transporter is localised in the ER and not the plasma or vacuolar membrane, very likely excluding a direct role in ion uptake or sequestration. Similarly, the Arabidopsis potassium transporter CHX20 is also exclusively localised in the ER and contributes to stomata aperture regulation. *chx20* knockout mutants show a significant increase in water loss compared to the wildtype, suggesting a role of CHX20 in drought tolerance. Likewise, Near Isogenic Lines (NILs) in soybean, which differ only in one transposon in *GmSALT3*, show severe differences in salt tolerance, accompanied by differences in foliar potassium content. We aim to decipher the cellular role of ER-localised CHX proteins and to connect them to mechanisms conferring abiotic stress tolerance and plant nutrition. Through biochemical assays, we found that the large cytosolic domain of CHX proteins likely has a regulatory role connecting the transporters to cellular energy levels. Experiments using genetically encoded ion sensors as well as gene expression studies are ongoing.

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Modulation of the developmental seminal root lifespan in barley

C. E. Tunc<sup>1</sup>, N. von Wirén<sup>1</sup>

<sup>1</sup>Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Plant roots serve important functions in water and nutrient uptake and in anchoring above-ground plant organs in the soil. The physiological contribution of roots to plant performance, however, changes over the life span of a plant. Here, root senescence is an important factor, as radial water and nutrient transport as well as respiration decline with the progression of root tissue age and cortical senescence (Schneider et al, 2017; Wang et al., 2022). Recently, it has been shown that roots also undergo developmental senescence in barley (Liu et al, 2019). However, it has remained unclear whether senescence-related processes in seminal roots are dependent on other organs (i.e. nodal roots). In an attempt to understand to what extent nodal root development modulates seminal root senescence, we removed nodal roots daily from their emergence on and monitored root senescence-related traits (e.g. root growth, elongation and nitrogen uptake capacity) in seminal roots. Excision of nodal roots delayed the onset of seminal root senescence - albeit to a certain extent, suggesting that other endogenous signals act on top in determining the senescence of seminal roots. In parallel, to exploit the role of phytohormones during the progression of root senescence, we grew transgenic barley lines with enhanced cytokinin (CK) degradation in roots and monitored physiological and molecular senescence-related processes. Our results reveal that lower CK levels in roots can prolong the physiological activity and longevity of seminal roots. Taken together, our work provides evidence that root senescence in barley is a plastic developmental process that is subject to hormonal regulation.

References

- Liu, Z., Marella, C. B., Hartmann, A., Hajirezaei, M. R., and von Wirén, N. (2019) An age-dependent sequence of physiological processes defines developmental root senescence. *Plant Physiology*, 181, 993-1007.
- Schneider, H. M., Wojciechowski, T., Postma, J. A., Brown, K. M., Lücke, A., Zeisler, V., Schreiber, L. and Lynch, J. P. (2017) Root cortical senescence decreases root respiration, nutrient content and radial water and nutrient transport in barley. *Plant, Cell and Environment*, 40, 1392-1408.
- Wang, X., Xu, C., Xiong, D., Yao, X., Chen, T., Jiang, Q., Jia, L., Fan, A. and Chen, G. (2022). Root age-related response of fine root respiration of Chinese fir seedlings to soil warming. *Tree Physiology*, 42, 1177–1187.

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### The potential green light receptor GRS affects plant development

S. Frank<sup>1</sup>, H. Tekin<sup>1</sup>, U. Vothknecht<sup>1</sup><sup>1</sup>University of Bonn, Plant Cell Biology, Bonn, Germany

In land plants, several light receptors are known, such as phytochromes for red light, phototropins, cryptochromes and LOV domain containing F-box proteins for blue light, and UVR8 for UV-B light (Paik and Huq, 2019). However, these receptors do not cover all wavelengths of light that influence plants. In our search for additional receptors, we found a potential green light sensor (GRS) that might close the gap.

The GRS orthologs from *Hordeum vulgare* and *Arabidopsis thaliana* are highly similar in their amino acid sequence and the predictions of the subcellular localization of these proteins via bioinformatical tools are very diverse, including plasma membrane, mitochondria, cytoplasm and nucleus. An analysis of the GRS transcripts in *Arabidopsis* and barley showed a very constant expression in different situations like deetiolation, circadian rhythm, drought or senescence. Only in the first days of development, a down-regulation of the GRS gene expression could be seen in barley plants. Furthermore, the phenotype of an *Arabidopsis* mutant line (*atgrs*) in comparison to Col-0 wild-type plants showed differences in growth, that were dependent on light intensity as well as light quality.

Although our studies so far cannot provide proof that GRS functions as a light receptor, we already collected several hints that support this hypothesis. Regardless of this, the expression profile of the GRS gene and the difference in growth observed for the *atgrs* mutant line indicate a function of GRS in plant development.

#### Reference:

Paik, I., & Huq, E. (2019). Plant photoreceptors: Multi-functional sensory proteins and their signaling networks. In *Seminars in cell & developmental biology* (Vol. 92, pp. 114-121). Academic Press.

P 169

### Molecular characterisation of cryptochrome 1-mediated suppression of the Arabidopsis COP1/SPA E3 ubiquitin ligase

L. Trimborn<sup>1</sup>, P. Yu<sup>1</sup>, J. Ponnu<sup>1</sup>, U. Hoecker<sup>1</sup><sup>1</sup>University of Cologne, Institute of Plant Sciences, Köln, Germany

Plants must dynamically adapt to changes in ambient light conditions. Such adaptive mechanisms are underpinned by complex regulatory networks for the detection and transduction of light signals. A central regulator of light signaling in *Arabidopsis* is the E3 ubiquitin ligase COP1/SPA. COP1/SPA is primarily active in the dark when it polyubiquitinates key transcription factors essential for light responses, thereby causing their degradation. These transcription factors share a valine-proline (VP) motif that is bound by the COP1/SPA complex. In the light, photoreceptors directly interact with COP1/SPA, thereby suppressing its activity, resulting in the accumulation of light-responsive transcription factors.

Here, we will present mechanistic insight into the blue light-induced inhibition of COP1/SPA activity by the cryptochrome 1 (CRY1) photoreceptor. We previously demonstrated that only blue light-activated CRY1 interacts with COP1/SPA. Here we show that, upon binding of CRY1 to COP1/SPA, substrates are competitively displaced from COP1 and SPA1, thereby preventing their polyubiquitination and subsequent degradation. This competitive binding arises from the shared utilization of a VP motif by both CRY1 and substrates to engage with COP1/SPA. Furthermore, we demonstrate that this mechanism is essential for blue light-induced seedling deetiolation and anthocyanin biosynthesis in *Arabidopsis*.

Taken together, our study provides mechanistic insights into the regulation of COP1/SPA by CRY1, shedding light on fundamental processes governing plant photomorphogenesis.



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The tissue-specific functions of trehalose 6-phosphate in coordinating plant metabolism with development

L. Müller<sup>1,2</sup>, M. G. Annunziata<sup>3</sup>, J. Lunn<sup>3</sup>, O. Ebenhö<sup>2,4</sup>, F. Fichtner<sup>1,2</sup>

<sup>1</sup>Heinrich Heine University, Institute of Plant Biochemistry, Düsseldorf, Germany

<sup>2</sup>Cluster of Excellence on Plant Sciences (CEPLAS), Düsseldorf, Germany

<sup>3</sup>Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

<sup>4</sup>Heinrich Heine University, Institute of Quantitative and Theoretical Biology, Düsseldorf, Germany

The sucrose-specific signalling metabolite trehalose 6-phosphate (Tre6P) is an essential low-abundance metabolite in plants that is in control over metabolic processes, sucrose allocation, and developmental decisions such as flowering, embryogenesis, and shoot branching. Tre6P plays a vital role in linking growth to carbon status and balancing sucrose levels in the plant. The sucrose-Tre6P nexus model describes the mechanism how Tre6P is both a signal and a negative feedback regulator of sucrose levels. The enzymes Tre6P synthase (TPS) and Tre6P phosphatase (TPP) play a key role in this nexus model through increasing or decreasing Tre6P levels in relation to the respective sucrose levels. In *Arabidopsis* the main Tre6P synthase (AtTPS1) has been reported to be expressed in guard cells and the vasculature including phloem parenchyma, companion, bundle sheath and xylem parenchyma cells. Furthermore, it is assumed that Tre6P has a direct or indirect effect on stomatal conductance since mutants with lesion in trehalose metabolism have impaired stomatal opening. Since the tissue-specific functions of Tre6P are largely unknown, cell-specific promoters are used to alter Tre6P levels in the vasculature and in guard cells. The generated data of Tre6P effects on the development and metabolome will elucidate the precise tissue-specific functions of Tre6P in the shoot and can thereby enhance the development of a more comprehensive model.

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Patterning the Shoot Meristem Stem Cell Niche by mir394 Signalling

F. Garbsch<sup>1</sup>, F. Liu<sup>1</sup>, C. Wang<sup>1</sup>, T. Laux<sup>1</sup>

<sup>1</sup>University of Freiburg, Institute of Biology III, Freiburg i. Br., Germany

Stem cell niches (SCN) are a reserve of undifferentiated cells that provide a steady supply of precursor cells to develop differentiated tissues. Despite constant rapid divisions and the continuous exchange of resident cells, the internal structure of the shoot apical meristem (SAM) and the spatial pattern of cell identities remain largely unaltered throughout development. The maintenance of the SAM is largely controlled by a feedback loop between the WUSCHEL (WUS) transcription factor and the small signaling peptide CLAVATA 3 (CLV3) (Schoof et al., 2000). Additionally, an epidermal microRNA394 (MIR394B) signal is required to acquire SC competence in the three outermost cell layers. A mir394 gradient acts as a mobile signal to repress the F-box protein LEAF CURLING RESPONSIVENESS (LCR) which potentiates the WUS gradient from the organizing center (OC) (Knauer et al., 2013). This mechanism requires the precise expression of the MIR394b gene in the L1 layer. In our results, we identified a 150bp region in the promoter of MIR394b that is sufficient to establish a wildtype-like expression domain. Using truncated promoter constructs and a linker-scanning approach, two cis-regulatory elements were identified. An AGAAAC-motif confers ubiquitous expression and is combined with repression from the inner cell layers by a 2nd element we designated the BigBox to achieve the specific L1 expression of the *MIR394b*. To identify the regulatory transcription factors binding the identified motifs, we screened for possible interactors using the yeast-one-hybrid (Y1H) system. Promising candidate regulators have been identified and are currently being confirmed. Altogether, these results provide a regulatory framework for stem cell niche patterning.

References

Knauer, S., Holt, A. L., Rubio-Somoza, I., Tucker, E. J., Hinze, A., Pisch, M., Javelle, M., Timmermans, M. C., Tucker, M. R., & Laux, T. (2013). A Protodermal miR394 Signal Defines a Region of Stem Cell Competence in the Arabidopsis Shoot Meristem. *Developmental Cell*, 24(2), 125–132. <https://doi.org/10.1016/j.devcel.2012.12.009>

Schoof, H., Lenhard, M., Haecker, A., Mayer, K. F. X., Jürgens, G., & Laux, T. (2000). The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell*, 100(6), 635–644. [https://doi.org/10.1016/S0092-8674\(00\)80700-X](https://doi.org/10.1016/S0092-8674(00)80700-X)

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The vascular lay-out of the barley rachis: implications for transport and spikelet connection

T. Rutten<sup>1</sup>, V. Thirulogachandar<sup>2</sup>, Y. Huang<sup>1</sup>, N. Shanmugara<sup>3</sup>, R. Koppolu<sup>1</sup>, S. Ortleb<sup>1</sup>, G. Hensel<sup>2</sup>, J. Kumlehn<sup>1</sup>, M. Melzer<sup>1</sup>  
T. Schnurbusch<sup>1</sup>

<sup>1</sup>Leibniz Institute for Plant Genetics and Crop Plant Research, Seeland / OT Gatersleben, Germany

<sup>2</sup>Heinrich Heine University, Düsseldorf, Germany

<sup>3</sup>Cold Spring Harbor, New York, NY, United States

Question: Vascular patterning is intimately related to plant form and function. Using barley (*Hordeum vulgare*) as a model, we studied the vascular anatomy of the spike-type inflorescence. Our aim was to clarify the relationship between rachis (spike axis) vasculature and spike size, define vascular dynamics and the implications for transport capacity and spikelet supply.

Methods: We employed serial transversal internode sectioning on rachises of several barley lines. To analyze internode area, vascular area, and vein number along the rachis, sections were scanned with an UV laser. The autofluorescence images thus recorded were analyzed using the open-source Fiji software.

Results: The results reveal that internode area and total vascular area show a clear positive correlation with spike size, whereas vascular number is only weakly correlated.

While the side wings of the rachis harbors large veins of fairly constant size, the central part of the rachis is occupied by small probably immature veins. This suggests a spatial separation between transport and distribution. By assigning bulk transport to the large mature veins in lateral rachis wings, transport resistance is minimized while the staggered array of immature vein endings could serve to create an accumulation of metabolites in the central rachis as previously shown by Melkus et al. (2013). Spikelet-derived veins entering the rachis often merge with the small immature veins in the rachis center but never merge with the large mature veins in the rachis wings. An increase in floret fertility through the conversion of a two-rowed barley into an isogenic six-rowed line, as well as a decrease in floret fertility due to enhanced pre-anthesis tip degeneration caused by the mutation *tip sterile 2.b (tst2.b)* significantly affected vein size, but had limited to no effects on vein number or internode area.

Conclusions: Our observations lead us to conclude that the rachis vasculature is the result of a two-step process involving an initial layout followed by size adjustment according to floret fertility/spike size. The identification of spikelet-derived veins entering the rachis without fusing with its vasculature indicates that a vascular continuity between rachis and spikelets may be non-essential.

Reference: Melkus et al. (2011). Plant Biotechnological Journal 9: 1022-1037.

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Photoperiod-dependent modulation of plant development and physiology by mitochondrial metabolic signals

M. D. P. Martinez<sup>1</sup>, I. Nica<sup>1</sup>, K. Zheng<sup>2</sup>, M. Schwarzländer<sup>2</sup>, V. G. Maurino-Larcher<sup>1</sup>

<sup>1</sup>Institute of Cellular and Molecular Botany (IZMB), University of Bonn, Molecular Plant Physiology, Bonn, Germany

<sup>2</sup>Institute of Plant Biology and Biotechnology (IBBP), University of Münster, Plant Energy Biology, Münster, Germany

Malate is an essential carboxylic acid in plant metabolism. Among other functions, it is involved in the tricarboxylic acid (TCA) cycle, and in shuttles that exchange redox cofactors between mitochondria, cytosol, and chloroplasts. Within the mitochondria, it is metabolized by the NAD-malic enzyme (NAD-ME) and malate dehydrogenase (mMDH). mMDH is a homodimer present as two isoforms, whereas NAD-ME is mostly active as heterodimer, although the separate subunits 1 and 2 also form active dimers. To study the effect of altered mitochondrial malate utilization, we constructed combinations of loss-of-function lines of NAD-ME and mMDH and analyzed them under different photoperiods and light intensities. In long days, no phenotypic differences were observed between the genotypes. In short days under low light intensity, only the double mutant of mMDH1 and NAD-ME2 (*mmdh1\*nad-me2*) show retarded growth and yellowing. The double mutants have a lower photosynthetic capacity and respiration rate. Not only do they suffer from severe metabolic limitations, but they also remain small, are unable to produce seeds, and have an extended lifespan. The most significant changes in gene expression were observed during the light period, when the expression of *NAD-ME1* was increased. Interestingly, transferring double mutants grown on short days to long days allows recovery of normal development. Furthermore, the absence of NAD-ME1 in the background of the double mutants (*mmdh1\*nad-me2\*nad-me1*) also allows recovery of normal development in short days. Our work suggests that in the absence of *NAD-ME2* and *mMDH1*, a metabolic signal generated by the action of NAD-ME1 in a photoperiod and light intensity dependent manner can reprogram plant development and physiology.

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Tissue-specific profiling of *Arabidopsis* Zn<sup>2+</sup>-dependent histone deacetylases revealed that histone deacetylase 8 is a class II HDAC in roots

F. Kotnik<sup>1</sup>, P. Tilak<sup>1</sup>, M. Gasper<sup>1</sup>, J. Eirich<sup>1</sup>, J. Kovacs<sup>2</sup>, J. Seidel<sup>3,4</sup>, C. Becker<sup>2,5</sup>, D. Schwarzer<sup>3</sup>, I. Finkemeier<sup>1</sup>

<sup>1</sup>University Muenster, Münster, Germany

<sup>2</sup>Austrian Academy of Sciences, Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria

<sup>3</sup>Interfaculty Institute of Biochemistry (IFIB), University of Tübingen, Tübingen, Germany

<sup>4</sup>Institute for Organic and Macromolecular Chemistry, Friedrich Schiller University Jena, Jena, Germany

<sup>5</sup>Faculty of Biology, Ludwig-Maximilians-University, München, Germany

Histone deacetylases (HDACs) are erasures of lysine acetylation in histone and non-histone proteins. They are known to regulate numerous cellular and developmental processes in all eukaryotes. In plants they have functions in seed germination, light signaling, morphogenesis, stress response and regulation of photosynthesis among others. The presence of the multigenic histone deacetylase family in the model plant *Arabidopsis thaliana* raises the question, whether they are present and active in all tissues. Here we report the profiling of *Arabidopsis* RPD3/HDA1-like histone deacetylases with the use of an  $\alpha$ -aminosuberic acid  $\omega$ -hydroxamate probe in different *Arabidopsis* tissues. While some HDACs show tissue-specific probe recruitment others show activities in all analyzed tissue. These results provide new insights into tissue-specific HDAC functions and will provide the basis for future analysis of selected HDAC mutants.

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PRC2 rewires developmental and metabolic programs during seedling emergence before and after the initiation of photosynthesis

N. Samo<sup>1,2</sup>, M. Guadalupe Trejo-Arellano<sup>1</sup>, F. Aflaki<sup>1</sup>, A. Ebert<sup>3</sup>, A. Erban<sup>3</sup>, L. Gahurová<sup>2</sup>, J. Kubásek<sup>2</sup>, H. Hönig Mondeková<sup>1,2</sup>, A. Schlereth<sup>3</sup>, Q. Riviere<sup>1</sup>, M. Zhou<sup>1</sup>, D. Bouyer<sup>4</sup>, J. Šantrůček<sup>2</sup>, O. Novák<sup>5</sup>, J. Kopka<sup>3</sup>, I. Mozgová<sup>1,2</sup>

<sup>1</sup>Biology Centre, CAS, České Budějovice, Czech Republic

<sup>2</sup>university of South Bohemia, České Budějovice, Czech Republic

<sup>3</sup>Max Planck Institute of Molecular Plant Physiology, Potsdam, Potsdam, Germany

<sup>4</sup>ESN, Lyon, France

<sup>5</sup>Laboratory of Growth Regulators, Faculty of Science of Palacký University & Institute of Experimental Botany of the Czech Academy of Sciences, Olomouc, Czech Republic

The progression from seed to seedling represents a key developmental and metabolic transition in plants. It is controlled by Polycomb repressive complex 2 (PRC2), but the coordination of PRC2 activity and its contribution to gene reprogramming during seedling establishment is unknown. We analysed transcription and H3K27me3 distribution in shoot and root tissues of heterotrophic and photoautotrophic seedlings at two developmental timepoints, revealing two phases of PRC2-mediated gene repression. The first phase is independent of light and photosynthesis and ensures the repression of the embryo maturation programme associated with heterotrophy and storage molecule biosynthesis. The second phase, in which H3K27me3 deposition is sensitive to photosynthesis inhibition, is associated with the repression of metabolic pathways related to seed germination and early light responses. This underscores a key role of PRC2 in establishing the identities of the shoot and root as metabolic source and sink tissues and in driving the transition from heterotrophy to photoautotrophy in emerging seedlings.

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## Genetic and molecular mechanisms of root-mediated nitrate use efficiency in wheat and barley

M. N. Siddiqui<sup>1</sup>, J. Léon<sup>1</sup>, A. Ballvora<sup>1</sup><sup>1</sup>University of Bonn, Bonn, Germany

Questions: What genetic factors and molecular mechanism regulates nitrate transport and sensing across crop species that ultimately improved nitrogen (N)-use efficiency (NUE)?

Methods: In this study, we performed a genome-wide scan using wheat and barley accessions characterized under low and high N inputs under field traits and controlled conditions, respectively.

Results: By a genome-wide association study, we uncovered a syntenic gene, *NPF2.12* for low-affinity nitrate transport. Phylogenetic analysis revealed that *NPF2.12* encodes a specific MAJOR FACILITATOR SUPERFAMILY domain-containing protein highly similar between wheat and barley with transporter activity. Here we show that the variation in *NPF2.12* promoter positively associated with root growth and root-to-shoot nitrate transport by decreasing its expression under low nitrate availability. Further, loss-of-function mutant *npf2.12* specifically transactivates nitrate reductase *NIA1* gene at low nitrate concentrations resulted an elevated levels of nitric oxide production leading to higher root growth and nitrate transportation compared to wild-type. Notably, multiple field trials revealed that the elite allele *TaNPF2.12TT* significantly enhanced N uptake, root-to-shoot transport that subsequently increased NUE under minimum N.

Conclusion: Our study uncovers a conserved genetic regulator of nitrate sensor in wheat and barley and *NPF2.12-NIA1* signaling cascade may provide a new route to improve NUE underlying root growth to limited N availability.

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## A metathesis-derived library of 12-OPDA variants for scrutinizing and tuning OPDA functions

M. S. Knieper<sup>1</sup>, M. Bittmann<sup>2</sup>, T. L. Guntelmann<sup>2</sup>, A. Viehhauser<sup>1</sup>, H. Gröger<sup>2</sup>, K. J. Dietz<sup>1</sup><sup>1</sup>University Bielefeld, Biochemistry and Physiology of Plants, Bielefeld, Germany<sup>2</sup>University Bielefeld, Industrial Organic Chemistry and Biotechnology, Bielefeld, Germany

(*Cis*)-12-oxophytodienoic acid (12-OPDA) is a reactive oxylipin derived from  $\alpha$ -linolenic acid in a three-step-reaction catalyzed by 13-lipoxygenase, allene oxide synthase and allene oxide cyclase. While 12-OPDA serves as a precursor of the phytohormone jasmonic acid, it has gained attention as an independent signaling and regulatory molecule over the past two decades, affecting major processes such as germination and stress defense. Although the mechanisms of 12-OPDA signaling have not been completely unraveled yet, it is proposed to either act as a ligand or to covalently bind to free thiol groups as a Michael acceptor due to its cyclopentenone moiety.

The process of covalent binding is termed OPDAylation. It affects several enzymes of the plant redox regulatory network. Binding of OPDA as ligand is reported for the stromal cyclophilin 20-3 (Cyp20-3) and stimulates cysteine synthesis, thereby enhancing antioxidant levels under stress conditions such as high light. In addition, adduct formation of GSH and OPDA, catalyzed by glutathione-S-transferases, might serve as a detoxification mechanism preventing cellular damage due to excessive OPDA levels.

Employing a library of OPDA derivatives obtained by olefin metathesis using Hoveyda-Grubbs 2nd generation catalysts, *in vitro* studies were conducted, assessing changes in either the conversion of OPDA to OPC-8:0 by OPR3 or affecting target proteins such as Cyp20-3. In addition, derivatives were assessed for OPDA-mediated inhibition of germination and root growth in *Arabidopsis* seeds. Distinct differences could be observed for all derivatives, uncovering novel insights into the structural base of 12-OPDA signaling.

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Rooting for *Lotus japonicus* WHIRLY2: a new player in root development

T. L. Nguyen<sup>1</sup>, E. Roitsch<sup>2</sup>, K. Humbeck<sup>1</sup>

<sup>1</sup>Martin-Luther-University Halle-Wittenberg, Plant Physiology, Halle a. d. Saale, Germany

<sup>2</sup>Leibniz Institute of Plant Biochemistry (IPB), Molecular Signal Processing, Halle a. d. Saale, Germany

The root system is crucial for land plants as it is their main contact point with soluble nutrients. Adaptation of root growth to nutrient availability is therefore vital for plant plasticity and environmental responsiveness [1]. Understanding the intricated signaling network governing the root system architecture is essential for enhancing above-ground plant growth and consequently plant productivity. The plant-specific WHIRLY protein family, known for its nucleic acid binding abilities and diverse subcellular localizations, plays multiple regulatory roles. These include maintaining organellar function, regulating nuclear gene expression, and facilitating organelle communication [2]. In legume *Lotus japonicus*, three genes encode WHIRLY proteins: *LjWHIRLY1* and *LjWHIRLY3* likely encode plastid-targeted proteins, while *LjWHIRLY2* is predicted to localize in mitochondria. In this work, a comprehensive phenotypic analysis of the LORE1-insertion mutant of *LjWHIRLY2*, denoted as *why2-1*, revealed for the first time *LjWHIRLY2*'s role in root system architecture. The *why2-1* mutant displayed a significant reduction in primary root growth but an increase in lateral root development, especially higher-order lateral roots. As a result, shoot biomass in the mutant decreased compared to the wild type Gifu. Notably, the root phenotype was more pronounced when plants were inoculated with the symbiotic bacterium *Mesorhizobium loti*. Additionally, a transcriptomic analysis of *why2-1* and Gifu seedlings provided insights into how *LjWHIRLY2* coordinates various signaling pathways to regulate root development.

References

[1] Ryan PR, Delhaize E, Watt M, Richardson AE. Plant roots: understanding structure and function in an ocean of complexity. *Ann Bot.* 2016 Oct;118(4):555–9. doi: 10.1093/aob/mcw192. Epub 2016 Aug 19. PMID: PMC5055641.

[2] Krupinska K, Desel C, Frank S, Hensel G. WHIRLIES are multifunctional DNA-binding proteins with impact on plant development and stress resistance. *Front Plant Sci.* 2022 Apr 21;13:880423. doi: 10.3389/fpls.2022.880423. PMID: 35528945; PMCID: PMC9070903.

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A role of jasmonate in root elongation of wheat

L. Wang<sup>1</sup>, M. Meier<sup>1</sup>, M. Mascher<sup>1</sup>, N. von Wirén<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

When growing under adverse soil conditions or limiting resources, plants alter their root system architecture (RSA) to explore further soil layers and to enhance nutrient and water acquisition. To date, adaptive root responses to challenging soil conditions have remained poorly investigated despite the high importance of wheat for human nutrition. In this study, we characterize a wheat line from a double haploid population, designated as line 98, notable for its reduced tiller number and delayed development of seminal roots. In a series of different germination assays, we observed that the seminal roots of line 98 are highly sensitive to physical resistance during germination, causing delayed root elongation. This went along with altered jasmonic acid (JA) concentrations in roots compared to reference lines. In addition, RNA-Seq analysis showed that genes related to JA synthesis and signaling were significantly enriched. Pharmacological approaches showed that root elongation of line 98 is less sensitive to externally applied MeJA but more sensitive to IBU, an inhibitor of JA biosynthesis, indicating disturbed JA homeostasis in line 98. While an inhibitory function of JA overproduction or JA application on root growth has been shown, our results provide a new link between germination conditions, JA homeostasis and root elongation in a cereal crop.

Keywords: wheat, JA, seminal root elongation

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### Unveiling the role of Trehalose 6-phosphate in root development

M. Göbel<sup>1,2</sup>, J. Foster<sup>3</sup>, F. Fichtner<sup>1,3,2</sup>

<sup>1</sup>Heinrich-Heine-University Düsseldorf, Plant Biochemistry, Düsseldorf, Germany

<sup>2</sup>Cluster of Excellence on Plant Sciences (CEPLAS), Düsseldorf, Germany

<sup>3</sup>University of Queensland, School of Biological Sciences, Brisbane, Australia

Trehalose 6-phosphate (Tre6P) is a sucrose-specific signalling molecule and thus a signal for the plant's carbon status. Tre6P has been demonstrated to influence a large variety of plant metabolic and developmental processes, including the regulation of primary metabolism, embryogenesis, shoot branching, and flowering. Despite this, little is known about the impact of Tre6P on root development. In order to unravel the underlying mechanisms, root growth phenotypes in Arabidopsis lines with increased or decreased Tre6P levels were examined, focusing on a broad range of root growth parameters. Altering Tre6P levels led to significant changes in root architecture. Notably, Tre6P had an opposite effect on root development when compared with shoot development. These findings imply that Tre6P might function as a signal in regulating source-sink relations and carbon allocation. Taken together, similar to its role in shoots, Tre6P functions as a key regulator of root growth. Grafting analyses, RNA sequencing data, and combinatorial mutant analyses further helped us to elucidate the Tre6P signalling network in roots which seems to differ significantly from that of shoots.

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### A zinc finger protein is involved in the response to RES and ROS and the tolerance of abiotic stress

A. Fössel<sup>1</sup>, M. Fuchs<sup>2</sup>, L. Titze<sup>1</sup>, S. Berger<sup>1</sup>

<sup>1</sup>University Würzburg, Pharmaceutical Biology, Würzburg, Germany

<sup>2</sup>Fraunhofer Institut, Hannover, Germany

Biologically active oxylipins comprise well-studied lipid mediators derived from jasmonic acid as well as reactive electrophile species (RES)-oxylipins like 12-oxo-phytodienoic acid (OPDA) which possess an  $\alpha,\beta$ -unsaturated carbonyl structure. OPDA and other RES-oxylipins accumulate upon stresses such as drought and induce stomatal closure and the expression of stress-responsive genes. However, little is known on the COI1-independent signaling pathways mediating the response to RES-oxylipins. Using a forward genetic approach, a zinc finger protein was identified as a putative signaling component. Mutants defective in this protein show less induction of genes related to stress responses. Therefore, the sensitivity of this mutant to different stress factors was investigated. The mutant is more sensitive to the reactive oxygen species butyl hydroperoxide and the xenobiotic triodobenzoic acid. Also the sensitivity to salt was increased in leaves. In contrast, the mutant performed better than the wild type upon extended darkness and drought.

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### Dual function of HvSWEET11b in transporting sugars and cytokinins governs the grain filling in barley

V. Radchuk<sup>1</sup>, Z. M. Belew<sup>2</sup>, A. Gündel<sup>1</sup>, S. Mayer<sup>3,1</sup>, G. Hensel<sup>4</sup>, H. Rolletschek<sup>1</sup>, H. H. Nour-Eldin<sup>2</sup>, L. Borisjuk<sup>1</sup>

<sup>1</sup>Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

<sup>2</sup>University of Copenhagen, Faculty of Science, Frederiksberg C, Denmark

<sup>3</sup>University of Würzburg, Würzburg, Germany

<sup>4</sup>Heinrich-Heine-Universität, Centre for Plant Genome Engineering, Düsseldorf, Germany

Sugars Will Eventually be Exported Transporters (SWEETs) have been found to transport not only sugars but also phytohormones, making them important regulators of plant's growth and development. Of 23 SWEET genes in barley (*Hordeum vulgare*), only *HvSWEET11b*, *HvSWEET15a* and *HvSWEET4* are highly active in the developing grains. In *Xenopus* oocytes, *HvSWEET11b* transported not only sucrose and glucose but also the transporting forms of the phytohormone cytokinin. Vegetative development and fertilization of plants carrying knockout homozygous mutations of *HvSWEET11b* were largely not affected but the developing grains died during the early seed filling period due to the inability of *hvsweet11b* caryopses to accumulate nutrients. The number of endosperm cells was drastically reduced. The partial repression of *HvSWEET11b* transcription by RNAi-technology altered the allocation of both sucrose and cytokinin in the grains and resulted in fewer endosperm cells, lower starch and protein accumulation and a reduction of the grain size at maturity. As analysed by magnetic resonance imaging (MRI), less sucrose was released toward the developing endosperm in transgenic grains, resulting in sucrose build-up in the maternal seed tissues and its redirection toward lateral spikelets. FTIR micro-spectroscopy revealed significantly higher accumulation of trans-zeatin-riboside in maternal seed tissues of *HvSWEET11b*-suppressed grains compared to wild type. The endosperm of transgenic grains further displayed a marked decrease in amylopectin content. Metabolic and transcriptome analyses supported the hypothesis that *HvSWEET11b* mediates the movement of both sucrose and cytokinin across the maternal-filial boundary in barley grains. Because sucrose and cytokinin are transferred by single *HvSWEET11b* transporter in barley grains, they might exert a synergistic effect on maternal-filial relationships and balance seed growth.

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Deciphering the Regulation of Abscisic Acid Transport and Homeostasis under Water Deficit in Arabidopsis

R. Waadt<sup>1</sup>, R. Rasiah<sup>1</sup>

<sup>1</sup>University of Münster, Institute of Plant Biology and Biotechnology, Münster, Germany

The phytohormone abscisic acid (ABA) plays a central role in the response and adaptation of plants to water deficit. In Arabidopsis, drought stress induces the biosynthesis of ABA in leaf vascular tissues via the induction of the *NCED3* gene, which product catalyzes the rate limiting step in the ABA biosynthesis pathway. How ABA is transported from leaf vascular tissues to the root is currently not well understood. Several ABA transporters have been identified in Arabidopsis, including ABCG-, NPF- and DTX/MATE-type transporters. Among them, only a few have been functionally characterized. We are interested in identifying and characterizing the ABA transporters that mediate the translocation of ABA to and within root tissues. For this, we have isolated several ABA transporter loss-of-function mutants, that we are characterizing in terms of altered drought responses in roots. In addition, we have established a heterologous HEK293T cell assay, in which we employ ABA biosensors to measure ABA transport in real-time. Here, we will present our results from these experiments.

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Type-B response regulators (RRs) direct initial steps of barley endosperm differentiation

C. Hertig<sup>1</sup>, I. Mora-Ramirez<sup>1</sup>, T. Rutten<sup>1</sup>, J. Kumlehn<sup>1</sup>, M. Melzer<sup>1</sup>, J. Schippers<sup>1</sup>, J. Thiel<sup>1</sup>

<sup>1</sup>IPK Gatersleben, Molecular Genetics, Gatersleben, Germany

Assimilate supply to reproductive organs has a large impact on grain yield in cereal crops like barley and wheat. Identification of molecular mechanisms triggering differentiation of distinct transfer tissues for nutrient uptake in the endosperm is a prerequisite for improvement of yield potential. Tissue-specific transcriptome profiling and functional studies identified two-component signaling (TCS) phosphorelays as major signal transduction pathway in differentiating endosperm transfer cells (ETCs) of barley. Here, we present effects of CRISPR/Cas9-induced mutations in type-B response regulators (RRs) on barley grain development. Mutants (*rr8*, *rr11* and *rr14*) in different combinations depict defects in ETC specification and adjacent endosperm tissues, finally leading to smaller grains with reduced seed weight and starch content. As type-B RR work as transcription factors, a DNA affinity purification (DAP)-seq approach was used to identify targets of HvRR11 and HvRR14. For both RRs a high number of common target genes was identified showing an enrichment in signaling, vesicle and transmembrane transport processes. Comparison with RNA-seq data of ETCs from *rr11/rr14*-double mutant grains revealed partial commonalities with targets that are directly affected in their expression. Analyses identified potential key elements for transfer cell specification in barley grains and thus, provides new avenues for improvement of yield formation in cereals.

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## Dissecting jasmonic acid and auxin cross-talk in tomato flower development and fruit set

M. Friesch<sup>1</sup>, H. Smits<sup>1</sup>, N. Ori<sup>2</sup>, B. Hause<sup>1</sup><sup>1</sup>Leibniz Institute of Plant Biochemistry, Jasmonate Function & Mycorrhiza, Halle a. d. Saale, Germany<sup>2</sup>Hebrew University of Jerusalem, Rehovot, Israel

Plant reproduction involves regulatory processes during flowering, fertilization, and fruit development, relying on phytohormones like jasmonic acid (JA) and auxin. In *Arabidopsis*, JA-Ile is perceived by the F-box protein COI1 and regulates stamen development, as *coi1* mutants are male sterile. The tomato mutant defective in the COI1 ortholog SlJAI1 (*jai1*) is, however, female sterile. Transcriptomic analyses using ovules from *jai1* identified the two JA-induced R2R3-MYB transcription factors SIMYB21 and SIMYB24 as key regulators in gynoecia development [1]. *Slmyb21* flowers show strong alterations in flower development, which is more severely affected in the *Slmyb21xSlmyb24* double mutant as visible by non-opened flowers, infertile ovules, and aborted fruits. A role of auxin in controlling flower development was shown for *Arabidopsis*, since the mutation of the AUXIN RESPONSE FACTORS 8/6 leads to male sterility [2]. The tomato orthologs SIARF8A/B, however, were shown to control stigma development and thereby control female fertility [3]. These findings point to a complex regulatory network of flower development controlled by the interplay between TFs associated to JA and auxin signaling. To identify the molecular interactions between SIARF8A/B, SIMYB21, and SIMYB24 in gynoecia and stigma development, different molecular methods will be employed. Using *trans*-activation assays, transcriptional activity of all four TFs on their respective promoters and those of possible target genes associated to flower development will be analyzed. Additionally, characterization of different multiple mutants will give insights into the interaction of the four TFs during flower development. Transcriptomic data from the mutants will be complemented by the identification of SIMYB21 and SIMYB24 target genes using a modified *in vitro* ChIP (DAP-seq [4]). This approach will be extended to *Arabidopsis* MYB21 and MYB24. Identification of the MYB21/24 target genes in both species will help to explain how the loss of MYB21 activity results in female and male sterility in tomato and *Arabidopsis*, respectively. Answering these questions will further expand our knowledge about the regulation of flower development in tomato and *Arabidopsis* by the interplay of JA and auxin.

## References

- [1] Schubert et al. (2019) *Plant Cell* 31, 1043-1062.
- [2] Nagpal et al. (2005) *Development* 132, 4107-4118.
- [3] Israeli et al. (2023) *Plant Physiol.* 192, 2336–2355.
- [4] Bartlett et al. (2017) *Nature Protocols* 12, 1659-1672

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Impact of *FLC* genes on flowering time in spring oilseed rapeS. Duveneck<sup>1</sup>, S. Melzer<sup>1</sup><sup>1</sup>Kiel University, Botanical Institute, Kiel, Germany

Winter and spring oilseed rape (*Brassica napus*) are different growth types that need or don't need vernalization, which is an extended time of cold exposure as a requirement for flowering. In *Arabidopsis thaliana*, as well as in other Brassicaceae, *FLOWERING LOCUS C (FLC)* is the major regulator of the vernalization response. FLC is a floral repressor and prevents the transition to flowering by directly blocking the activity of several flowering time pathways. The cold-induced stable epigenetic repression of *FLC* by vernalization commits plants to flower. Since *B. napus* is closely related to *A. thaliana* a similar flowering time regulation is expected. However, due to the allotetraploid nature of the *B. napus* genome that retained the triplicated ancestral genomes of *B. rapa* and *B. oleracea*, many homologous genes are expected for one *Arabidopsis* ortholog. Therefore, a complex pattern of how different *FLC* alleles are epigenetically repressed in cold to confer different vernalization responses can be expected. *B. napus* has nine *FLC* homologs and we have started to characterize those genes by expression profiling and CRISPR/Cas9 knockout studies in winter as well as in spring oilseed rape. *FLC* mutants for the spring-type Westar differed in their genotype due to varying combinations of up to nine mutated *FLC* genes. Phenotypic analyses have shown that also in spring oilseed rape the *FLC* genes have a profound impact on flowering time, with certain mutations more important than others. How the downregulation of the *FLC* genes in *B. napus* is controlled and whether this is accompanied by histone modifications is currently under study.



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Regulation of *Arabidopsis thaliana* 13-LOX enzymes involved in root jasmonate biosynthesis

F. Rößler<sup>1</sup>, D. Gasperini<sup>1</sup>, Y. Ma<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry, Molecular Signal Processing, Halle a. d. Saale, Germany

The jasmonate (JA) pathway plays vital roles in plant growth and environmental responses. Following tissue damage provoked by insect herbivory or mechanical wounding, levels of the pro-hormone JA and its bioactive conjugate JA-Ile increase rapidly with resulting sensing and signalling mechanisms leading to transcriptional, proteomic and metabolomic reprogramming to ultimately grant plant acclimation. Biosynthesis of JA-Ile requires the activity of plastidial 13-LIPOXYGENASE (13-LOX) enzymes catalysing the oxygenation of  $\alpha$ -linolenic acid. However, it remains unclear what are the molecular mechanisms regulating 13-LOX activation specifically in root tissues. *Arabidopsis thaliana* harbours four 13-LOX genes (*LOX2*, *LOX3*, *LOX4* and *LOX6*), with *LOX6* being essential, yet not sufficient, for root JA-Ile production following local root wounding. The aim of my PhD project is therefore to uncover how is root JA-Ile biosynthesis initiated. As root JA-Ile levels are much lower than in green tissues, I will first establish a root wounding method for robust JA-Ile induction based on micro-needling and osmotic changes. The method will be assessed by quantitative hormone profiling by LC-MS and the expression of JA-Ile marker genes. I will then investigate the mode of *LOX6* enzyme activation by examining point mutants impacting specific regulatory domains as well as putative allosteric sites. Furthermore, I will determine which of the remaining three 13-LOXs is involved in root JA-Ile biosynthesis and how are they regulated. Our preliminary analyses point towards a post-transcriptional regulation which will be investigated further. Overall, my PhD project aims to uncover early events leading to JA-Ile biosynthesis in roots, which has thus far been poorly characterized.

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Plastid development and proteomics: potentials and challenges: A review

A. Kumar<sup>1</sup>

<sup>1</sup>University of Rajasthan, Jaipur 302004, Department of Botany and Biotechnology, Jaipur, India

Climate change resulting in increasing global warming, frequent droughts and floods and alarming world population highlights the urgent need for development of climate resilient crops. Plastid differentiation and specialization is regulated by environmental signals and plastid proteomics is influenced by abiotic and biotic stresses. The omics technologies i.e., proteomics, transcriptomics, metabolomics, genomics, provide information that helps us improve the stress tolerance in plants. Development of plastids *in vitro* provides an opportunity to correlate physiological and morphological status of plastids with the external and internal environmental conditions. Plastid proteomics during development, stress and senescence has provided valuable data to understand proteome dynamics and complexity. Metabolites that are synthesized in chloroplasts are important for plant interactions with their environment e.g. responses to heat, salt, drought, light etc. and their defense against invading pathogens. Thus, chloroplasts serve as metabolic centers in cellular reactions to signals and respond via retrograde signaling. Plastids are key environmental sensors. Plastids interconnect cellular metabolism with mitochondria and nucleus to regulate and stress responses. The terrestrial stressors are key triggers of plastid-to-nucleus retrograde signaling. The chloroplast genome encodes many key proteins that are involved in photosynthesis and other metabolic processes. Ultrastructural development of plastids in cultured plant cells shall be elucidated and role of proteomics shall be presented as mini review.

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*Arabidopsis thaliana* adapts its sphingolipid profile to different temperatures

F. Waller<sup>1</sup>, K. Erwardt<sup>1</sup>, P. Korte<sup>1</sup>, A. Voss<sup>1</sup>, M. Krischke<sup>1</sup>, A. Fekete<sup>1</sup>, M. J. Mueller<sup>1</sup>

<sup>1</sup>Julius-Maximilians-Universität Würzburg, Biocenter. Julius-von-Sachs-Institute, Pharmaceutical Biology, Würzburg, Germany

Sphingolipids make up a significant proportion of the plasma membrane of plant cells. They are, together with Sterols, predominantly localized in membrane micro- or nanodomains. Each of the two sphingolipid classes Glycosylceramides (GlcCers) and Glucosylinositolphosphorylceramides (GIPCs) consists of many different species. These species have different properties due to different combinations of head group, sphingolipid long-chain base and acyl chain of different lengths, degree of desaturation and addition of hydroxy groups.

While functional studies in *Arabidopsis* have shown that specific sphingolipids can be required for receptor or transmembrane protein functions, little is known about adaptations of the sphingolipid profile to different environmental conditions. As a first step towards a better understanding of cellular adaptations to different temperature conditions, we determined the sphingolipid profile of *Arabidopsis thaliana* during different temperature regimes. We analyzed the levels of GlcCers and GIPCs, and their precursors, Ceramides and Hydroxyceramides, using UPLC-MS/MS. We observed that temperature-specific changes in the sphingolipid profile occurred as early as 4 h after subjected to moderate heat stress (37°C). We also analyzed sphingolipid profiles in leaves of *Arabidopsis* plants grown at four different temperatures for several days. Again, significant differences in the sphingolipid profile were observed, including sphingolipid species showing a similar regulation at an elevated temperature of 28°C as during moderate heat stress. For major membrane lipids, such as Glycerophospholids, it is known that lower temperatures correlate with a higher degree of acyl chain desaturation, which serves to maintain membrane function by increasing membrane fluidity. Here, we observed a similar pattern for some, but not all sphingolipid species. We hypothesize that GlcCer and GIPC composition is adapted to maintain membrane properties in a temperature-dependent manner, while specific sphingolipid species might be required for functions independent of temperature conditions.

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**Multiple paclobutrazol and nitrogen treatments increase potato tuber formation**S. Mubarok<sup>1</sup>, J. S. Hamdai<sup>1</sup>, A. Nuraini<sup>1</sup>, K. Kusumiyati<sup>1</sup><sup>1</sup>Universitas Padjadjaran, Agronomy, Sumedang, Indonesia

Development of G0 potato seeds in medium-altitude has been constrained by the deficiency of nutrients for plant growth and high temperatures which increased the biosynthesis of gibberellic acid, which inhibited optimal plant production. The effort to increase the production of G0 potato seeds was by the addition of nitrogen fertilizer and paclobutrazol (PBZ) application. The study aimed to determine the interaction between the dose of nitrogen fertilizer and the frequency of PBZ application on the plant growth and yield of G0 potato seeds in medium-altitude. The following experimental design was a Randomized Complete Block Design (RCDB) in a factorial arrangement with two factors. The first factor was the dose of nitrogen fertilizer (n1=60, n2=120, and n3=180 kg/ha) and the second factor was the frequency of PBZ application (p1 = 1, p2 = 2, and p3 = 3 times), applied starting at 40 DAP and repeated every 10 days with the concentration of 100 ppm. The results showed that there was a significant interaction between the dose of nitrogen fertilizer and the frequency of PBZ application on plant dry weight. The 120 kg/ha of nitrogen fertilizer (n2) showed higher yields on plant height and the number of stolons per plant. The multiple frequencies of 3 times PBZ application at 40, 50, and 60 DAP (p3) showed the best independent results on plant height, tuber growth rate, and the number and weight of tuber per plant. The highest number and weight of tuber per plant were 9.26 and 83.97 g (p3).

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**Changes of leaf water relations during severe drought stress through silicon application to spring wheat (*Triticum aestivum* L.)**T. Selzer<sup>1</sup>, M. Pérez Rodriguez<sup>1,2</sup>, J. Santner<sup>1</sup><sup>1</sup>Justus Liebig University, Institute of Plant Nutrition, Gießen, Germany<sup>2</sup>Universidad Politecnica de Valencia, Valencia, Spain

Silicon (Si), although not considered an essential element for plant nutrition, has been shown to influence a plants' ability to cope with biotic and abiotic stress positively [1]. Especially monocots take up large amounts of Si in the form of silicic acid (H<sub>4</sub>SiO<sub>4</sub>) [2]. Various studies have reported an increased drought resistance of grain crops such as rice and wheat after Si application [3]. However, the underlying mechanisms have not been identified yet.

Increasing evidence suggests that Si does not have a direct biochemical effect [1], since Si quickly precipitates at the cell wall and in the apoplast of leaf cells in the form of silica (SiO<sub>2</sub>), a process called biosilicification [4]. Due to the well-known hygroscopic characteristics of silica gel we hypothesized that the additional silica layer in the leaf exerts a matrix potential which is absent in plants without Si nutrition. This additional matrix potential could increase the plants' ability to retain water in the leaves under drought conditions, resulting in a higher drought resilience. Spring wheat (*Triticum aestivum* L. cv. Expectum) was grown under controlled conditions in 2.8 L pots with a modified Hoagland nutrient solution without Si (Si0) and with Si (Si1 = 0.5 mmol L<sup>-1</sup>, Si2 = 1.75 mmol L<sup>-1</sup>) for a total of 35 days. On day 27, drought stress was induced using 20% polyethylene glycol (PEG 6000). A control treatment (no PEG) was included for each Si treatment. 34 days after sowing (DAS), water-related physiological parameters (osmotic potential, water potential, relative water content, photosynthesis) were determined for the youngest fully developed leaf. Biomass of shoot and roots was determined 35 DAS. Element concentrations (Si, N, P, K) were determined using ICP-OES and an elemental analyzer.

Results of the experiment will be displayed on the poster focusing on the influence of Si nutrition on leaf water retention.

## References

- [1] Coskun et al. (2019) The controversies of silicon's role in plant biology. *New Phytologist* 221:67-85.
- [2] Liang et al. (2015) *Silicon in Agriculture*. Springer Science+Business Media. Dordrecht.
- [3] Bukhari et al. (2021) Silicon mitigates drought stress in wheat (*Triticum aestivum* L.) through improving photosynthetic pigments, biochemical and yield characters. *Silicon* 13, 4757–4772.
- [4] Xu et al. (2023) Functions of silicon and phytolith in higher plants. *Plant Signaling & Behavior* 18, e2198848.

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**Arabidopsis NADP-malic enzyme 1 involved in abscisic acid response during drought stress and seed development**

Y. Fu<sup>1</sup>, M. Klamke<sup>1</sup>, S. Rameshkumar<sup>1</sup>, V. G. Maurino-Larcher<sup>1</sup>

<sup>1</sup>University of Bonn, Institut für Zelluläre & Molekulare Botanik, Bonn, Germany

In the natural environment, plants are exposed to various abiotic stresses such as salinity, heat, and drought. These environmental stresses often occur in combination and are serious factors limiting plant growth and productivity. In our previous transcriptome analysis in *Arabidopsis thaliana* exposed to single and combined stresses<sup>1</sup>, we found that NADP-malic enzyme1 (NADP-ME1) was specifically highly expressed after osmotic stress applied alone and also in combination with other stresses. The *A. thaliana* genome contains four NADP-malic enzyme isoforms (NADP-ME1 to 4) that catalyze the oxidative decarboxylation of L-malate to pyruvate, CO<sub>2</sub>, and NADPH. Our in silico analysis revealed that the promoter region of AtNADP-ME1 contains the abscisic acid (ABA)-responsive element (ABRE) and the dehydration-responsive element (DRE). Many abiotic stress- and ABA-inducible genes contain these two cis-elements in their promoter region. Additionally, we found NADP-ME1 to be co-expressed with ABA-related genes, and these genes were also significantly induced under various abiotic stresses<sup>1</sup>. The phytohormone ABA, known as the “stress hormone,” rapidly accumulates in plants in response to abiotic stress and plays an important role in stress adaptation and plant growth regulation. NADP-ME1 is normally expressed in mature seeds and roots but not in leaves<sup>2</sup>. However, we found that NADP-ME1 is significantly induced in leaves after drought stress, while the expression of AtNADP-ME2 to 4 is not significantly altered. To further investigate the link between NADP-ME1 and ABA signaling, we analyzed germination and postgerminative development of NADP-ME1 knockout mutants (*me1*) in the presence of ABA. Interestingly, we found that *me1* lines germinate like wild-type plants in the presence of ABA, but are subsequently able to elongate the primary root, whereas root development of wild-type plants is arrested. Taken together, these results suggest that NADP-ME1 is involved in the ABA network that controls responses to drought and root development.

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**Differential expression of Methanol Inducible Protein, Pectin Methylesterases, and Pectin Methylesterase Inhibitor/s induced by Hormetic level of Cadmium in *Solanum lycopersicum***

M. Yadav<sup>1</sup>, R. Ravi<sup>1</sup>, S. R. Kanade<sup>1</sup>

<sup>1</sup>University of Hyderabad, Plant Sciences, School of Life Sciences, Hyderabad, India

Hormesis suggests that low concentrations of toxic elements can benefit plant growth, but high concentrations are lethal. Heavy metals, such as cadmium (Cd), have a fatal effect on the growth and development of plants at lethal levels. Recent studies suggest that Cd has a hormetic effect even though its precise function in plant development has not been reported. The hormetic effect is represented by a reverse U-shaped curve and this hormetic responses have recently attracted the interests of scientists all around. In the present study, we have observed that the morphological parameters such as root length, shoot length, fresh weight, dry weight of tomato plants grown under low concentration were seen to be increased and in high concentration they were decreased. We also found improved plant growth having better photosynthetic activities and more dense root architecture at low concentrations of Cd with reduced stress markers like total proline and Malondialdehyde content. Pectin methylesterase (PME) is involved in the biogenesis and remodelling of cell walls throughout overall plant development. PME aids in the demethylation of pectin, cleaving methyl ester groups into carboxyl groups which alters the pH and ion equilibrium within the primary cell wall and contributes to methanol production inside cell. While the exact fraction of methanol produced that undergoes recycling through metabolism in plants remains unclear, but it is certain that plants can metabolize methanol and can utilize it as a specific signaling molecule within the plant and plant-to-plant communication. The transcriptomic analysis of the roots and leaves of plants treated with low and high cadmium were performed and gene expression of MIP was validated using RT-PCR. While numerous studies have explored stress and developmental responses in plants regarding PMEs, there remains a substantial need for further investigation to comprehensively map PMEs, MIP and their potential involvement in hormesis. This research could facilitate the utilization of PMEs and MIP to cultivate crops with enhanced adaptability to extreme environmental conditions.

Keywords: Heavy metals, Cadmium, Hormesis, MIP, Pectin, PME

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## Elucidation of Non-Histone Protein Acetylation in Arabidopsis Under High Light Stress

J. Shen<sup>1</sup>, J. Eirich<sup>1</sup>, I. Finkemeier<sup>1</sup><sup>1</sup>University of Muenster, Münster, Germany

Light serves as the primary energy source for photosynthesis and plays a pivotal role in various developmental processes in plants. However, exposure to high light (HL) levels exceeding the energy requirements for photosynthesis can induce stress responses in plants. Previous research has highlighted the involvement of transcriptional regulatory networks in mediating HL signaling and regulating the expression of specific genes. Nonetheless, the precise molecular mechanisms governing epigenetic regulation in HL stress-induced plant development remain elusive. In this study, we explore the role of non-histone protein N-ε-lysine acetylation, a dynamic post-translational modification (PTM), in Arabidopsis development under HL stress conditions, and explore whether acetylation patterns persist beyond the stress period, potentially serving as an epigenetic memory mechanism. N-ε-lysine acetylation is a dynamic PTM crucial for modulating various physiological functions of proteins, including localization, stability, enzyme activity, protein-protein interactions, transcriptional regulation, and signal transduction. By employing LC-MS/MS-dependent proteomics, we identified protein acetylation sites induced by HL, thereby enhancing our understanding of the underlying epigenetic mechanisms involved in non-histone protein acetylation during HL stress processes. Additionally, molecular biology techniques are utilized to specifically modify lysine residues on selected proteins, further elucidating the functional implications of non-histone protein acetylation in response to HL stress. This research aims to understand how acetylation contributes to plant adaptation and survival under HL conditions and bridges the gap between epigenetics, HL stress signaling, and plant development. We hope to provide valuable insights for sustainable crop improvement strategies in agricultural systems.

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## Towards a better understanding of ethylene metabolism using a novel UPLC-MS/MS based quantification method

T. Depaepe<sup>1</sup>, D. Cao<sup>1</sup>, R. Sanchez-Muñoz<sup>1</sup>, H. Janssens<sup>1</sup>, F. Lemièrè<sup>2</sup>, T. Willems<sup>2</sup>, J. Winne<sup>1</sup>, E. Prinsen<sup>2</sup>, D. Van Der Straeten<sup>1</sup><sup>1</sup>Ghent University, Biology, Gent, Belgium<sup>2</sup>Antwerp University, Antwerp, Belgium

The balance of plant hormone levels is critical for optimal responses to internal and external stimuli, and is maintained through biosynthesis, transport, conjugation, and catabolism. Ethylene, a volatile key regulator of growth and defence, has a well-characterized biosynthetic pathway, but its transport and conjugation remain poorly understood. Its direct and soluble precursor 1-aminocyclopropane-1-carboxylic acid (ACC), was proposed to be conjugated *in planta* to malonyl-ACC (MACC), glutamyl-ACC (GACC), and jasmonyl-ACC (JA-ACC). Moreover, the recent discovery of ACC acting as an independent signal from ethylene in both vegetative and generative growth stages underscores the need for further research into its roles. To that end, precise quantification of ACC and its conjugates is essential. However, no combined method currently exists for this purpose. We developed and validated a high-throughput method to simultaneously quantify ACC, MACC, GACC, and JA-ACC with a straightforward extraction process, eliminating the need for derivatization or additional concentration steps. Using this method, we explored the roles of ACC and its conjugates in ethylene metabolism and under abiotic stress conditions. Using mutants, pharmacological approaches, and stress assays, our findings unveiled significant physiological insights, particularly regarding the metabolic equilibrium of ACC and its major conjugate MACC. Contrary to previous research, GACC and JA-ACC were undetectable *in vivo*, casting doubt on their presence in Arabidopsis. However, GACC was identified *in vitro*, suggesting it might accumulate in specific subcellular compartments or be rapidly converted into another metabolite after synthesis. This new method paves the way for novel explorations in ethylene and ACC metabolism, offering a powerful tool for future research into their regulatory mechanisms and environmental stress responses.

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Grain yield of wheat as affected by individual and combined heat and drought stress during different growth stages  
with emphasis on source-sink relations

D. Ehrhardt<sup>1</sup>, J. Santner<sup>1</sup>, B. Hütsch<sup>1</sup>

<sup>1</sup>Institute of Plant Nutrition, Justus-Liebig University, Gießen, Germany

In the future, temperatures are expected to rise worldwide and water supply is expected to decrease, which will affect plant productivity [1]. Yet, the impact of combined heat and drought stress on plants has not been sufficiently investigated. For wheat (*Triticum aestivum* L.), a heat-sensitive crop, previous studies have mainly focused on the grain-filling phase; however, the combined effects of heat and drought stress during vegetative growth and flowering have been rarely studied [2; 3].

A previous study showed that under continuous heat stress the plants were able to strongly increase the number of ear-bearing tillers. The yield potential could not be fully utilized (kernel setting reduced, but set kernels were well filled). No source limitation occurred but the sink capacity (fewer and smaller grains) was reduced. [2].

The aim of this project is to identify the limiting factors for grain yield formation under heat and drought stress at vegetative growth, flowering, and grain-filling. Two wheat varieties are going to be stressed in five trials, both individually and in combination of heat and drought, at certain phenological stages of plant development (vegetative growth, flowering, and grain-filling). The analyses focus on the source-sink relations, which are characterized by measurements on living plants and by analyses of various plant organs harvested at flowering, grain-filling stage or full maturity. In addition, physiological processes (photosynthesis, starch synthesis) and enzyme activities (acid invertase, plasma-membrane H<sup>+</sup>-ATPase, starch synthase) involved are investigated.

The results help to identify traits that can improve the heat and/or drought resistance of wheat leading to a more stable yield formation under stress conditions and can be integrated into breeding programs and thus improve the sustainability of wheat cultivation.

References

- [1] Uprety, D.C.; Reddy, V.R (2016) Crop Responses to Global Warming. Springer Science+Business Media Singapore
- [2] Hütsch, B.W.; Jahn, D.; Schubert, S. (2019) Grain yield of wheat (*Triticum aestivum* L.) under long-term heat stress is sink limited with stronger inhibition of kernel setting than grain filling. *Journal of Agronomy and Crop Science* 205, 22-32
- [3] Kaur, J.; Sandhu, S.S.; Sharma, S. (2020) Yield response of wheat grown under periodic heat stress imposed by using mini heat tents. *Journal of Agronomy and Crop Science* 207, 285-303

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ABA-signaling differs between the extremophyte *Eutrema salsugineum* and its  
close glycophytic relative *Arabidopsis thaliana*

L. Spiller<sup>1</sup>, S. Endres<sup>1</sup>, C. Weiste<sup>2</sup>, R. Hedrich<sup>1</sup>, D. Geiger<sup>1</sup>

<sup>1</sup>Julius-Maximilians-Universität Würzburg, Department of Botany 1 - Plant-Physiology and Biophysics, Würzburg, Germany

<sup>2</sup>Julius-Maximilians-Universität Würzburg, Chair of Pharmaceutical Biology, Würzburg, Germany

As the global climate change progresses, the frequency and intensity of biotic and abiotic stressors, such as herbivory, pathogen infestation, drought, salinized soils, and extreme periods of heat, cold, and drought, plants are confronted with, is increasing. With abiotic stress playing a major role in agricultural crop loss, it is becoming ever more important to understand the molecular mechanisms behind stress responses in plants, to ensure sustainable crop yields through targeted breeding or transgenic plants to sustain the growing population. The phytohormone abscisic acid (ABA) plays a crucial role in stress response and, though the signal pathway has been widely studied, quantitative data describing the massive protein-protein-interaction rearrangements within the core signaling complex are widely lacking to this day. This project serves to advance the understanding of protein-protein interaction dynamics within the core ABA-signaling complex through comparative characterization of ABA receptors between the glycophyte *Arabidopsis thaliana* and its close halophytic relative *Eutrema salsugineum*. An optimized bimolecular luminescence complementation (BiLC) assay was established and applied to study and quantify the interactions between ABA-receptors (PYLs) and the PP2C phosphatase (ABI1). The obtained insights into the quantified dynamics and affinities revealed a significant difference between the two model plants. Interestingly, the interaction of EsPYL3 with ABI1 showed a 5-fold higher ABA-affinity than the *Arabidopsis thaliana* orthologue. Accompanying structure-function research identified the structural basis of the differing ABA-affinities. To which extent the increase in ABA-affinity has contributed to the stress tolerance of *Eutrema salsugineum*, will move into focus of following phenotyping studies.

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### Molecular signatures in the induction of UV protection by chlorogenic acid accumulation in sunflower leaves

J. Stelzner<sup>1</sup>, E. M. Molin<sup>2</sup>, H. P. Mock<sup>3</sup>, W. Bilger<sup>1</sup>

<sup>1</sup>Christian-Albrechts Universität zu Kiel, Botanisches Institut, Kiel, Germany

<sup>2</sup>AIT Austrian Institute of Technology, Center for Health & Bioresources, Tulln an der Donau, Austria

<sup>3</sup>Leibniz-Institute for Plant Genetics and Crop Plant Research, Seeland, Germany

Phenolic compounds act as UV-screening compounds in higher plants and their accumulation is induced not only by UV-B radiation but also by other environmental factors such as high photon flux density (PFD) or low temperature. While in most plants these compounds belong to both groups, flavonoids and hydroxycinnamic acid derivatives (HCAs), sunflower (*Helianthus annuus* L.) leaves contain only compounds of the latter group, mainly chlorogenic acid (CGA) derivatives. In order to better understand the specific signalling pathways that dynamically induce CGA accumulation while avoiding the induction of flavonoids, we exposed young sunflower plants to sudden changes in environmental conditions by transferring them from low PFD, 21°C, to either high PFD (350  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) or low temperature (9°C) or UV-B radiation (50  $\text{mW m}^{-2}$ ).

After transfer, epidermal UV-A absorbance, indicating the formation of CGA derivatives, increased already after 24 h in all conditions, correlating well with the results of HPLC analyses. Six h (except for low temperature), 12 h, 24 h and 72 h after the transfer, samples were taken from the youngest mature leaf and RNA was extracted for RNAseq analysis. Especially the first time points after the transfer (6 h and 12 h) showed a higher number of differentially expressed genes in all three environmental conditions compared to the initial time point before the transfer, whereas later time points (24 h and 72 h) came closer to the initial pattern of gene expression. At 12 h and 24 h after transfer, 302 and 50 differentially expressed genes, respectively, were shared by all three environmental conditions. However, the differential expression of all these genes was in the same direction for exposure to high PFD and low temperature, whereas there was a surprisingly high discrepancy between induction by UV-B and high PFD. 183 of the 302 genes differentially regulated after 12 h were downregulated by UV-B radiation but upregulated by high PFD. When focusing on hydroxycinnamoyl quinate transferases (HQT), which are central enzymes in the biosynthesis of CGA, HQT2 and HQT3 were highly expressed under all conditions. The nature of the differentially expressed genes will be further analysed to provide a deeper understanding of the molecular mechanisms underlying the UV protection mechanism in sunflower based on accumulation of hydroxycinnamic acid conjugates.

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### Unraveling Photoprotective Mechanisms: Insights from Green Algae to Vascular Plants

P. Redekop<sup>1</sup>, E. Sanz-Luque<sup>2</sup>, A. Grossman<sup>3</sup>, M. Baier<sup>1,3</sup>

<sup>1</sup>Freie Universität Berlin, Biology, Chemistry, Pharmacy, Berlin, Germany

<sup>2</sup>University, Biochemistry and Molecular Biology, Cordoba, Spain

<sup>3</sup>Carnegie Institution of Science, Plant Biology, Stanford, CA, United States

Understanding how photosynthetic organisms dynamically respond to changing light conditions is crucial for elucidating their adaptive strategies. Building upon investigations into the highlight stress perception of the green alga *Chlamydomonas reinhardtii*, which revealed the activation of key photoprotective genes, we now shift our focus to vascular plants and include the impact of redox signaling.

In our previous work with *C. reinhardtii*, we uncovered a nuanced interplay of factors influencing gene induction, even at low light intensities. Notably, distinct influences from blue and ultraviolet B radiation, as well as carbon dioxide levels, were found to modulate gene expression patterns before highlight stress has occurred. These findings underscore the proactive approach of cells in preventing photodamage and point out the sophisticated mechanisms by which green algae adapt to changing light environments.

In our current research with *A. thaliana*, we are investigating the regulation of thylakoid Ascorbate peroxidase (tAPX), a crucial component of the antioxidative defense system. Through transcript analysis of tAPX in different mutants lacking various components of the photoprotective system, we aim to unravel the regulatory networks between the antioxidative defense system and photoprotection in vascular plants.

By bridging the gap between green algae and vascular plants, our research offers a comprehensive perspective on the molecular underpinnings of photosynthetic resilience. We anticipate that our findings will not only deepen our understanding of photoprotective mechanisms but also pave the way for innovative strategies to enhance crop resilience in fluctuating light environments.

P 201

**Myrosinase TGG1 regulates guard cell glucosinolate levels with significance for drought-related stomatal closure**

B. Franzisky<sup>1</sup>, G. Bárdos<sup>2</sup>, K. Witzel<sup>3</sup>, F. Hanschen<sup>3</sup>, B. Kroschewski<sup>4</sup>, C. Zörb<sup>2</sup>, C. M. Geilfus<sup>1</sup>

<sup>1</sup>Geisenheim University, Soil Science & Plant Nutrition, Geisenheim, Germany

<sup>2</sup>University of Hohenheim, Crop Science, Hohenheim, Germany

<sup>3</sup>Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren, Germany

<sup>4</sup>Humboldt-University Berlin, Chair of Statistics, Berlin, Germany

Myrosinase in guard cells of *Brassicaceae* is suspected to be involved in the drought response by regulating stomatal closure to minimize water loss. Specifically, THIOGLUCOSIDE GLUCOHYDROLASE1 (TGG1) and TGG2 hydrolyze glucosinolates (GLSs), with the degradation products triggering stomatal closure (Zhao *et al.*, 2008; Khokon *et al.*, 2011; Aihara *et al.*, 2023).

We explore the role of TGG1 in drought tolerance in *Arabidopsis thaliana* by comparing wild-type (Col-0) plants with *tgg1-1* mutants under progressive soil desiccation. In *tgg1-1* mutants, the drought-induced reduction in stomatal pore area was less pronounced, while relative leaf water content decreased and membrane leakage increased compared to Col-0. These observations suggest that TGG1 is relevant for effective stomatal closure, which is vital for preserving leaf hydration and membrane integrity during drought. Quantitative glucosinolate profiling unveiled similar proportions of alkyl and indol GLSs in guard cells, with higher concentrations of these GLSs in *tgg1-1* mutants also under drought, implying a role for TGG1-mediated GLS breakdown in stomatal regulation. These findings underscore the importance of TGG1 for drought tolerance, possibly through GLS breakdown-mediated stomatal closure, and provide new insights into the complex interplay between plant chemical defense and abiotic stress responses.

References

Aihara Y, Maeda B, Goto K, Takahashi K, Nomoto M, Toh S, Ye W, Toda Y, Uchida M, Asai E *et al.* 2023. Identification and improvement of isothiocyanate-based inhibitors on stomatal opening to act as drought tolerance-conferring agrochemicals. *Nature communications* 14: 2665.  
 Khokon MAR, Jahan MS, Rahman T, Hossain MA, Muroyama D, Minami I, Munemasa S, Mori IC, Nakamura Y, Murata Y. 2011. Allyl isothiocyanate (AITC) induces stomatal closure in *Arabidopsis*. *Plant, cell & environment* 34: 1900–1906.  
 Zhao Z, Zhang W, Stanley BA, Assmann SM. 2008. Functional proteomics of *Arabidopsis thaliana* guard cells uncovers new stomatal signaling pathways. *The Plant cell* 20: 3210–3226.

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**When Temperatures Rise: Exploring Root Thermomorphogenesis Through Single Cell Transcriptomics**

T. Jacob<sup>1</sup>, O. Maciel Rodrigues Jr.<sup>1</sup>, H. Ai<sup>1</sup>, B. Hause<sup>2</sup>, L. Eschen-Lippold<sup>1</sup>, C. Delker<sup>1</sup>, M. Quint<sup>1</sup>

<sup>1</sup>Institute for Agricultural and Nutritional Sciences, Martin-Luther-University Halle-Wittenberg, Department of Crop Physiology, Halle a. d. Saale, Germany

<sup>2</sup>Leibniz Institute for Plant Biochemistry, Department of Cell and Metabolic Biology, Halle a. d. Saale, Germany

Plant growth and development are highly plastic, allowing for acclimation to a changing environment, such as the global rise in temperature. Root thermomorphogenesis describes root growth responses to elevated ambient temperatures and is characterized by increased primary root growth, possibly to reach deeper soil layers in anticipation of drought stress [1]. During early seedling development, it mainly relies on increased cell divisions, which are conveyed by elevated auxin levels in the root apical meristem [2]. However, we are still far from comprehensively understanding the underlying molecular mechanisms that connect the auxin signal with cell cycle acceleration. Therefore, we performed single-cell RNA sequencing of root apical meristems (RAMs) grown either at constant 20°C or 28°C, or subjected to a short-term shift from 20°C to 28°C prior to sampling. Overall, we detected more than 100,000 high-quality cells expressing a median of roughly 1,500 genes per cell, which represent more than 22,000 genes in total. Further in-depth analysis of this dataset will elucidate expression patterns of genes regulating cell cycle activity and polar auxin transport in response to elevated temperatures at cellular resolution. A possible candidate for the regulation of cell cycle activity in root thermomorphogenesis is the kinase TARGET OF RAPAMYCIN (TOR). TOR is a central growth regulator throughout the eukaryotic kingdom, which in plants integrates external stresses as well as internal energy, nutrient, and hormone levels [3]. TOR promotes meristem activation by phosphorylation of the S-phase-promoting transcription factor E2FA [4]. A combination of genetic and pharmaceutical root growth assays, as well as microscopical analyses of the RAM, demonstrated that TOR is involved in the regulation of primary root elongation in response to elevated ambient temperatures. Specifically, seedlings impaired in TOR function did not show increased cell proliferation in the RAM at warm temperatures, in contrast to the wildtype. Elucidating this previously unknown function of the TOR kinase further contributes to an advanced understanding of root thermomorphogenesis.

References:

[1] de Lima *et al.* 2021, *J. Exp. Bot.* (72).  
 [2] Ai *et al.* 2023, *EMBO J.* (42).  
 [3] Morales-Herrera *et al.* 2024, *Trends Plant Sci.* (in press).  
 [4] Xiong *et al.* 2013, *Nature* (496).

P 203

Na<sup>+</sup>-preferential ion transporter HKT1;1 mediates salt tolerance in blueberry

H. Song<sup>1</sup>

<sup>1</sup>University of Würzburg, Würzburg, Germany

Soil salinity is a major environmental factor constraining growth and productivity of highbush blueberry (*Vaccinium corymbosum*). Leaf Na<sup>+</sup> content is associated with variation in salt tolerance among blueberry cultivars; however, the determinants and mechanisms conferring leaf Na<sup>+</sup> exclusion are unknown. Here, we observed that the blueberry cultivar “Duke” was more tolerant than “Sweetheart” and accumulated less Na<sup>+</sup> in leaves under salt stress conditions. Through transcript profiling, we identified a member of the High-Affinity K<sup>+</sup> Transporter (HKT) family in blueberry, *VcHKT1;1*, as a candidate gene involved in leaf Na<sup>+</sup> exclusion and salt tolerance. *VcHKT1;1* encodes a Na<sup>+</sup>-preferential transporter localized to the plasma membrane and is preferentially expressed in the root stele. Heterologous expression of *VcHKT1;1* in *Arabidopsis* (*Arabidopsis thaliana*) rescued the salt hypersensitivity phenotype of the *athkt1* mutant. Decreased *VcHKT1;1* transcript levels in blueberry plants expressing antisense-*VcHKT1;1* led to increased Na<sup>+</sup> concentrations in xylem sap and higher leaf Na<sup>+</sup> contents compared with wild-type plants, indicating that *VcHKT1;1* promotes leaf Na<sup>+</sup> exclusion by retrieving Na<sup>+</sup> from xylem sap. A naturally occurring 8-bp insertion in the promoter increased the transcription level of *VcHKT1;1*, thus promoting leaf Na<sup>+</sup> exclusion and blueberry salt tolerance. Collectively, we provide evidence that *VcHKT1;1* promotes leaf Na<sup>+</sup> exclusion and propose natural variation in *VcHKT1;1* will be valuable for breeding Na<sup>+</sup>-tolerant blueberry cultivars in the future.

P 205

PGPR-induced *OsNAM2* gene provides salt tolerance in *Arabidopsis* by AFP2 and SUS protein interaction

H. Joshi<sup>1</sup>, K. Harter<sup>2</sup>, P. S. Chauhan<sup>1</sup>

<sup>1</sup>CSIR-NBRI, Microbial Technologies Division, Lucknow, India

<sup>2</sup>University of Tübingen, Tübingen, Germany

Research Question: How does the *OsNAM2* transcription factor contribute to salt stress response in plants, particularly *Arabidopsis*, and its regulatory pathways?

Methods: In silico analysis determined *OsNAM2* gene properties. Amplification and cloning in pCambia1303 were verified via PCR and sequencing. Agrobacterium-mediated transformation introduced *OsNAM2* into *A. thaliana*. Phenotyping assessed growth under NaCl. Membrane integrity, enzymatic activities, nutrient analysis, metabolite profiling, and chromatographic analysis quantified plant hormones. Expression profiling examined stress-responsive genes. Protein-protein interactions were studied using BiFC and FRET-FLIM.

Results: Overexpression of *OsNAM2* leads to improved germination percentage, seedling growth, root length, and biomass accumulation under high NaCl concentrations compared to WT plants. Moreover, *OsNAM2* overexpression enhances relative water content and reduces electrolyte leakage and malondialdehyde accumulation, indicating better membrane integrity and stress tolerance. Additionally, *OsNAM2* modulates the expression of key metabolic genes involved in glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle, facilitating metabolic adjustments crucial for plant adaptation to salt stress. The study also reveals the regulatory roles of *OsNAM2* in ABA signaling pathways, demonstrating enhanced transactivation activity on the promoters of ABA-responsive genes and significant protein-protein interactions with key regulatory proteins involved in ABA signaling and stress responses. Fluorescence lifetime imaging microscopy highlights strong interactions between *OsNAM2* and various putative interactors within the nucleus, emphasizing their significance in mediating transcriptional responses to salt stress. Overall, the findings provide comprehensive insights into the molecular mechanisms underlying salt stress responses in plants and underscore the potential of *OsNAM2* as a candidate gene for enhancing salt tolerance in crops.

Conclusions: *OsNAM2* plays a crucial role in enhancing salt stress tolerance in plants. Its multifaceted effects on growth, stress response, and metabolic adjustments underscore its potential for crop improvement. Understanding *OsNAM2*'s regulatory mechanisms is vital for developing stress-tolerant crop cultivars, crucial for agricultural sustainability amid environmental challenges.



P 207

Physiological and genetic mechanisms underlying silicon-mediated drought tolerance in barley

G. de Oliveira Ragazzo<sup>1</sup>, S. Beier<sup>1</sup>, A. Hartmann<sup>1</sup>, N. von Wirén<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Physiology and Cell Biology, Gatersleben, Germany

Silicon (Si) has been shown to enhance plant growth and alleviate various stresses, including drought. However, the underlying mechanisms by which Si improves drought tolerance are unclear. Here, we investigated the role of Si in enhancing drought tolerance in two barley genotypes, Golden Promise and Steptoe, which were grown in pots with or without Si fertilization for 21 days under optimal conditions, followed by a 14-day drought stress period. Although plants from both genotypes had similar Si accumulation in shoots, only Golden Promise plants exhibited increased biomass, number of tillers and relative water content in response to Si supplementation. In this genotype, the concentration of the drought-responsive hormone abscisic acid (ABA) in leaves remained significantly lower than in Steptoe, indicating that the effectiveness of Si in modulating ABA levels and drought stress signaling depends on the genotype. RNA-seq data revealed that Si supplementation increased the expression of genes involved in photosynthesis and water transport in Golden Promise, while ABA-responsive genes were down-regulated. Our results provide new insights into genotype-specific mechanisms of Si-mediated drought stress tolerance in barley, which can help to develop strategies to mitigate yield losses under water-scarce conditions.

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A New Player in Nitric Oxide Modulation and Hypoxia Response: Amidoxime Reducing Component (ARC)

V. Safavi-Rizi<sup>1</sup>, F. Lutter<sup>1</sup>, M. Herde<sup>1</sup>, K. Hölscher<sup>1</sup>, C. Stöhr<sup>1</sup>, C. P. Witte<sup>1</sup>

<sup>1</sup>Universität Leipzig, Leipzig, Germany

Global warming exacerbates flooding and hypoxia (low oxygen concentration) which reduces plant yield and endangers food security<sup>1</sup>. Therefore, understanding the physiological and molecular mechanism of plant hypoxia tolerance and the signaling molecules involved in this response is essential to overcome the future food crisis due to progressive global warming.

Nitric oxide (NO) is a multi-tasking signal molecule playing a key role in hypoxia tolerance<sup>2</sup>. In the model plant *Arabidopsis thaliana*, hypoxia sensing and signaling are conducted by the oxygen- and NO-dependent N-degron pathway<sup>2</sup>. However, the exact role of NO in hypoxia tolerance is still elusive. In plant cells, NO is synthesized via various enzymatic routes and in different organelles. Cytosolic nitrate reductase (NR), the key enzyme involved in nitrate reduction to nitrite, is shown to catalyze the conversion of nitrite to NO and is considered as one of the major NO producers in plant cells<sup>3</sup>.

A recent discovery in the green algae *Chlamydomonas reinhardtii* showed that a new enzyme called ARC (Amidoxime Reducing Component) collaborates with NR and produces NO<sup>4</sup>. It is suggested that NR transfer electrons from NAD(P)H over its homeodomain to the MoCo domain of ARC and these electrons are used for the reduction of nitrite to NO by ARC<sup>4</sup>. Whether ARC plays the same role in land plants is not yet clear. We utilized CRISPR-Cas technology to generate *arc* mutants in *Arabidopsis thaliana*, targeting both *ARC1* and *ARC2* genes, to probe ARC function in land plants. The resulting mutant lines demonstrated altered levels of NO and exhibited changes in hypoxia response, as evidenced by the differential expression of hypoxia marker genes under both control and hypoxic conditions. These data indicate that ARC might be a new player involved in the hypoxic response in land plants. Further investigations are underway to elucidate the functional mechanism of ARC in regulating the hypoxia response and flooding tolerance.

References:

1. Ezin *et al.*, 2010. *J Plant Physiol.* 2010;22(2):131–142.
2. Gibbs *et al.*, 2011. *Nature.* 479(7373):415–418.4.
3. Rockel *et al.*, 2002. *J. Exp. Bot.* 53(366): 103-110.
4. Chamizu-Ampudia *et al.*, 2016. *Plant Cell Environ.* 39(10):2097-107.

P 209

**Proteome alterations in bread wheat cultivars differing in their drought tolerance**O. Lakhneko<sup>1,2</sup>, O. Stasik<sup>3</sup>, L. Škultéty<sup>4</sup>, D. Kiriziy<sup>3</sup>, O. Sokolovska-Sergiienko<sup>3</sup>, M. Kovalenko<sup>5</sup>, M. Danchenko<sup>1</sup><sup>1</sup>Plant Science and Biodiversity Center, Slovak Academy of Sciences, Institute of Plant Genetics and Biotechnology, Nitra, Slovakia<sup>2</sup>Institute of Cell Biology and Genetic Engineering, National Academy of Sciences of Ukraine, Kyiv, Ukraine<sup>3</sup>Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine<sup>4</sup>Biomedical Research Centre, Slovak Academy of Sciences, Institute of Virology, Bratislava, Slovakia<sup>5</sup>Taras Shevchenko National University of Kyiv, Educational and Scientific Centre (ESC) "Institute of Biology and Medicine", Kyiv, Ukraine

Water shortage at the beginning of anthesis considerably impacts bread wheat production. We estimated the relation to the drought of two bread wheat cultivars by quantifying photosynthesis, water status, and oxidative stress-related parameters at the flowering stage of development after a transient drought. The sensitive cultivar (Darunok Podillia) showed ineffective water management and a more severe decline in photosynthesis. Apparently, the tolerant genotype (Odeska 267) used photorespiration to dissipate excessive light energy. The tolerant cultivar sooner activated superoxide dismutase and showed less inhibited photosynthesis. Such protective effect resulted in less affected yield and seed proteome profile. Proteomic analysis revealed a more stable composition of grain proteins with nutrient reservoir activities in the tolerant genotype accompanied by a lower magnitude of differential accumulation of allergenic proteins. Water deficit caused the accumulation of medically relevant proteins—mainly components of gluten in the sensitive cultivar and metabolic proteins in the tolerant cultivar. We suggest specific proteins as indicators of drought tolerance for guiding effective breeding for more sustainable bread wheat production: thaumatin-like protein, 1-Cys peroxiredoxin, 14-3-3 protein, peroxidase, FBD domain protein, and AP2/ERF plus B3 domain protein (Lakhneko et al., 2023). Our follow-up study is focused on the evaluation of the contrasting proteomic response in flag leaves, including redox post-translational modifications, under drought during anthesis with the subsequent recovery.

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## References

Lakhneko O, Stasik O, Škultéty L, Kiriziy D, Sokolovska-Sergiienko O, Kovalenko M., Danchenko M. 2023. Transient drought during flowering modifies the grain proteome of bread winter wheat. *Frontiers in Plant Science* 14, 1181834. doi: 10.3389/fpls.2023.1181834

P 210

**Two liverworts from same habitats developed many similar but few distinct seasonal adaptive strategies:****Insights from a transcriptomic approach**S. Basu<sup>1</sup>, S. Yadav<sup>1</sup>, V. Jha<sup>1</sup>, Y. Mishra<sup>1</sup><sup>1</sup>Science, Botany, Varanasi, India

Liverworts were among the first land plants to experience seasonal changes after terrestrialization. Since liverworts experience four distinct seasons in India (pre-monsoon, monsoon, post-monsoon, and fruiting season), examining the changes in their gene expression during these growing seasons can provide a molecular overview of seasonal acclimations. Considering this, we performed a seasonal transcriptome analysis of *Dumortiera hirsuta* and *Plagiochasma appendiculatum*, two representative liverwort species from India that coexist in the same habitat but diverged at different times during evolution. Phylogenetic trees and evolutionary timescale analyses showed that *D. hirsuta* is primitive compared to *P. appendiculatum*. Of the four seasons, the fruiting season and the post-monsoon season are the most challenging for both liverworts due to reduced temperature, precipitation, nutrients availability, and day length. The RNA-seq analysis of both liverworts during each of their four growing seasons showed that *D. hirsuta* primarily modifies its transcriptome in the post-monsoon season but mainly induces specific genes in the fruiting season, most likely to develop reproductive organs and to adapt strategically by conserving energy in the fruiting season to deal with the harsh environmental conditions of both seasons. Conversely, *P. appendiculatum* exhibited significant transcriptome variability during both the fruiting and post-monsoon seasons, albeit to a lesser degree than *D. hirsuta*. This suggests that, in order to survive the harsh conditions of both seasons, it strategically modulated its necessary gene expression levels over an extended period of time while taking energy conservation into consideration. Like *D. hirsuta*, it also induces most unique genes during the fruiting season. To the best of our knowledge, this is the first work to examine the seasonal transcriptome of liverworts, and offers insights into how two liverworts that share a habitat but diverged at different times endure seasonal fluctuations.

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**Sugar and salt: How seagrass cell walls adapted to the marine habitat**

L. Pfeifer<sup>1</sup>, B. Classen<sup>1</sup>

<sup>1</sup>University of Kiel, Pharm. Biology, Kiel, Germany

Seagrasses evolved from monocotyledonous land plants that returned to the marine habitat. This transition was accomplished by substantial changes in cell wall composition. We investigated polysaccharide composition of nine seagrass species from the Baltic, Mediterranean, Red Sea and Indian Ocean<sup>1</sup>. Sequential extraction revealed a seagrass cell wall composition comparable to terrestrial angiosperms with pectins and different hemicelluloses, especially xylans and/or xyloglucans. However, the pectic fractions were characterized by high amounts of apiose, suggesting unusual apiogalacturonans are a common feature of seagrass cell walls.

Whether arabinogalactan-proteins (AGPs), important signalling molecules of land plants, are present in seagrass cell walls is of evolutionary interest. AGPs of *Zostera* were structurally characterised by analytical and bioinformatic methods as well as by ELISA with different anti-AGP antibodies<sup>2</sup>. Although the common backbone structure of land plant AGPs is conserved in *Z. marina*, the glycan structures exhibit unique features, including a high degree of branching and an unusually high content of terminating 4-OMe GlcA residues. Calcium-binding of *Zostera* AGPs was studied by ITC and microscopy. The high Calcium-binding capacity due to the polyanionic surface is possibly involved in adaptation to the marine environment.

References

<sup>1</sup>Pfeifer L. et al. 2022. Profiling the cell walls of seagrasses from A (*Amphibolis*) to Z (*Zostera*). *BMC Plant Biology* 1322: 63.

<sup>2</sup>Pfeifer L. et al. 2020. Arabinogalactan-proteins of *Zostera marina* L. contain unique glycan structures and provide insight into adaption processes to saline environments.

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P 212

**Distinct guard cell shaped strategies for coping with repeated drought stress**

P. P. Lehr<sup>1</sup>, A. Erban<sup>2</sup>, R. Hartwig<sup>1</sup>, M. Wimmer<sup>1</sup>, J. Kopka<sup>2</sup>, C. Zöhr<sup>1</sup>

<sup>1</sup>University of Hohenheim, Quality of Plant Products, Stuttgart, Germany

<sup>2</sup>Max-Planck-Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

The adaptation of crops to recurrent drought stress is of paramount importance for maintaining agricultural productivity and achieving food security in the context of a changing climate. The metabolic dynamics of guard cells under drought stress remain poorly understood, particularly in grapevine, a prominent crop grown in arid regions, and maize, an essential staple crop with substantial water requirements. In this study, the metabolic differences in guard cells during drought and repeated drought stress between grapevine and maize were investigated by performing physiological and metabolomic analyses. The results demonstrated that under drought conditions, the metabolite profiles of primed plants, which had experienced their second drought event, were less altered compared to non-primed plants. This suggests that priming could be a viable approach for enhancing crop productivity. Metabolites of the arginine and proline pathway, as well as the glycine, serine, and threonine pathway, were less impacted by drought stress in maize guard cells compared to mesophyll cells. This suggests that plants prioritize maintaining stable guard cell metabolomes for functional integrity. Metabolomic analysis revealed differential responses in amino acids and sugar patterns. Grapevine guard cells displayed greater stability in amino acid signatures, while maize showed marked increases in sugar levels. These findings suggest the existence of two distinct adaptive strategies, namely a vigorous acclimation of guard cells, as observed in maize, and a muted acclimation of guard cells, as shown in grapevine. Consequently, we provide a mechanistic explanation of guard cell physiology under drought, with the aim of enhancing drought resilience in agricultural systems.

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Synergistic effects of root-associated arbuscular mycorrhizal fungi and green compost on the growth and salt stress tolerance of tomato plants

S. Zaidi<sup>1</sup>, M. R. Hajirezaei<sup>1</sup>, N. von Wirén<sup>1</sup>

<sup>1</sup>The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Physiology and cell biology, Gatersleben, Germany

The growth of tomato (*Solanum lycopersicum*) is limited by soil salinity. While the use of arbuscular mycorrhizal fungi (AMF) can improve the tolerance of tomato to salinity, the combination of AMF with other biostimulants, such as green compost, and the underlying mechanisms are not yet fully understood. In this study we investigated the effect of combined application of AMF and compost on plant growth and salt tolerance. To this end, an experiment with four treatments was designed: control, AMF, compost, and the combination of AMF and compost at three salt levels (0, 150, and 300 mM). Our results indicate that the combined application decreased Na uptake and led to improved shoot accumulation of P, S, and B. This changes in ionic composition were accompanied by increased shoot length and biomass across all salinity levels. Moreover, both AMF and compost applications affected positively the concentration of chlorophyll, and the accumulation of osmolytes such as sucrose, glucose, and starch, as well as various amino acids including proline, GABA, and alanine. Altogether, this study demonstrates that AMF and compost can mitigate salinity stress in tomato, a response that can be further boosted when both biostimulants are combined.

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Dynamic growth QTL action in diverse light environments:  
characterisation of light regime-specific and stable QTL in *Arabidopsis*

R. Meyer<sup>1</sup>, K. Weigelt-Fischer<sup>1</sup>, H. Tschiersch<sup>1</sup>, G. Topali<sup>1</sup>, L. Altschmied<sup>1</sup>, M. Heuermann<sup>1</sup>, D. Knoch<sup>1</sup>  
M. Kuhlmann<sup>1</sup>, Y. Zhao<sup>2</sup>, T. Altmann<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research, Molecular Genetics, Gatersleben, Germany

<sup>2</sup>Leibniz Institute of Plant Genetics and Crop Plant Research, Breeding Research, Gatersleben, Germany

Plant growth is a complex process shaped by many genetic and environmental factors and their complex interactions. In our study, we aimed to identify (epi)genetic factors that influence plant performance under different environmental conditions. Using non-invasive high-throughput phenotyping, we performed daily automated imaging of 382 *Arabidopsis thaliana* accessions, providing growth data with high temporal resolution throughout the developmental progression under constant or fluctuating light intensities.

Genome-wide association studies identified quantitative trait loci (QTL) for projected leaf area, relative growth rate and photosystem II operating efficiency ( $\Phi$ PSII), which showed predominantly condition-specific patterns and different temporal activity profiles. These QTL exhibited dynamic activity phases lasting between two and nine days, emphasising the temporal variation of their effects. Within the detected QTL regions, we identified eighteen protein-coding genes and one miRNA gene as potential candidates, which were consistently found under both light regimes. The time-resolved phenotypic measurements allowed us to fit a logistic growth model to the projected leaf area data and to evaluate the derived parameters “inflection point”, “final value”, “initial value” and “b” (position on the time axis), which increases the possibility to detect relevant genetic variations.

Further investigations included analysing the expression patterns of putative candidate genes affecting projected leaf area through time-series experiments in accessions exhibiting different vegetative growth rates. In addition, accessions with extreme  $\Phi$ PSII levels were subjected to kinetic fluorescence measurements three times a day under constant or fluctuating light throughout the vegetative growth period.

Our results underline the importance of considering both environmental and temporal dynamics in QTL/allele actions and argue for detailed time-resolved analyses under well-defined environmental conditions. Such approaches are essential to decipher the intricate and stage-specific contributions of genes that influence plant growth processes at different developmental stages.

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**Systemic effects of combined arsenic and hypoxia exposure in *Arabidopsis thaliana* L. roots**

L. Vogelsang<sup>1</sup>, V. Kumar<sup>1</sup>, M. Persicke<sup>2</sup>, I. Finkemeier<sup>3</sup>, K. J. Dietz<sup>1</sup>

<sup>1</sup>University Bielefeld, Biochemistry and Physiology of Plants, Bielefeld, Germany

<sup>2</sup>University Bielefeld, Microbial Genomic and Biotechnology, CeBiTec, Bielefeld, Germany

<sup>3</sup>University of Münster, Plant Physiology, Münster, Germany

Plant development in nature often is influenced by change of multiple and not isolated abiotic factors. This crosstalk of environmental factors determines accumulation, tolerance or relative toxicity of heavy metal(oids) like arsenic (As) in crops or tolerant plants. In the context of natural combination of abiotic factors, co-occurrence of As-contamination and flooding induced root hypoxia is common. In previous studies we have established the unique responses of hydroponically grown *Arabidopsis* plants to combined arsenic and hypoxia root exposure. In comparison to the individual stresses, the combination i.e., HpxAs-effects comprised seized root growth, strong accumulation of sugars in root and leaves as well as strong accumulation of anthocyanins implicating perturbed C/N ratio. Further, a very characteristic oxidation of root cell cytosol leading to inhibited recovery on reaeration becomes apparent. Root transcriptomic analysis and root growth phenotype indicate a unique hypoxia as well as As-induced phosphate starvation response that leads to increased root hair growth as an energy efficient mechanism for increasing root surface area. Although low oxygen reduced As-accumulation in these plants, the toxicity increased. Important stress hormone ABA and JA-precursor cis-OPDA (12-oxo-phytodienoic acid) accumulation in leaves indicated their potential role in systemic signaling.

The present study focuses on the systemic effects of HpxAs. Rapid impact on Fd redox state within 4h of combined stress exposure indicated efficient long-distance propagation of stress signals. A reasonably higher dominance of hypoxia-induced changes in CO<sub>2</sub>-assimilation rates, stomatal conductance, and transpiration rates were recorded for combined stressed plants, with an appreciable recovery of assimilation on reaeration. Further, combined stress effects showed comparatively stronger impact on PSI function than PSII. Shoot proteomic investigation highlighted inhibition of primary chloroplastic processes like assembly of electron transport chain complexes, branched chain amino acid synthesis, xanthophyll cycle etc. along with perturbation in starch-sucrose homeostasis and protein synthesis. Interestingly, changes in the relative nuclear and chloroplastic contribution to plastid proteins along with stress specific effects on metabolic steps like MEP (methylerythritol 4-phosphate)-pathway in plastids appear to have adaptive value for stress acclimation and recovery.

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**Targeted expression of a maize NADP-Malic enzyme as a metabolic engineering tool for enhanced drought tolerance in tobacco**

P. Oitaven<sup>1</sup>, M. F. Guindón<sup>1</sup>, Y. Fu<sup>2</sup>, K. Verma<sup>2</sup>, V. G. Maurino-Larcher<sup>2</sup>, M. F. Drincovich<sup>1</sup>

<sup>1</sup>Centro de Estudios Fotosintéticos y Bioquímicos (CEFOTI-CONICET), Universidad Nacional de Rosario, Rosario, Argentina

<sup>2</sup>Institut für Zelluläre & Molekulare Botanik (IZMB), Molecular Plant Physiology, Bonn, Germany

Sustainable agriculture is crucial in the face of climate change and global population growth. To address this challenge and consider the role of malate in stomatal dynamics and sugar export, we employed targeted metabolic engineering in tobacco (*Nicotiana tabacum*). Our aim was to modify malate levels in a localised manner by expressing the maize plastidic non-photosynthetic NADP-Malic enzyme (NADP-ME) specifically in phloem companion cells and guard cells using the potassium channel 1 promoter. This manipulation significantly improved water use efficiency (WUE), biomass production, and CO<sub>2</sub> uptake, and shortened the plant life cycle. The enhanced WUE in transgenic plants (TP) was due to a 68–77% reduction in stomatal aperture, resulting in 71–89% less water usage while producing almost the same biomass as wild-type (WT) plants under well-watered conditions (95% field capacity of soil moisture). After 30 days of drought stress followed by 7-day rewatering, the TP recovered faster and flowered before the WT. Remarkably, the TP can survive up to 45 days of drought without adversely affecting seed production, whereas WT plants perished. Stomatal conductance (SC) was 2-fold lower in TP at 95% FC. Our study found that the SC reduction in the TP was independent of abscisic acid (ABA), indicating a “biochemical override” of ABA-induced stomatal closure. Although the TP remained responsive to ABA under drought stress, the increase in ABA was much less—12–16-fold compared to a 30-fold increase in WT. The enhanced NADP-ME activity in guard cells and surrounding vascular cells may have altered ion fluxes, particularly involving malate, modulating stomatal dynamics and ion transport, leading to the observed phenotype. Our results highlight the potential of targeted metabolic engineering to offer novel strategies for improving crop yield and stress tolerance to cope with environmental challenges.

References

Müller G. L., Lara M. V., Oitaven P., Andreo C. S., Maurino V. G., & Drincovich M. F. (2018). Improved water use efficiency and shorter life cycle of *Nicotiana tabacum* due to modification of guard and vascular companion cells. *Scientific Reports*, 8(1), Article 1. <https://doi.org/10.1038/s41598-018-22431-5>.

Oitaven, M.F. Guindón, G.L. Müller, E. Margarit, C. Saper, M.S. Srebot, Y. Fu, K. Verma, V. Wewer, S. Metzger, G.M. Estavillo, V.G. Maurino, M.F. Drincovich. (2024). Improved drought resistance coupled with high yield in tobacco with modified malate metabolism in guard and vascular companion cells. Submitted.

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**Taurine: the key plant regulator in cadmium hormesis**R. Ravi<sup>1</sup>, M. Yadav<sup>1</sup>, S. R. Kanade<sup>1</sup><sup>1</sup>University of Hyderabad, Plant Sciences, School of Life Sciences, Gopanpalle, India

Hormesis refers to the adaptive mechanism of organisms in response to environmental challenges. It is a dose-response phenomenon where a lower dose induces an improvement in functionality and overall development and a higher dose endangers even the existence of the organism. The scientific community in the past decades has initiated efforts to widen the concept of hormesis, interpret the basic activity, and exploit their practical applications for better agricultural practices and enhanced productivity. In our study, we examined the morphological and biochemical implications of Cadmium hormesis in *Solanum lycopersicum* and tried to decode the underlying molecular mechanisms. Our results showed all growth parameters and biochemical responses exhibiting a “U” or “inverted U” shaped graph confirming the hormetic behavior upon cadmium exposure. Plants treated with 1 μM Cd presented more promising outcomes in terms of growth and development and 50 μM led to growth retardation. To get a comprehensive understanding, comparative transcriptomic profiling of 20-day-old tomato plants treated with 1 μM and 50 μM Cd for 5 days was performed against the control. The GO analysis of Differentially Expressed Genes (DEGs) suggested that genes for oxidoreductase activity, signaling, and cell cycle were differentially expressed in 1 μM condition. Among the DEGs, GAD (Glutamate decarboxylase), uniquely expressed in 1 μM aroused interest due to its similarity to the GADL1, producing taurine in animals. Taurine, a rare amino acid found in plants improves growth, nutrient uptake, and photosynthetic pigments by regulating ROS production, secondary metabolism, and ion homeostasis. The physiological implications of exogenous taurine on plants were highly consistent with the physiological changes in 1 μM Cd plants. The LC-MS analysis quantitatively confirmed taurine in 1 μM Cd-treated plants. However, the taurine biosynthetic pathway is yet to be discovered and investigated in plants. The gene expression analysis of GAD and PCO (Plant Cysteine Oxidase) suggested that the putative taurine synthetic genes in plants are upregulated in 1 μM condition. In light of all these findings, a taurine biosynthetic pathway is proposed and is regarded as one of the factors behind cadmium hormesis. An in-depth study of taurine metabolism will give a detailed idea of hormesis and help in utilizing this for generating crops with higher productivity.

Keywords: hormesis; cadmium; taurine; GAD; PCO

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**Sediment archives as time-series of millennial phytoplankton adaption**S. Bolius<sup>1</sup>, A. Schmidt<sup>2</sup>, J. Kaiser<sup>1</sup>, O. Dellwig<sup>1</sup>, H. Arz<sup>1</sup>, L. Epp<sup>2</sup>, A. Kremp<sup>1</sup><sup>1</sup>Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Biology, Rostock, Germany<sup>2</sup>University of Konstanz, Biology, Konstanz, Germany

Climate change and the intensification of human impact since the early 1950s have led to a profound decrease in marine biodiversity and changes in species composition of phytoplankton communities in the Baltic Sea since the last century. The Baltic Sea also experienced fundamental environmental changes throughout its Holocene history (last ~ 10000 years), which were triggered, e.g., by glacial rebound, alternating warmer and cooler periods, and varying salinity and nutrient availability.

Some phytoplankton species are capable of forming resistant resting stages, which allow long-term dormancy. Following the annual blooms, the resting stages settle on sea floor, where they accumulate in distinct sediment layers. These resting stages can serve as natural archives of phytoplankton communities and populations representing different time points in history. Thus, they offer a unique opportunity to trace changes in population structure and functional traits through time under varying environmental conditions. From a composite core comprising modern and Holocene sediments from the central Baltic Sea, we could re-germinate up to 7000-year-old temporal cohorts of the spring-bloom diatom *Skeletonema marinoi*.

Growth experiments under differing temperature and salinity were conducted in the laboratory in order to study the adaptation potential of these temporal cohorts from different ambient environments to changing temperature and salinity, to see if and how these strains from different stages of the Baltic Sea's history react to these abiotic conditions. The results suggest that the re-germinated *S. marinoi* strains exhibit differences in their growth temperature optima and trait changes in relation to the possible ambient environment. These differences suggest that 1) phenotypic adaptation and 2) trait changes have occurred over the past ~7000 years.

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The dynamic function of the cytosolic redox regulatory network and the sensory role of the type II peroxiredoxins B/C/D

Kishan Gurjar<sup>1</sup>, Lara Vogelsang<sup>2</sup>, Jürgen Eirich<sup>1</sup>, Karl-Josef Dietz<sup>2</sup>, Iris Finkemeier<sup>1</sup>

<sup>1</sup>Institute of Plant Biology and Biotechnology (IBBP), University of Münster, Schlossplatz 7, Münster, D-48149, Germany.

<sup>2</sup>Biochemistry and Physiology of Plants, Faculty of Biology, Bielefeld University, Bielefeld, Germany.

Peroxiredoxin II B (PRXII B) is a thiol peroxidase and a member of the type II peroxiredoxin family. It has a single peroxidatic cysteine residue, which is essential for its antioxidant enzyme activity. In the presence of Reactive Oxygen Species (ROS), the peroxidatic cysteine is oxidised and forms an intramolecular disulphide bond with a second resolving cysteine residue to protect cellular biomolecules from damage and to maintain cellular redox homeostasis. Cytosolic PRXII B has a wide substrate preferences, including hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), tert-butyl hydroperoxide (t-BOOH) and cumene peroxide (CuOOH) (Vogelsang et al., 2023). Recent studies have shown that PRXII B expression in *Brachypodium distachyon* varies by tissue under abiotic stress, having higher expression specifically in roots during salt and dehydration stress as well as higher expression levels in leaves in response to H<sub>2</sub>O<sub>2</sub> (Farjallah et al., 2024). PRXII B also plays a pivotal role in biotic stress by forming an intermolecular complex with the phosphatase ABA-insensitive 2 (ABI2) and causing its oxidation in an H<sub>2</sub>O<sub>2</sub> dependent manner. This mechanism inhibits its phosphatase activity, leading to stomata closure (Guozhi et al., 2022). Additionally, PRXII B was found to interact with the cytosolic NHR2A protein, that provides plant immunity from potential pathogens (Singh et al., 2020). Considering the significant role of PRXII B in stress conditions, diverse substrate range and interaction with ABA signalling components, our aim is to investigate the functional role of PRXII B in redox sensing and identify the potential interacting partner to understand the cytosolic redox network. For this we employ pull down assays and proximity labelling using the biotin ligase Turbo-ID fused with the open reading frame of PRXII B and stably integrated into *Arabidopsis*. Here, we will present our experimental approach and initial findings.

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Evaluating Transpiration and Chlorophyll Dynamics alongside Stomatal Density and Size in Drought-Impacted Winter Wheat Genotypes

E. Ganji<sup>1</sup>, E. Villar Alegria<sup>2</sup>, M. M. Mabrouk Ahmed<sup>2</sup>, T. W. Chen<sup>2</sup>, A. Matros<sup>1</sup>, A. Stahl<sup>1</sup>

<sup>1</sup>Julius Kühn Institute (JKI)- Institute for Resistance Research and Stress Tolerance, Research Group Crop-Environment Interactions, Quedlinburg, Germany

<sup>2</sup>Humboldt Universität zu Berlin, Albrecht Daniel Thaer Institut of Agricultural and Horticultural Science, Groupe of Intensive Plant Food Systems, Berlin, Germany

Global warming and changes in precipitation patterns threaten crops like winter wheat. Therefore, breeding varieties with efficient water utilization is a top priority in agricultural research. Most water loss in plants occurs through the stomata during transpiration, which can be regulated by adjusting the apertures of stomatal pores. Additionally, plants may modulate stomatal development, including the size and density, which in turn affects plant water use efficiency, photosynthesis rate, and yield.

The objective of this study was to gain insights into 1) The transpiration response to soil water deficit and its relationship with vapor pressure deficit (VPD) among 30 selected winter wheat genotypes at early growth stages, 2) The variation and genetic basis of stomatal density, size, and their association with transpiration, and 3) The dynamics of chlorophyll in response to drought stress. The setup was a pot experiment with an alpha-lattice design consisting of two irrigation scenarios, representing 70% (well-watered) and 35% (stress) soil water capacity, with two replicates, starting with 50 plants per pot. The pots were kept at 4°C for vernalization for 8 weeks and then moved to an advanced gravimetric phenotyping platform (Plantarray®). Before implementing the 35% soil water capacity treatment, the number of plants per pot was reduced to 10. In addition to the automatic measurements of transpiration rate and VPD by Plantarray®, a portable high-throughput microscope with a 400x magnification lens (ProScope HR5) was used for stomata imaging on both sides of the 3rd leaf of a marked plant in each pot before water stress, and the 6th leaf after water stress. SPAD measurements were conducted twice per week to track chlorophyll. The experiment ended at the stem elongation stage, and the above-ground biomass was then measured. The results of this research provides more information to understand the complex relationships involved in drought stress tolerance. Imaging can show if different varieties can adapt their stomatal morphology and distribution depending on water availability and from the calibrated model for chlorophyll dynamics, it can be understood if different varieties have different strategies for acclimating their photosynthetic capacity to their environment.

Keywords: evapotranspiration, abiotic stress, stomatal regulation, water loss dynamics, phenotyping, winter wheat

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The impact of drought stress on growth and cucurbitacins accumulation in different lines of *C. pepo* subsp. *pepo*

A. Wiese-Klinkenberg<sup>1</sup>, F. Genzel<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich GmbH, IBG-4, Jülich, Germany

The increasing occurrence of extreme weather phenomena like droughts impairs the horticultural production and can cause severe reduction of yield and quality of fruits of zucchini (*Cucurbita pepo* subsp. *pepo*), an economically important vegetable crop. The availability of stress tolerant cultivars is a crucial aspect for the minimization of losses. Non-invasive phenotyping techniques were used to investigate the drought stress response of 20 selected cultivars/lines of *C. pepo* in young plants, aiming at an early identification of varieties with improved drought tolerance. Although drought stress conditions caused a significant growth reduction in all studied cultivars/lines, results indicate differences in tolerance. Also, abiotic stressors like drought can induce an accumulation of secondary metabolites in plants (1). These metabolites include high-value health-promoting compounds, but also undesired toxic and bitter compounds like the cucurbitacins in the family of the *Cucurbitaceae*. Recently, a connection between extreme weather conditions and increased amounts of cucurbitacins in zucchini has been suggested (2). Due to the high toxicity of cucurbitacins (3), the project QCuK aims at investigating the impact of drought stress on cucurbitacin biosynthesis in the zucchini cultivars/lines. First results show drought stress resulted in an increased expression of genes involved in the biosynthesis of cucurbitacins. For instance, expression of a gene coding a cucurbitadienol 11-hydroxylase was significantly increased after drought treatment in cotyledons of different lines of zucchini. In following experiments, it will be investigated whether the drought-induced biosynthesis of cucurbitacins does also occur in leaves of older plants. Furthermore, cucurbitacin levels will be investigated in fruits by the project partners. Thus, the project aims at contributing to a more secure horticultural production of zucchini.

References

1 Akula, R.; Ravishankar, G. A. Plant Signaling Behav. 2011, 6,1720–1731

2 Pfab, R.; Öfele, H.; Pietsch, J.; Kapp, T.; Zellner, T.; Eyer, F. Revenge of the Zucchini – Is This the Result of Climate Change?; Clinical Toxicology 2016 54/4 p.501-502.

3 Rehm, S. Poisonous Principles in Vegetables – the Case of the Cucurbitacins. J. Agric. Trop. Subtrop. Beitr. Zur Trop. Landwirtschaft. Veterinärmedizin 1985, 86 (2)

P 222

Linking the drought response to the regulation of inducible crassulacean acid metabolism

K. Schiller<sup>1,2</sup>, M. Bosse<sup>1</sup>, P. Viehöver<sup>2,3</sup>, N. Perron<sup>4</sup>, J. Hartwell<sup>5</sup>, A. Bräutigam<sup>1,2</sup>

<sup>1</sup>Bielefeld University, Computational Biology, Bielefeld, Germany

<sup>2</sup>CeBiTec, Bielefeld, Germany

<sup>3</sup>Bielefeld University, Genetics and Genomics of Plants, Bielefeld, Germany

<sup>4</sup>University of Florida, Plant Molecular and Cellular Biology, Gainesville, FL, United States

<sup>5</sup>University of Liverpool, Institute of Systems, Molecular and Integrative Biology, Liverpool, United Kingdom

Plants evolved crassulacean acid metabolism (CAM) in environments where water availability is limited and/or extremely seasonal. By temporal separation of CO<sub>2</sub> uptake and fixation via Rubisco, plants improve their water use efficiency. This add-on metabolism can be found in about 6% of flowering plants. Some plants are able to switch back and forth between C<sub>3</sub> and CAM photosynthesis mode depending on environmental cues. This facultative, weak CAM is often induced by drought or water deficit stress. We therefore hypothesize that transcription factors (TFs) that bind promoters of drought responsive genes can also bind to promoters of CAM genes providing a linkage of the two networks.

Based on drought stress responses in C<sub>3</sub> and CAM species, we chose orthologous TFs from the C<sub>3</sub> plant *Arabidopsis thaliana* and the facultative CAM plant *Kalanchoë gracilipes* for analysis. To determine their binding sites we apply DNA Affinity Purification sequencing (DAPseq) on the genomes of C<sub>3</sub>, obligate CAM, and facultative CAM species.

We show that binding motifs of orthologous transcription factors between *Arabidopsis* and *K. gracilipes* are conserved and are highly similar on all tested genomes. By defining a core drought module and a CAM module we are able to analyze whether binding sites are also conserved between the two orthologous transcription factors and between the different genomes. Testing whether binding of the TFs differs on the presented genomes, especially depending on the photosynthetic mode of the genome donor, will allow conclusions about adaptations of promoter regions for specialized transcriptional regulation linking the induction of CAM and drought stress response. Similarly, testing whether binding behavior differs between the two orthologous transcription factors from *Arabidopsis* and *Kalanchoë* allows conclusions about adaptations of the transcription factor itself between the C<sub>3</sub> and CAM species which might be linking the drought stress response to the induction of CAM



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Transcriptional reprogramming during developmental and stress-induced leaf senescence in barley

W. Zschiesche<sup>1,2</sup>, C. Mohr<sup>1</sup>, N. Fataftha<sup>1</sup>, K. Humbeck<sup>1,2</sup>

<sup>1</sup>Biology/MLU, Plant Physiology, Halle a. d. Saale, Germany

<sup>2</sup>Agrochemical Institute Piesteritz, Wittenberg, Germany

Leaf function dynamically alters during development and in response to environmental cues, from being a source of assimilates in photosynthetically active leaves to a source of valuable resources in senescing leaves. In this work we analyze reprogramming of gene expression at early and late stages of developmental and stress-induced senescence, i.e., drought stress-induced senescence and N-deficiency-induced senescence. The data show that there is a hierarchical structure of the pathways leading to senescence. At early stages, overlap between the different conditions is low and most genes regulated are specific for the different stress-conditions, e.g., early down-regulation of photosynthesis related genes at onset of developmental senescence, induction of genes encoding osmotic regulators after decrease in soil water content and regulation of genes involved in N-metabolism when availability of N is decreasing. Nevertheless, there are few genes which are already at this early stage commonly regulated. At later stages, specific pathways flow into a common senescence pathway which involves typical senescence-associated genes.

Knowledge about the structure and function of the complex, dynamic, environment-sensitive and highly flexible regulatory senescence-networks helps to identify key factors of plant performance under changing environment.

P 224

Salt-priming induces salt tolerance in young tomato plants

S. Beier<sup>1</sup>, F. Genzel<sup>1</sup>, A. Wiese-Klinkenberg<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, Institut für Bio- und Geowissenschaften IBG-4: Bioinformatik, Jülich, Germany

Crops are increasingly exposed to a broad range of stresses that require strategies for improving tolerance. Besides breeding techniques, priming is a mechanism that induces plant tolerance against abiotic and biotic stresses without the need for chemical intervention. The project HortiPrimed aims to establish abiotic stress priming on young tomato plants as a crop protection method in tomato production.

Young tomato plants were subjected to salt stress to induce priming and after a following 5 days or 10 days lasting recovery phase plants were exposed to a repeated salt stress treatment.

Plants' defense responses in each phase of experiment were analyzed by image-based phenotyping using a fotobox combined with image segmentation method, quantification of secondary metabolites and gene expression analysis.

Young tomato plants had reduced size and relative growth rate due to the priming stress-treatments, but salt-primed plants established a significantly higher relative growth rate under second following salt stress treatments. Quantification of secondary metabolites revealed that salt-priming induced an improved antioxidant defense response against subsequent stress indicated by a higher content of total phenolics and anthocyanins in leaves. Accumulation of these metabolites in salt-stressed plants was accompanied by higher expression of dihydroflavonol 4-reductase (DFR) gene in leaves. A genome wide gene expression analysis by RNA-sequencing revealed significant differences in transcription of genes in primed compared to unprimed plants during salt stress treatment. In response to salt, primed plants showed overrepresentation of early upregulated genes in functional groups (BINs) for example related to phytohormone, chromatin organization, cell division, and in plant secondary metabolism related to phenolics/flavonoid biosynthesis.

The applied salt-priming improved growth and enhanced metabolic stress responses by inducing corresponding genes under salt stress. By further understanding the underlying mechanisms and the durability of the priming memory, priming might serve as a plant protection method in tomato production in future.

P 225

Arabidopsis Shrs in the Accommodation of the Downey Mildew Pathogen *Hyaloperonospora Arabidopsidis*

A. Makris<sup>1</sup>, L. Caggegi<sup>1</sup>, M. Schmidt<sup>1</sup>, J. Ziegler<sup>1</sup>, M. K. Ried-Lasi<sup>1</sup>

<sup>1</sup>IPB Halle, Halle a. d. Saale, Germany

Plants are constantly exposed to numerous microorganisms and engage in beneficial as well as detrimental interactions with many of them. The leucine-like domain leucine-rich repeat receptor-like kinase (MLD-LRR-RLK) SymRK plays a crucial role in establishing arbuscular mycorrhiza (AM) and nitrogen-fixing root nodule symbiosis. We have previously identified two SymRK homologs, SHRK1 and SHRK2, in *Arabidopsis thaliana*, which significantly affect the reproductive success of the downy mildew oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*). Additionally, we discovered a third homolog, SHRK3, which, along with SHRK1 and SHRK2, which also influences *Hpa* spore and sporangio-phore production. Here, we show that ER-localized cell death suppressor Bax Inhibitor-1 (BI-1) interacts SymRK/SHRKs in yeast. We finally investigated the metabolic response to *Hpa* infection, hypothesizing that *Arabidopsis* produces specialized metabolites upon pathogen detection. Through a preliminary untargeted metabolomics approach across various time points, we discerned a distinct metabolic profile between *Hpa*-treated and mock-treated seedlings, identifying several metabolites potentially associated with the response to *Hpa*.

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Abiotic conditions play a key role in the maturation of *Chlamydomonas reinhardtii* zygotes into dormant zygosporesM. Cardador<sup>1</sup>, S. Krüger<sup>2</sup>, T. Jakob<sup>1</sup>, R. Goss<sup>1</sup>, S. Sasso<sup>1</sup><sup>1</sup>Leipzig University, Plant Physiology, Leipzig, Germany<sup>2</sup>Martin Luther University Halle-Wittenberg, Biozentrum, Halle a. d. Saale, Germany

*Chlamydomonas reinhardtii* is a terrestrial green microalga capable of reproducing asexually and sexually. The fusion of sexually compatible, haploid gametes leads to the formation of a diploid zygote. While under suitable conditions the zygote will germinate to release progeny cells, it will mature into a dormant, highly resistant zygospore in the absence of light or an adequate nitrogen source. During this transition the cell synthesizes a new multi-layered cell wall, accumulates storage lipids, and degrades most of its chlorophyll. Additionally, new ketocarotenoids are also produced, which are exclusive to this stage of the alga's life cycle.

Surprisingly, the influence of abiotic conditions on such a complex process has not yet received much attention. To address this gap of knowledge, we analyzed how zygotic maturation is affected by differences in organic carbon availability, temperature and light intensity. In our present experiments, organic carbon was found to be a crucial prerequisite for zygospore maturation. The absence of acetate from the maturation medium prevented the full development of the zygotic cell wall, which is essential for the resistance to abiotic stressors. Furthermore, the zygotes were found to remain metabolically active, both with regard to respiration and photosynthesis. Withholding organic carbon prevented pigment remodeling and ketocarotenoid biosynthesis in zygotes.

Temperature and light intensity also impacted the pigment profile of maturing *C. reinhardtii* zygotes. Zygotes matured at 28 °C were found to more rapidly accumulate ketocarotenoids and degrade chlorophyll when compared with zygotes matured at 20 °C or 10 °C. On the other hand, zygotes kept at 5 °C did not produce any ketocarotenoids at all. Exposure to light promoted ketocarotenoid synthesis and chlorophyll degradation. Of the tested conditions, a light intensity of 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  resulted in the greatest accumulation of ketocarotenoids, while the higher light intensity of 200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  led to the most complete degradation of chlorophyll. In conclusion, our results demonstrate that the maturation of *C. reinhardtii* zygotes into resistant zygospores depends on several environmental parameters such as organic carbon availability, light and temperature.

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Characterization of CPK*Ti* in signal propagation after woundingS. Lederer<sup>1</sup>, J. Bortlik<sup>2</sup>, V. Handrick<sup>1</sup>, P. Weckwerth<sup>1</sup>, S. Matschi<sup>1</sup>, T. Romeis<sup>1,2,3</sup><sup>1</sup>Leibniz-Institute for Plant Biochemistry (IPB), Biochemistry of Plant Interactions (BPI), Halle a. d. Saale, Germany<sup>2</sup>Freie Universität Berlin, Dahlem Centre of Plant Sciences, Berlin, Germany<sup>3</sup>Martin-Luther-Universität, Institute of Biology, Halle a. d. Saale, Germany

Wounding plants and herbivore feeding induce a response in local but also in distal tissues. One of the most rapid cellular responses is a transient increase in intracellular calcium concentrations at the wounding site, which propagates as a calcium wave to distal parts of the plant. Glutamate-like receptors (GLRs) have been identified as key components, which are required for wound-induced electric and calcium signal propagation in *Arabidopsis thaliana*. Here, we identified a calcium-dependent protein kinase (CPK*Ti*) as part of the signal propagation network after wounding. CPKs are modular enzymes which can sense calcium changes and translate them into phosphorylation events to modulate target protein function. The respective *cpkTi* mutant shows a reduced wound-induced gene expression in distal, but not in local, leaves, comparable to the *glr3.3a;glr3.6a* double mutant. Furthermore, in *cpkTi* mutant lines, the rapid wound-induced calcium increase in distal leaves is impaired and the velocity of the propagating calcium signal from the wounding site into the petiole is slower. To address the question whether the impaired signalling affects caterpillar feeding behaviour in distal leaves after wounding the local leaf 8, we established a two-choice assay between leaf 13 of two genotypes. This feeding assay showed that local wounding leads to an increased resistance of distal leaves against feeding insects in wildtype plants, whereas the *glr3.3a;3.6a* and *cpkTi-1* mutants are more susceptible than the wildtype. Our data identify CPK*Ti* as an important player in the propagation of a "warning" signal to establish a timely and specific systemic anti-herbivory response.

P 228

Unraveling the link between rain stimuli and flooding-induced hypoxia acclimation in plants

R. Chaudhury<sup>1</sup>, A. Maric<sup>1</sup>, S. Hartman<sup>1</sup>

<sup>1</sup>University of Freiburg, Faculty of Biology, Freiburg i. Br., Germany

Flooding events impact agricultural productivity and are expected to increase due to climate change. Plant flooding survival is highly dependent on their capacity to overcome hypoxia stress and early entrapment of the plant hormone ethylene promotes hypoxia survival (Hartman et al., 2019). Calcium and jasmonate signalling are also involved in hypoxia acclimation (Singh et al. 2023; Yuan et al., 2017). Interestingly, mechano-perception of rain falling on plant leaves, triggers rapid calcium, ethylene and jasmonate signaling (Chehab et al., 2012; Matsumara et al., 2022). In addition, both rain-triggered calcium signalling and jasmonate signalling are shown to increase tolerance to incoming biotic stress (Chehab et al., 2012; Matsumara et al., 2022). Our results show that rain-simulation prior to submergence enhances flooding stress resilience in *Arabidopsis*. We currently investigate how rain duration and plant developmental stage determine rain-induced flooding tolerance. In addition, we aim to unravel the spatiotemporal dynamics, hierarchy and dependency of calcium, jasmonate and ethylene signalling during rain-induced flooding acclimation.

P 229

Clarifying the molecular defense mechanisms of tomato against dodder

A. Blaquiere<sup>1</sup>, P. Slaby<sup>1</sup>, R. Burggraf<sup>1</sup>, I. Albert<sup>1</sup>, M. Albert<sup>1</sup>

<sup>1</sup>Friedrich-Alexander-University, Biology, Molecular Plant Physiology, Erlangen, Germany

In addition to microbes and herbivores, also parasitic plants endanger terrestrial plants and cause massive agronomic problems worldwide. The genus *Cuscuta* comprises about 150 species that all live as holoparasites. *Cuscuta spp.* grow as a vine around the stems of host plants and penetrates host tissues via multicellular organs called haustoria. By establishing cell-cell connections with the host's vascular tissue, the parasitic plant is able to extract water, nutrients and carbohydrates. The receptors and molecular signals involved in this plant-plant dialogue remain mainly unknown.

While almost all dicot plants are susceptible hosts to *Cuscuta spp.*, *Solanum lycopersicum* (cultivated tomato) is one of the few exceptions that shows active resistance specifically against *Cuscuta reflexa*. In tomato, the plasma membrane-bound *Cuscuta* receptor 1 (CuRe1) has been identified, which recognizes a glycine-rich cell wall protein of dodder as a pathogen-associated molecular pattern and induces defense-related responses. In contrast, the wild tomato species *Solanum pennellii* is a susceptible tomato species and does not show defense responses.

In our work, we phenotyped library of *S. lycopersicum* x *S. pennellii* introgression lines for susceptibility and/or hypersensitive response (HR) occurring at haustorium penetration sites on the tomato stem. Within the tomato genome, we mapped a region around 172 kbps on chromosome x that is relevant for the HR-based defense against *C. reflexa*. By further deleting smaller chromosomal parts with CRISPR-Cas9, we reduced the number of candidate genes to ~20, which are now under detailed investigations.

In a second project part, we are attempting to identify the defense trigger of *C. reflexa*. we purified an yet unknown protein from *C. reflexa* extract by using fast protein liquid chromatography techniques. When infiltrated, this protein-containing pre-purified extract induces HR only in *S. lycopersicum* leaves not in susceptible species. Deciphering the molecular mechanisms of tomato resistance against parasitic *Cuscuta spp.* will help to create robust crops for the future.

P 230

**Breeding for improved chilling tolerance in Sorghum to boost its cultivation in Germany**C. Seiler<sup>1</sup>, A. Eltigani<sup>2</sup>, A. Hajjarpoor<sup>3</sup>, C. Balko<sup>4</sup>, D. Perovic<sup>1</sup>, S. Windpassinger<sup>5</sup>, A. Matros<sup>1</sup>, L. Kottmann<sup>2</sup>, T. Feike<sup>3</sup>, G. Wehner<sup>1</sup>, A. Stahl<sup>1</sup><sup>1</sup>Julius Kühn-Institut, Quedlinburg, Germany<sup>2</sup>Julius Kühn-Institut, Braunschweig, Germany<sup>3</sup>Julius Kühn-Institut, Kleinmachnow, Germany<sup>4</sup>Julius Kühn-Institut, Sanitz, Germany<sup>5</sup>Justus-Liebig-Universität, Gießen, Germany

The production of established crops is expected to become more challenging in West Europe as drought and heat events are increasing in frequency and intensity. It is necessary to introduce new crops that can cope with such conditions. Sorghum (*Sorghum bicolor* L.) is the fifth most important cereal crop worldwide and is increasingly cultivated in Germany in recent years, but is still at a very low level. Sorghum is characterised by a deep root system, high photosynthetic efficiency and by high water use efficiency. These characteristics make it more drought and heat tolerant and a promising alternative to maize.

The SORGHUM project aims to investigate its potential as a supplemental C4 crop. Since the major challenges of larger Sorghum cultivation in Germany are insufficient cold tolerance and non-adapted maturation, the project focuses on the genetic base of these two traits towards identification of desirable allelic variants, heterotic potential and development of molecular breeding tools. Moreover, we test whether an increased humus reproduction and respective active carbon sequestration supports an improved greenhouse gas balance of crop production.

200 F1-test hybrids were produced from 53 preselected restorer and four mother lines. The selection is based on previous phenotypic data of recombinant inbred lines populations that had already been adapted to the photoperiodic conditions in Germany. Field experiments at different locations along a north-south-axis of Germany are performed to study the cold tolerance and yield performance of the test hybrids to finally identify superior performing lines. 10 selected hybrids are studied under drought conditions to assess the soil carbon fixation under well-watered and drought conditions. Results indicate high correlation of yield-related traits between the locations ( $r = 0.6 - 0.9$ ). Heritability ranges between 0.5 (number of plants, number of panicles, yield), 0.84 (flowering time) and 0.93 (plant height). The phenotypic data will be applied to process-based crop models to conduct comprehensive simulations of sorghum vs. maize under current and future climatic scenarios.

In addition, germination assays in a controlled environment have been carried out on the parental lines and selected test hybrids. Finally, genomic prediction models for chilling tolerance, early maturity, yield, carbon fixation and biomass of crop residues will be developed.

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**Degradation of plant cell wall components by type II-secreted enzymes – how phytopathogenic bacteria turn the plant apoplast into a habitat**S. Goll<sup>1</sup>, J. Erickson<sup>1</sup>, D. Büttner<sup>2</sup><sup>1</sup>Leibniz-Institut of Plant Biochemistry, Biochemistry of Plant Interactions, Halle a. d. Saale, Germany<sup>2</sup>Martin-Luther-University Halle-Wittenberg, Genetics, Halle a. d. Saale, Germany

The plant apoplast and actively manipulated by colonizing pathogens to increase nutrient availability and avoid detection by the host. Many bacteria achieve this by secreting degradative enzymes via type II secretion (T2S) systems. T2S systems are conserved virulence factors of major crop pathogens including *Xylella* and *Xanthomonas* species and mediate protein secretion from the bacterial periplasm into the extracellular milieu. We previously reported that the Xps-T2S system from the tomato and pepper pathogen *Xanthomonas euvesicatoria* contributes to bacterial pathogenicity by secreting xylanases, a protease and a lipase. T2S substrates likely manipulate plant cell wall components to promote bacterial survival and proliferation however, their precise functions and plant targets are largely unknown. Here, we show that the T2S system from *X. euvesicatoria* and related pathogens is required for bacterial *in vitro* growth in minimal medium containing plant cell wall extracts or highly abundant cell wall-derived carbohydrates such as cellulose or the hemicellulose xylan as sole carbon source. The T2S system is also required for extracellular protease activity. Together, this suggests that T2S substrates degrade plant cell wall and proteins during the infection process. In agreement with these observations, mass spectrometry analyses of apoplastic fluid from infected tomato leaves identified xylanases, polygalacturonases, cellulases and numerous proteases as T2S substrates from *X. euvesicatoria*. Additional *in vitro* assays confirmed type II-dependent secretion and enzyme activity of all identified substrates. Our studies provide insight into potential roles of T2S substrates from *Xanthomonas* species in nutrient acquisition and bacterial colonization of the plant apoplast.

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Remarkable phenolic iron complexes in glacier ice algae (*Ancylonema* spp., Zygnematophyceae) contribute to protection against harmful irradiation

L. Procházková<sup>1</sup>, P. Mojzeš<sup>2</sup>, L. Nedbalová<sup>1</sup>, D. Remias<sup>3</sup>

<sup>1</sup>Charles University, Faculty of Science, Department of Ecology, Praha, Czech Republic

<sup>2</sup>Charles University, Faculty of Mathematics and Physics, Institute of Physics, Prague, Czech Republic

<sup>3</sup>Paris Lodron University of Salzburg, Department of Environment and Biodiversity, Salzburg, Austria

**Introduction:** During summer, glacier surfaces are darkened by blooms of *Ancylonema* spp. microalgae. This phenomenon decreases the ice albedo and thus significantly enhances melting processes. The intracellular brownish secondary pigment was earlier described as a water soluble purpurogallin derivative.

**Questions:** How are these phenolic pigments distributed within the cells? Why do vacuoles appear dark but the putative main compound, when isolated by LC/FC, is only yellowish? Why is there a clear discrepancy in the qualitative VIS spectral absorbance between the isolated compound compared to aqueous raw extracts of the algae?

**Methods:** *Ancylonema* cells freshly harvested from glaciers, Inductively coupled plasma - optical emission spectrometry (ICP/OES), Raman Spectroscopy (RS), High Performance Liquid Chromatography (HPLC), and colorimetric assays.

**Results:** First, regardless of the excitation wavelength used for RS (532, 647, and 785 nm), dark vacuoles from field cells exhibited *in vivo* similar Raman spectra, all of them congruent with the RS features of the main fractions gained by LC/FC. Second, in above mentioned spectra, to our surprise, bands characteristic for iron complexes formed *in vitro* with a purpurogallin standard, were found. Third, to some extent phenols were already present in green cells of an *Ancylonema* laboratorial strain. Finally, ICP/OES performed with aqueous extracts of dark cells confirmed the presence of significant amounts of iron.

**Conclusions:** Besides the glycolized polyphenols, so far unreported, putative Fe-complexes of purpurogallin contribute significantly to the strong UV/VIS spectral absorption of the glacier ice algae, which points to a sophisticated protection strategy in these streptophytic green algae

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Differential proteomics and post-translational modifications in the defense response of *Zea mays* to multitrophic biotic stresses

A. Penteriche<sup>1,2</sup>, D. Gallan<sup>2</sup>, G. Veronez<sup>2</sup>, J. Eirich<sup>1</sup>, I. Finkemeier<sup>1</sup>, M. D. C. Silva Filho<sup>2</sup>

<sup>1</sup>Universität Munster, IBBP, Münster, Germany

<sup>2</sup>University of Sao Paulo, Department of Genetics, Piracicaba, Brazil

*Fusarium verticillioides* is a phytopathogen that highly impacts crop yields of maize (*Zea mays*) and other grasses. It causes economic losses associated to stalk and ear rot, and due to the production of mycotoxins. Recent studies revealed its intimate association with the borer *Diatraea saccharalis*, an insect pest of great relevance to the same crops. Plants infected by *F. verticillioides* produce volatile organic compounds that increase *D. saccharalis* attraction and oviposition. In addition, *F. verticillioides* is capable of being transmitted vertically during *D. saccharalis* life cycle and manipulates the insect behavior to increase its dissemination. In order to defend against pests and pathogens, plants must modulate a vast repertoire of proteins and metabolic pathways that lead to tolerance or susceptibility. In the field, plants are exposed to a great variety of simultaneous multitrophic biological interactions, which can influence or alter the plants defense response as a whole. Studies that seek to understand the plants response to interactions with more than two biological components better reflect the environment field crops are exposed to, and represent a promising strategy for the discovery of new candidates for breeding programs. Knowledge of how these interactions happen at the protein level is scarce and poorly explored. Hence, the present work aims to explore changes in the proteome of *Z. mays* challenged with agronomical relevant multitrophic interactions. With that aim, stem tissue of *Z. mays* genotype B73 was exposed to four different treatments, namely *F. verticillioides* infection (FV), *D. saccharalis* herbivory (DS), and the combination of both FV and DS (FVDS), together with a Mock (MK) condition. A section around the damage was collected and proteins were extracted for mass spectrometry-based proteomic analyses, in addition to the analysis of post-translational protein modifications. Our results indicate that different hormonal signaling pathways are activated in each condition, and many pathogenesis-related proteins seem to be exclusive to the multitrophic interaction. Based on this analysis, functional studies will be carried out to validate interesting candidate proteins that show a different expression profile in each condition and might play a role in this multitrophic interaction.

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**Root hairs are involved in stress recovery of maize plants after water limitation**  
 M. Wimmer<sup>1</sup>, R. Hartwig<sup>1</sup>, L. Käsbauer<sup>1</sup>, I. Engel<sup>1</sup>, S. Wienkoop<sup>2</sup>, C. Perez-Rizquez<sup>2</sup>

<sup>1</sup>University of Hohenheim, Quality of Plant Products, Stuttgart, Germany

<sup>2</sup>University of Vienna, Functional and Evolution Ecology, Vienna, Austria

**Background:** The role of root hairs under conditions of water limitation is under discussion. A benefit by increasing the root surface (Haling et al. 2013) might be offset by a rapid shrinking of root hairs under water limitation (Duddek et al. 2022). The involvement of root hairs in water uptake is still not clearly established (Bienert et al. 2021).

**Objective:** Using a split-root system, we tested the hypothesis that root hairs are beneficial during post-stress recovery after drought, rather than during the water limitation itself.

**Materials and Methods:** The root-hairless maize mutant (*rth3*) and its corresponding wildtype B73 (WT) were grown in split-root rhizoboxes under greenhouse conditions. Plants were exposed to water limitation for 10 days, and then re-watered locally (one root half) or fully (both root halves). Roots and shoots were analyzed for physiological stress responses, proteome and metabolome, and expression of several aquaporins.

**Results:** Due to its smaller biomass, *rth3* experienced a less severe final stress level compared to WT. Upon local re-watering, WT seemed to recover better than *rth3*. While the expression of aquaporins was unchanged during the drought stress in both genotypes, an up-regulation occurred during recovery. Interestingly, this effect was more pronounced in the non-watered root half of the WT, while it occurred only in the watered root half of *rth3*. Proteomic and metabolomic data are currently being evaluated and will be included to identify pathways involved in these reactions in both genotypes.

**Conclusions:** The root-hairless mutant *rth3* recovered less well after a period of water limitation compared to the WT, even though its stress level was not as severe. Under local re-watering, the non-watered root half of WT recovered partly, while this was not the case in *rth3*. This might indicate in *rth3* (i) a systemic problem in signal transduction from one root half to the other, and/or (ii) an impaired water uptake due to the lack of root hairs.

**References**

Bienert et al. (2021) *Biochem Soc Trans* 49: 1133-1146; <https://doi.org/10.1042/BST20200716>.

Duddek et al. (2022) *Plant Physiol* 189: 1232-1236; <https://doi.org/10.1093/plphys/kiac144>.

Haling et al. (2013) *J Exp Bot* 64: 3711-3721; [doi:10.1093/jxb/ert200](https://doi.org/10.1093/jxb/ert200).

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**Elucidation of the molecular networks of *Arabidopsis thaliana* SHRKs**

A. Bannmüller<sup>1</sup>, A. Makris<sup>1</sup>, M. K. Ried-Lasi<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry Halle (Saale), Molecular Signal Processing, Halle a. d. Saale, Germany

The plant model organism *Arabidopsis thaliana* can be infected by *Hyaloperonospora arabidopsidis* (*Hpa*), an obligate biotrophic oomycete and causative of downy mildew disease (Coates and Beynon, 2010; Asai et al., 2023). In contrast to most land plants, *Arabidopsis* is not able to establish symbiotic interactions with arbuscular mycorrhiza fungi, as specific genes required for their accommodation inside plant cells were lost (Favre et al., 2014; Delaux et al., 2013; Delaux et al., 2014; Bravo et al., 2016; Ried et al., 2019). *Symbiosis Receptor-like Kinase* (*SymRK*), a member of the LRR-I subfamily of leucine-rich repeat receptor-like kinases, is one of the genes essential for the establishment of fungal and bacterial root endosymbiosis in plants (Endre et al., 2002; Gherbi et al., 2008; Shiu and Bleeker, 2001; Stracke et al., 2002). *Arabidopsis* *SymRK*-homologous Receptor-like Kinase 1 (SHRK1), SHRK2 and SHRK3 were identified to be closely related to *SymRK* (Ried et al., 2019). Interestingly, *Arabidopsis shrk1*, 2 and 3 single and triple mutants display a reduction in the reproductive success of *Hpa* compared to the wildtype, thus indicating an involvement of SHRKs in the accommodation of the oomycete (Ried et al., 2019; Makris and Ried, unpublished). Similarly to *SymRK*, SHRKs seem to be cleaved in their ectodomain region (ECD) *in planta* (Makris and Ried, unpublished; Antolin-Llovera et al., 2014). However, the molecular mechanisms responsible for cleavage of the ECD are not known. Furthermore, we could demonstrate that *Arabidopsis* SHRK1, 2 and 3 are involved in salt stress responses (Makris and Ried, unpublished). To date, little is known regarding the regulation of SHRKs, SHRK-specific interactors and their downstream signaling. Therefore, we will elucidate the molecular networks of SHRKs in *Arabidopsis*, in the context of *Hpa* infection and salt stress. This includes the analysis of the SHRK intracellular interactomes as well as SHRK-associated metabolomic and transcriptional changes.

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The role of the phloem in plant defence and protection

A. Furch<sup>1</sup>, M. Zimmermann<sup>1</sup>, G. Noll<sup>2</sup>, A. Mithöfer<sup>3</sup>, E. Peiter<sup>4</sup>, K. H. Kogel<sup>5</sup>, A. J. van Bel<sup>5</sup>

<sup>1</sup>Friedrich-Schiller-Universität Jena, Matthias-Schleiden-Institut für Genetik, Bioinformatik und molekulare Botanik, Jena, Germany

<sup>2</sup>University of Münster, Institute of Plant Biology and Biotechnology, Münster, Germany

<sup>3</sup>Max-Planck-Institute for Chemical Ecology, Research Group Plant Defense Physiology, Jena, Germany

<sup>4</sup>Martin Luther University of Halle-Wittenberg, 10Plant Nutrition Laboratory, Institute of Agricultural and Nutritional Sciences, Faculty of Natural Sciences III, Halle a. d. Saale, Germany

<sup>5</sup>Justus Liebig University Giessen, Institute of Phytopathology, Centre for BioSystems, Land Use and Nutrition, Gießen, Germany

Pathogen detection by pattern recognition receptors triggers a range of defense responses including the initiation of systemic alarm signals. We investigated how and where chemical information acquired upon perception of the microbe-associated molecular pattern (MAMP) flg22 is transformed into long-distance electrical potential waves crucial for the generation of local and systemic defense responses. In *Arabidopsis thaliana* leaves, the density of AtFLS2 receptors was high in upper epidermal cells, very low in cortex cells, abundant in phloem parenchyma and companion cells, but extremely low in sieve elements (SEs). VfFLS2 gene expression results and isolated SE protoplasts revealed a similar VfFLS2 deployment pattern in *Vicia faba*. Aequirin-based examinations disclosed cytosolic Ca<sup>2+</sup> peaks one and three min after flg22 application. These waves coincided with the propagation of two partly overlapping electrical messages from epidermis to SEs as revealed by voltage recordings. At the SE plasma membrane, the respective components were converted either into rapid long-range action potentials (APs) or slower short-range variation potentials (VPs). Modified levels of the phytohormones JA, JA-Ile, and SA demonstrated systemic effects of flg22-induced APs in distant *Arabidopsis* leaves. The role of VPs is less clear. Appreciable Ca<sup>2+</sup> influx associated with VPs was responsible for transient sieve-element occlusion (SEO) within a few cm from the site of flg22 perception in *Arabidopsis* and *Vicia*. This response was absent in *Atseor1/2* and *Atfls2* mutants. While the flg22 effects on SA downregulation were similar in wild-type and *Atseor1/2* mutants, JA upregulation was significantly higher in wild-type plants. The biological relevance of SEO was further tested by pathogen assays using *Atseor1/2* mutants, which turned out to be more susceptible to *Pseudomonas syringae* DC3000 than wild-type plants. Collectively, the data show that SEO is an integral part of the defense response rather than a futile side effect of a turgor wave associated with flg22-induced stomatal closure.

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Exploring the Molecular Mechanism and Functional Diversity of NEP1-like proteins in Plant Pathogenesis

G. Giuliani<sup>1</sup>, D. Geiger<sup>2</sup>, R. Roelfsema<sup>2</sup>, P. Dietrich<sup>1</sup>, T. Nürnberger<sup>3</sup>, I. Albert<sup>1</sup>

<sup>1</sup>Friedrich-Alexander Universität, Molecular Plant Physiology, Erlangen-Nürnberg, Germany

<sup>2</sup>Julius-Maximilians-Universität, Molecular Plant Physiology and Biophysics, Würzburg, Germany

<sup>3</sup>Eberhard-Karls-Universität, ZMBP Plant Biochemistry, Tübingen, Germany

NLPs (NEP1-Like Proteins, Necrosis and Ethylene-inducing proteins) are produced by numerous phytopathogenic fungi, oomycetes and bacteria with a necrotrophic lifestyle. Gene families often comprise multiple members, in some cases up to 80 genes per organism. NLPs are virulence-promoting proteinaceous toxins that trigger cell death and ethylene accumulation in many eudicot plants. Secreted by the pathogen into the extracellular space of host plants, some NLPs target plant-specific glycosylinositol phosphorylceramides (GIPCs) in the outer leaflet of the plasma membrane. Upon membrane binding, NLPs cause membrane rupture and cytolysis. Structural similarities between NLPs and pore-forming toxins from marine invertebrates suggest a pore-driven mechanism of cytolysis. However, the precise molecular mechanism underlying NLP cytolysis remains poorly understood.

To elucidate the molecular mechanism of cytolytic NLPs, we investigate the behavior of NLPs on membranes and their effects on host cells in more detail. Our studies focus on whether NLPs, like the structurally related actinoporins, also form oligomers and pores on plant membranes to exert cytolysis. Expanded NLP gene families often occur in pathogen species with a diverse host spectrum. Potentially, pathogens evolved specialized NLPs for different host lipids. To study the biological relevance and compare their functional behaviors, we examine various NLPs from *Phytophthora sojae*, *Phytophthora capsici*, and *Verticillium dahliae* under different conditions. Thereby, we aim to characterize the putative functional diversification among different NLP variants. Understanding the molecular mechanisms and functional diversity of NLPs is crucial for developing strategies to mitigate their virulent impact and enhance crop resistance.

References

- Ottmann et al., A common toxin fold mediates microbial attack and plant defense. Proc. Natl. Acad. Sci. USA 25: 10359-10364 (2009).  
 Lenarcic et al., Eudicot-specific sphingolipids determine host selectivity of microbial NLP cytolysins. Science 358: 1431-1434 (2017).  
 Pirc et al., An oomycete NLP cytolysin forms transient small pores in lipid membranes. Sci. Adv. 8: eabj (2022).

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**Novel Disease Prevention Strategy in Arabidopsis: Reducing Apoplastic Water Availability and Water Potential via Flagellin-Mediated AQP Inhibition to Restrict Bacterial Growth**

A. Dalal<sup>1,2</sup>, Z. Attia<sup>2</sup>, M. Moshelion<sup>2</sup>

<sup>1</sup>Heinrich-Heine-Universität Düsseldorf, Institute for Molecular Physiology, Düsseldorf, Germany

<sup>2</sup>The Hebrew University of Jerusalem, Institute of Plant Sciences and Genetics in Agriculture, Rehovot, Israel

Plants possess sophisticated defence mechanisms to combat pathogen attacks. A common early symptom of infection is the formation of water-soaked lesions, resulting from the disruption of plasma membrane or cell wall integrity by pathogen effector proteins. These lesions are characterized by a sudden increase in the water content at the site of infection, which facilitates further bacterial growth.

PAMP-triggered immunity (PTI) begins when plants recognize pathogen-associated molecular patterns (PAMPs), such as flagellin. Flagellin22 (flg22) plays a pivotal role in this defence strategy by binding to specific receptors on the plasma membrane, initiating a series of immune responses. In this study, we hypothesize that the flg22 signal transduction pathway also involves reducing Aquaporin (AQP) activity in the mesophyll and vascular bundle sheath cells, thus decreasing leaf and cellular water conductivity as a means to mitigate lesions needed for bacterial establishment.

Our experimental data show that flg22 significantly reduces cellular membrane osmotic water permeability (Pf) and leaf hydraulic conductivity (Kleaf), leading to a substantial decrease in leaf water potential ( $\psi_L$ ), in wild type Arabidopsis (WT) but not in the mutants of flg22 receptors (*bak1-4* and *fls2*) and AQPs (*pip1*).

Bacterial infiltration into WT leaves resulted in increase of both Pf and  $\psi_L$  along with water-soaked lesions by *Pseudomonas syringae* pv tomato (*Pst*DC3000 or *Pst*) but not by delta-hrcQ-U ( $\Delta hrc$ , T3SS mutant of *Pst*). Increase of  $\psi_L$  was also observed in *bak1-4*, *fls2* and *pip1* mutants by *Pst* but not by  $\Delta hrc$ .

Pre-infiltration with flg22 in WT prevents increase of  $\psi_L$  by *Pst*, and promotes further reduction of  $\psi_L$  by  $\Delta hrc$ , thus effectively limiting the growth of *Pst* and  $\Delta hrc$ . However, pre-infiltration with flg22 resulted in no change in  $\psi_L$  by *Pst* and  $\Delta hrc$ , which interestingly resulted in limited growth of *Pst* but not  $\Delta hrc$  in all the mutants.

We conclude that the binding of flagellin to its receptors reduces the water content in the intercellular spaces via AQP inhibition and creates a more negative water potential, leading to reduced water availability to bacteria in the apoplast. We suggest that this mechanism of cellular dehydration is a part of PTI, and a novel non-stomatal apoplastic prevention phase in plant immune response, critically restricting the bacterial growth and establishment in the intercellular spaces.

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**Ferricrocin: The concealed virulence factor of the corn anthracnose pathogen *C. graminicola***

L. Aliyeva-Schnorr<sup>1</sup>, H. B. Deising<sup>1</sup>, B. R. Fernando<sup>1</sup>, C. Goldbach<sup>1</sup>, R. Csuk<sup>2</sup>

<sup>1</sup>MLU Halle, Institute of Agricultural and Nutritional Sciences, Halle a. d. Saale, Germany

<sup>2</sup>MLU Halle, Institute of Chemistry, Halle a. d. Saale, Germany

Iron is an important nutrient that plays a prominent role in diverse biological functions in all organisms, including fungi. Due to its poor solubility, iron is a limiting factor in growth and development. On the other hand, an excess of iron leads to toxicity through the formation of hydroxyl radicals. In the maize anthracnose fungus *Colletotrichum graminicola*, the non-ribosomal peptide synthetase *Nps2* is responsible for the synthesis of the intracellular siderophore ferricrocin and thus indispensable for iron scavenging and for prevention of iron toxicity (Albarouki et al., 2014). Also, in *Magnaporthe oryzae* and *Alternaria alternata* *Nps2* is required for the biosynthesis of ferricrocin as an intracellular storage siderophore (Hof et al., 2007; Voss et al., 2020). Since the exact role of *Nps2* in *C. graminicola* is yet unknown, we generated *NPS2* deletion and green fluorescent protein (GFP) promoter fusions strains to elucidate the role of *NPS2* in intracellular siderophores biosynthesis and control of intracellular iron homeostasis under iron deficiency and iron surplus conditions. Our findings to date show that expression of *NPS2* occurs at each stage of pathogenesis. Although the differences in growth and development between WT and  $\Delta nps2$  strains appear to be marginal under standard conditions, the deletion mutants show higher susceptibility to oxidative stress. In addition, infection experiments on maize leaves, qPCR analyses, and quantification of infection structures revealed that *NPS2* is required for full virulence of *C. graminicola*.

**References**

- Albarouki, E., L. Schaffner, F. Ye, N. von Wiren, H. Haas, H.B. Deising, 2014: Biotrophy-specific downregulation of siderophore biosynthesis in *Colletotrichum graminicola* is required for modulation of immune responses of maize. *Mol Microbiol* 92 (2), 338-355
- Hof, C., K. Eisfeld, K. Welzel, L. Antelo, A.J. Foster, H. Anke, 2007: Ferricrocin synthesis in *Magnaporthe grisea* and its role in pathogenicity in rice. *Mol Plant Pathol* 8 (2), 163-172
- Voss, B., F. Kirschhofer, G. Brenner-Weiss, R. Fischer, 2020: *Alternaria alternata* uses two siderophore systems for iron acquisition. *Sci Rep* 10 (1), 3587



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## Calcium-dependent protein kinases in the regulation of intrinsically disordered transcription factors in plant immunity

J. Oehlschläger<sup>1</sup>, S. Lederer<sup>2</sup>, S. Matschi<sup>2</sup>, X. Jiang<sup>2</sup>, T. Romeis<sup>2</sup><sup>1</sup>Martin-Luther-University Halle-Wittenberg, Department of Pharmaceutical Chemistry & Bioanalytics, Halle a. d. Saale, Germany<sup>2</sup>Leibniz-Institute of Plant Biochemistry, Biochemistry of Plant Interactions, Halle a. d. Saale, Germany

During both abiotic and biotic stress responses, Calcium (Ca<sup>2+</sup>) plays a critical role as one of the most important second messengers in plants and eukaryotes in general. Decoding of the specific Ca<sup>2+</sup> signatures is mediated by a range of different protein families, among them the plant specific family of Calcium-dependent protein kinases (CDPKs). Combining both the Ca<sup>2+</sup>-binding and kinase effector domain in a single protein, CDPKs are activated upon cytosolic Ca<sup>2+</sup> increase and are involved in several signaling pathway. During plant immunity, several CDPKs are described as positive regulators both during local and systemic immune responses. Enhanced CDPK activity leads to increased resistance and enhanced immune memory in the form of Systemic Acquired Resistance (SAR). While the involvement of CDPKs in signaling pathways, for example in immune signaling, has been well described, the identification of respective phosphorylation target proteins and the definitive mechanistic characterization of target interactions in these pathways often remains elusive. This work highlights an interplay of Ca<sup>2+</sup>-regulated networks and systemic plant immunity via the CDPK-dependent phosphorylation of the key transcriptional regulator SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1 (SARD1) as an *in vivo* target.

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Identification and characterization of miR398GGT of *Gaeumannomyces graminis* var. *tritici* and its possible role in plant-fungus interactionsJ. Stehle<sup>1</sup>, M. Schemmel<sup>1</sup>, L. Han<sup>1</sup>, D. Cai<sup>1</sup><sup>1</sup>Christian-Albrechts-Universität zu Kiel, Molecular Phytopathology & Biotechnology, Kiel, Germany

Wheat is a globally significant crop but is exposed to the Take-all pathogen *Gaeumannomyces graminis* var. *tritici* (GGT), which can cause severe damage in wheat production worldwide. Take-all is a widespread wheat root disease that can lead to 40-60% yield losses. The fungus infects roots via hyphae that survive in the soil in root debris of wheat plants or on the field weeds. The hyphae penetrate root cortical cells, causing a root rot and gradually spread throughout the root and progress into the stem's base. As there is no plant genetic resistance and effective fungicides against this disease, molecular understanding of plant-pathogen interactions may provide alternative strategies to combat this pathogen. Bidirectional cross-kingdom RNA interference (RNAi) proved to play important roles in plant-pathogen interactions, in which both plants and pathogens can use small RNAs (sRNAs) to silence target genes. By a large-scale metagenomics approach in combination with smallRNA sequencing we identified 9.128 sRNAs from 1.009 fungal genomes, which are potentially able to interfere with plant gene expression. Among these, a homologue of wheat miR398, referred as to miR398GGT, was identified in the infected wheat roots. Here, we report the identification and characterization of the miR398 locus in the GGT genome and its expression as well as processing during the infection process. Transcript analyses indicate that miR398GGT is involved in post-transcriptional silencing SOD target genes, thus benefiting its infection process in wheat. Our data suggest that miR398GGT-mediated suppression of SOD genes in wheat may be an essential component of virulence of GGT to evade plant defence response by impairing ROS production. Further investigation on the mode of function of miR398GGT in wheat-fungus interactions is currently in progress.

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The *Ustilago maydis* GATA transcription factor Nit2 controls nitrate utilization of the pathogen during biotrophy and influences major amino acid metabolism in leaf galls

C. Schulz<sup>1</sup>, P. Lopinski<sup>1</sup>, N. Schäfer<sup>1</sup>, N. Braun<sup>1</sup>, A. Fischer<sup>1</sup>, L. Voll<sup>1</sup>

<sup>1</sup>Philipps-Universität Marburg, Molecular Plant Physiology, Marburg, Germany

The basidiomycete corn smut fungus *Ustilago maydis* leads a dimorphic lifestyle, in which two compatible sporidia fuse on the host surface to produce phytopathogenic filamentous hyphae that subsequently infect meristematic tissue of maize (*Zea mays*). In previous work with the solopathogenic SG200 strain, we have shown that the transcription factor Nit2 plays a major role for the utilization of non-favored nitrogen sources like nitrate, minor amino acids or nucleobases in saprotrophic sporidia.

While  $\Delta$ Nit2 mutants had shown delayed filamentation and strongly reduced pathogenicity in the solopathogenic SG200 strain, pathogenicity and host colonization were not reduced in the FB1 x FB2 background, which allowed us to study the impact of Nit2 on the reciprocal responses of host and pathogen on the molecular level and to compare the physiology of leaf galls during biotrophy of wild type and the  $\Delta$ Nit2 mutant. We have identified around 30 Nit2 target genes during biotrophy by RNA-Seq analysis, with one third of them being involved in nitrogen metabolism. While transport proteins for organic nitrogen almost completely differed among the Nit2 targets during saprotrophy and biotrophy, nitrate assimilation was consistently regulated in a Nit2-dependent fashion. Among the tested genes, nitrate and nitrite reductase were the only target genes that were completely dependent on Nit2 during biotrophy. Comparing closely defined nitrogen deficient versus nitrogen replete maize plants, substantial accumulation of nitrate was observed in  $\Delta$ Nit2 leaf galls under nitrogen limitation. In addition, the impact of  $\Delta$ Nit2 colonization on steady state levels of major amino acids was less pronounced compared to wild type galls. As for nitrate, the effect of Nit2 deficiency on amino acid metabolism was much more pronounced in nitrogen deplete than in nitrogen replete conditions. Given the 25% reduction in total protein content in  $\Delta$ Nit2 relative to wild type leaf galls in nitrogen deplete conditions, it will be interesting to conduct systemic N flux experiments by stable isotope labeling to reveal, why these extensive changes in gall physiology do not have a measurable impact on  $\Delta$ Nit2 pathogenicity. Our findings demonstrate that nitrate utilization is dispensable for *Ustilago maydis* during biotrophy and reinforces the role of organic nitrogen for the nutrition of the corn smut fungus *in planta*.

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Loss of *pgm*, *sweet11* and *sweet12* alters local and systemic carbohydrate allocation and pathogen susceptibility

J. Seufer<sup>1</sup>, S. Goll<sup>2</sup>, T. Engelsdorf<sup>1</sup>, A. Frey<sup>1</sup>, L. Voll<sup>1</sup>

<sup>1</sup>Philipps University of Marburg, Molecular plant physiology, Marburg, Germany

<sup>2</sup>Martin Luther University of Halle-Wittenberg, Department of Genetics, Halle a. d. Saale, Germany

The starch free phosphoglucomutase deficient *Arabidopsis* mutant *pgm* shows impaired carbohydrate availability especially at the end of the dark phase and is more susceptible towards *Colletotrichum higginsianum*. Early post-penetration establishment in *pgm* mutants is enhanced due to altered cell wall composition while during the transition to the necrotrophic phase, *pgm* exhibits dampened responsiveness of salicylic acid induced genes despite increased levels of free salicylic acid (Engelsdorf et al., 2013). On the other hand, the double mutant *sweet11/sweet12*, which lacks the two major phloem loading sucrose transporters, has been shown to be more resistant than the wildtype. The *sweet11/sweet12* mutant shows increased levels of free sugars, salicylic acid and increased responsiveness of salicylic acid dependent genes. We have generated *pgm/sweet11/sweet12* triple mutants to investigate, how hampered sucrose export (in *sweet11/sweet12*) and reduced carbohydrate availability (*pgm*) affect primary metabolism, SA accumulation, host defence and compatibility. *pgm/sweet11/sweet12* might relieve carbohydrate shortage in *pgm* plants, as we have now shown in leaf exudation experiments. The decreased carbohydrate export in the *pgm/sweet11/sweet12* background to sink tissue might revert the susceptibility phenotype of starch-deficient *pgm*. In comparison to *pgm*, the triple mutant exhibited alleviated hypersusceptibility towards *C. higginsianum*, which was accompanied by further increased accumulation of soluble sugars compared to the *pgm* parent and further enhanced premature SA accumulation compared to the *sweet11/sweet12* parent. Our data strongly favour the idea that carbohydrate availability and the defence response are increased in the triple mutant compared to *pgm*, because excess sucrose export to roots is prevented. Using the triple mutant as a tool, we currently investigate the crosstalk of sugar and defence signalling. In addition, we are using genetically encoded fluorescent nanosensors to assess the spatiotemporal organization of the defence response in the epidermis of the individual genotypes.

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Molecular signatures of quantitative disease resistance against *Sclerotinia sclerotiorum* in the *Brassica napus* genome assessed by genetic, genomic and transcriptomic approaches

H. Seide<sup>1</sup>, U. Riesterer<sup>1</sup>, W. Ye<sup>1</sup>, T. Bergmann<sup>2</sup>, S. Rietz<sup>2</sup>, D. Cai<sup>1</sup>

<sup>1</sup>Christian-Albrechts-Universität zu Kiel, Molecular Phytopathology and Biotechnology, Kiel, Germany

<sup>2</sup>NPZ Innovation GmbH, Holtsee, Germany

*Sclerotinia sclerotiorum*, the causal agent of Sclerotinia stem rot (SSR), has a vast host range spanning over 400 plant species. Among these species are economically important crops, including oilseed rape (*Brassica napus*) where SSR can result in devastating yield and quality losses. The control of *Sclerotinia* under field conditions remains challenging due to increasing restrictions of fungicide application. Effective SSR genetic resistance is missing within the *B. napus* gene pool. However, QTLs for a higher level SSR resistance have been identified in related species, like *B. villosa*, offering a unique opportunity for breeding SSR resistant *B. napus* lines through interspecies crossing. This study used two independent segregating populations of *B. napus* for SSR resistance.

Using a 19k Brassica SNP chip array, two high-density genetic maps were generated, which contain 2,285 and 1,329 SNP markers and comprise a total genetic length of 1,900 and 1,972 cM, respectively. QTL analysis identified four QTLs that can explain between 8.2 % and 19.5 % of the phenotypic variance. Genome sequencing revealed a large set of resistance-specific sequences within the QTLs. These include one NBS-LRR gene, several RLK (LecRK41, CRLK1, CRLK2, SOBIR1) and RLP genes (RLP26), MAPKs (MAPKKK15, MAPKKK19) and transcription factors known to be involved in plant defense responses, such as WRKY54 and MYB29.

To narrow down the candidates, we performed transcriptomic analysis. By comparing infected resistant and susceptible plants, the QTL/resistance-specific genes whose expression responded to *Sclerotinia* infection were identified. Many of them are potentially involved in the activation and regulation of the plant defense response, such as THE1, MYB46 and SWAP70. Furthermore, ethylene (ET)-activated signaling was strongly enhanced (ERF106, ERF038). Strikingly, several non-coding small RNAs (miRNAs and sRNAs) were expressed after *Sclerotinia* infection.

These data provide first insights into the genomic and molecular signatures of QTLs for SSR resistance in *B. napus* and offer the possibility to further decipher the underlying resistance mechanisms and to develop molecular markers for breeding resistant cultivars. The mode of action of genes and in particular the role of non-coding small RNAs in conferring SSR resistance in *B. napus* are currently under investigation.

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Role of calcium signalling in chitosan-induced immune response in *Arabidopsis* mesophyll cells

P. Tennakoon<sup>1</sup>, R. Roelfsema<sup>1</sup>

<sup>1</sup>University of Wuerzburg, Institute for Molecular Plant Physiology and Biophysics, Julius-von-Sachs-Institute, Würzburg, Germany

Plants possess a remarkable ability to defend themselves against pathogenic microorganisms, which can cause severe damage. Chitin is a fungal cell wall component which is perceived by the PRRs of CERK1 and LYK5. However, little is known about the early signalling events evoked by CERK1 and LYK5. In the presented study, we used intracellular microelectrodes and live cell imaging, to study the activation of SLAH3 by chitosan. Our results indicate that upon perception of chitosan by mesophylls rapidly activate SLAH3, via a Ca<sup>2+</sup>-independent signalling pathway. We propose a signalling pathway in which LYK5 acts via PBL27, to activate SLAH3, as proposed for guard cells by Liu *et al.*, 2019. The impact of this response on the ability of *Arabidopsis* to resist fungal pathogens will be evaluated in future experiments.

References

Liu Y., Maierhofer, T., Rybak K., Sklenar, J., Breakspear, A., Johnston, M.G., Fliegmann, J., Huang, S., Roelfsema, M.R.G., Felix, G., Faulkner, C., Menke, F.L.H., Geiger, D., Hedrich, R. and Robatzek, S. (2019). Anion channel SLAH3 is a regulatory target of chitin receptor-associated kinase PBL27 in microbial stomatal closure. *eLife* 8:e44474. DOI: <https://doi.org/10.7554/eLife.44474.024>

P 246

**An untargeted proteomics method reveals plant proteins that are ADP-ribosylated by pathogen effectors to promote infection**

S. Kaur<sup>1</sup>, T. Colby<sup>2</sup>, D. Thieme<sup>1</sup>, C. Proksch<sup>1</sup>, S. Matschi<sup>1</sup>, I. Matic<sup>2</sup>, L. Wirthmüller<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry, Dept. Biochemistry of Plant Interactions, Halle a. d. Saale, Germany

<sup>2</sup>Max Planck Institute for Biology of Ageing, Proteomics and ADP-ribosylation Signaling group, Köln, Germany

Protein ADP-ribosylation is a post-translational modification that plays a pivotal role in the molecular arms race between pathogens and plants. Pathogens promote virulence by translocating effector proteins with ADP-ribosyltransferase activity into host cells. On the plant side, endogenous ADP-ribosylation events contribute to a coordinated immune response. Substrate identification of plant and pathogen-derived ADP-ribosyltransferases remains technically challenging due to lack of efficient enrichment strategies, the short half-life time of the modification, and its lability under common LC-MS/MS conditions.

We present a proteomics workflow for enrichment of ADP-ribosylated proteins from total plant extracts followed by identification of the ADP-ribosylated peptides and amino acids by proteomics. As a proof-of-concept we applied the method to Arabidopsis plants expressing the *Pseudomonas syringae* type III effector AvrRpm1. Our untargeted method allowed mapping the ADP-ribosylation sites on the previously characterized AvrRpm1 substrate RIN4 and two of its homologs with high confidence. In the Col-0 accession, ADP-ribosylation of RIN4 activates the immune receptor RPM1. However, in genetic backgrounds in which AvrRpm1 is not recognized by RPM1, *RIN4* is genetically dispensable for the virulence function of the effector. This indicates that AvrRpm1 promotes virulence by ADP-ribosylating yet unknown plant proteins. We identified an additional set of proteins that show specific ADP-ribose affinity enrichment in plants expressing AvrRpm1, indicating that they might constitute virulence targets of the effector. This list includes the blue light receptor PHOTOTROPIN1, a phospholipase D, and the MEMBRANE-ASSOCIATED MANNITOL-INDUCED (MAMI) protein that shows homology to vesicle-associated membrane proteins. For MAMI and the phospholipase our method provided site-resolution of the modified amino acids.

To our knowledge, this is the first report of direct enrichment and unbiased detection of ADP-ribosylated peptides from total plant cell extracts. While our method still has limitations (e. g. expression of the pathogen effector as a transgene), we envisage that it will facilitate untargeted substrate identification for uncharacterized pathogen-derived ADP-ribosyltransferases from bacterial and filamentous pathogens. A better understanding of pathogen virulence strategies can inform approaches for breeding or engineering disease resistance in plants.

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**The phytosulfokine pathway as a target for host immune suppression by the wheat pathogenic fungus *Zymoseptoria tritici***

M. Faroux<sup>1,2</sup>, E. Thynne<sup>1,2</sup>, E. Holtgrewe-Stukenbrock<sup>1,2</sup>

<sup>1</sup>Botanical Institute, Christian-Albrechts University of Kiel, Environmental Genomics, Kiel, Germany

<sup>2</sup>Max Planck Institute for Evolutionary Biology, Plön, Germany

The plant immune system relies on various physiological pathways, with phytohormones playing a crucial role. Among these, the disulfated pentapeptide phytosulfokine (PSK) is perceived by leucine-rich repeat receptor-like kinases (LRR-RLKs), as described in *Arabidopsis*. PSK signaling regulates plant immunity by modulating salicylate and jasmonate pathways, conferring resistance to necrotrophic pathogens but susceptibility to bio- and hemibiotrophic pathogens. While PSK signaling is well-studied in dicots, its role in monocots remains less explored. Interestingly, several genomes of *Zymoseptoria* fungi, causing disease in grasses and cereal crops, encode the PSK YIYTQ motif, hereunder *Z. tritici* (*Zt*) and *Z. passerinii*, two hemibiotrophic pathogens of wheat and barley, respectively. Our primary hypothesis posits that wheat and barley possess functional PSK receptors (PSKR), and that these act as susceptibility factors to *Zymoseptoria* infection. To test this hypothesis, we firstly aim to structurally and physiologically characterize wheat and barley PSK receptors. Through protein sequence alignments and structure prediction analyses we observed significant homology between wheat and reference PSK-receptors of *Arabidopsis*, *Nicotiana* and rice, especially in the extracellular PSK-binding island domain and the intracellular kinase domain. We found that PSK down-regulates the immune-related genes *PR1* and *PAL3* in barley, supporting the functionality of PSK signaling in these monocot species. Subsequently, we used Virus-Induced Gene Silencing (VIGS) technology to down-silence *N. benthamiana* PSKRs, followed by over-expression of wheat and barley candidate PSKRs to confirm their activity in a heterologous system. Our ultimate goal for this project is to investigate whether *Zymoseptoria spp.* produces PSK to manipulate the plant immune system via PSK signaling. To explore this further, we genetically engineered *Zymoseptoria* to knock-out the PSK-encoding gene and thereby to assess its impact on virulence. Our study offers a dual-sided approach, shedding light on both plant and fungal physiology at a molecular level.

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Purification and characterization of a novel antifungal protein, CT-1 from seeds of *Clitoria ternatea*

M. Mishra<sup>1</sup>, H. Shirsat<sup>1</sup>

<sup>1</sup>Dr. Vishwanath Karad MIT-World Peace University, Department of Biosciences & Technology, Pune, India

The leguminous herb, *Clitoria ternatea* (butterfly pea) is known for its important agronomic traits as well as medicinal benefits in traditional knowledge systems. A repertoire of phytochemicals, ultra-stable macrocyclic peptides and seed proteins and peptides have been implicated as the bioactive agents responsible for its resistance to various pathogens and pests. In this study, a novel ~14 kDa protein (CT-1) was purified from dried seeds of *Clitoria ternatea* using ammonium sulfate precipitation and DEAE-cellulose. CT-1 was characterized for its thermostability, pH stability and resistance to proteolytic degradation/enzymatic degradation. CT-1 was evaluated for its fungistatic/fungicidal action against various important fungal pathogens of plants, namely *Botrytis cinerea*, *Sclerotium rolfsii* and *Fusarium oxysporum* by incorporating it in the Potato Dextrose Agar (PDA) growth medium. CT-1 exhibited significant antifungal activity against *Botrytis cinerea* and *Sclerotium rolfsii* with a minimum inhibitory concentration in the range of 25-100 µg/ml and up to 85.41% and 80% reduction in fungal biomass production, respectively. Furthermore, the morphological changes in *Botrytis cinerea* caused by CT-1 protein were studied using Scanning electron microscopy. CT-1 also exerted an inhibitory effect on the growth of *Fusarium oxysporum* with a minimum inhibitory concentration in the range of 50-200 µg/ml. CT-1 exhibited thermotolerance up to 90°C in its structure and fungistatic action. The present study adds to the literature on novel seed proteins which can be of significant use in agronomical applications. Methods involving use of CT-1 protein can be effective in biocontrol of phytopathogenic fungi in fields which lead to major crop losses worldwide.

References

1. Ajesh K and Sreejith K (2014) A Novel Antifungal Protein with Lysozyme-Like Activity from Seeds of *Clitoria ternatea*. *Appl Biochem Biotechnol.* 173:682–693.
2. Kelemu S, Cardona C and Segura G (2004) Antimicrobial and insecticidal protein isolated from seeds of *Clitoria ternatea*, a tropical forage legume. *Plant Physiology and Biochemistry* 42: 867–873.
3. Oguis GK, Gilding EK, Jackson MA and Craik DJ (2019) Butterfly Pea (*Clitoria ternatea*), a Cyclotide-Bearing Plant With Applications in Agriculture and Medicine. *Front. Plant Sci.* 10:645.

P 249

The role of Nitrogen in the context of *Vitis*-Esca crosstalk

E. Zareei<sup>1</sup>, P. Nick<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, KIT, Joseph Gottlieb Kölreuter Institute of Plant Sciences, Karlsruhe, Germany

Grapevine Trunk Diseases (GTDs) have rapidly expanded due to the impacts of climate change, posing a significant challenge to sustainable viticulture globally. Unfortunately, winegrowers currently lack effective control methods, and there are no grapevine cultivars that are completely resistant to these diseases. GTDs are primarily caused by normally harmless endophytic fungi, but they transform into destructive agents when the host plant experiences drought and heat stress (1). Based on plant immunity investigation, there is a correlation between the outbreak of disease and the chemical signaling between the plant and pathogen. Phenolic plant compounds, e.g. the phenylpropanoids, are one of the certain signals involved in this process, which serve as defense mechanisms against pathogens. They have diverse functions such as plant defense compounds (phytoalexins), or precursors of lignin, the fundamental structural component of wood. By the detection of ferulic acid, a precursor of lignin, the fungal organism is capable of assessing the condition of the host (2). Phenylpropanoid synthesis starts with the amino acid phenylalanine, which is converted to cinnamic acid, upstream of ferulic acid in the pathway, and ammonium by an enzyme called phenylalanine ammonium lyase (PAL). Ammonia is an important source in the Glutamate-Glutamine cycle leading to forming other acidic amino acids. As a result, there is a link between the synthesis of proteins and phenolic compounds. This study is going to find the impact of N on the regulation and restructuring of disease outbreak, the behavior of fungus after the N manipulation, and the molecular pathways involved in the plant-pathogen communication concerning defense responses. In this regard, the disease outbreak of Riesling plants exposed to altered levels of N resources alone and in combination with fungus, *Neofusicoccum parvum*, will be investigated. Finding the N signaling in this regard creates an opportunity to make woody plants more resistant to climate change, one of the challenges of the 21st century.

References

1. Flubacher, Noemi, et al. "The fungal metabolite 4-hydroxyphenylacetic acid from *Neofusicoccum parvum* modulates defence responses in grapevine." *Plant, Cell & Environment* 46.11 (2023): 3575-3591.
2. Khattab, Islam M., et al. "Ferulic acid is a putative surrender signal to stimulate programmed cell death in grapevines after infection with *Neofusicoccum parvum*." *Plant, Cell & Environment* 46.1 (2023): 339-358.

P 250

## Lessons to learn from a gall-inducing fungus

A. Djamei<sup>1</sup><sup>1</sup>University of Bonn, Plant Pathology, Bonn, Germany

Smut fungi form a large group among the basidiomycetes and are biotrophic specialists in infecting a diverse set of mainly grasses, among them important crops like sorghum, millet, barley and maize. The maize smut fungus *Ustilago maydis* serves as an important model for smuts fungi and induces prominent galls on all aerial parts of its host, reflecting a metabolic and developmental reprogramming of the plant. This massive manipulation of the host is achieved with the help of fungal-secreted molecules, so-called effectors. In a systematic approach we screened in the past decade hundreds of putative effector proteins to identify their specific place of action and their functions on the plant side. Here I will present our current molecular understanding of the fungal effectome and the biotrophic interaction between the fungus and its host plant maize. The main focus will be given to a group of effectors manipulating hormone signaling in plants, thereby explaining various central aspects of biotrophy.

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## Understanding the proteolytic processing of plant receptor like kinases

A. Acharya<sup>1</sup>, B. Ortel<sup>1</sup>, M. Schuster<sup>1</sup><sup>1</sup>IPB (Leibniz Institute of Plant Biochemistry), Receptor Biochemistry, Halle a. d. Saale, Germany

Cells have membrane-bound receptors capable of sensing signals from the surroundings. The regulation of the function of these receptors is therefore one of the fundamental processes to ensure survival. Receptor-like kinases (RLKs) are the main players of cell-to-cell communication in plants and many of them constitute key immune receptors. Understanding function and regulation of these receptors therefore promises to reveal new opportunities for plant breeding and crop protection.

Proteolytic cleavage, especially receptor ectodomain shedding (shedding) is a regulatory mechanism of membrane-bound receptors and it refers to the proteolytic release of the extracellular portion of a membrane-anchored protein. Shedding is therefore an immediate mechanism to i) generate local and systemic extracellular signals, ii) to shut down trigger perception and iii) to modulate cell-cell and cell-matrix interactions. [1]. Shedding is best understood in mammals, with hundreds of shedding events reported [2]. In plants, by contrast, only few ectodomain shedding events have been reported. Despite the essential functions of the processing of membrane proteins in animals, the biological significance of receptor processing in plants, and the fact that plant cells harbour many more receptors than their animal counterparts, receptor processing has yet to be studied systematically in plants. We have reported that RLK processing is widespread in *N. benthamiana* [3] and are currently investigating both, the identity of the proteases that cleave these receptors as well as the physiological consequences of receptor cleavage.

## References

1. Lichtenthaler SF, Lemberg MK, Fluhrer R: Proteolytic ectodomain shedding of membrane proteins in mammals—hardware, concepts, and recent developments. *The EMBO Journal* 2018, 37:e99456.
2. Tien W-S, Chen J-H, Wu K-P: SheddomeDB: the ectodomain shedding database for membrane-bound shed markers. *BMC bioinformatics* 2017, 18:31-48.
3. Zheng K, Lyu JC, Thomas EL, Schuster M, Sanguankiatichai N, Ninck S, Kaschani F, Kaiser M, Hoorn RALvd: The proteome of agroinfiltrated *Nicotiana benthamiana* is shaped by extensive protein processing. *bioRxiv* 2024:2023.2011.2002.565301.

P 252

Degradation of plant cell wall components by type II-secreted enzymes – how phytopathogenic bacteria turn the plant apoplast into a habitat

S. Goll<sup>1</sup>, J. Erickson<sup>2</sup>, D. Büttner<sup>1</sup>

<sup>1</sup>Martin-Luther-University Halle-Wittenberg, Genetics, Halle a. d. Saale, Germany

<sup>2</sup>Leibniz-Institut of Plant Biochemistry, Biochemistry of Plant Interactions, Halle a. d. Saale, Germany

The plant apoplast is actively manipulated by colonizing pathogens to increase nutrient availability and avoid detection by the host. Many bacteria achieve this by secreting degradative enzymes via type II secretion (T2S) systems. T2S systems are conserved virulence factors of major crop pathogens including *Xylella* and *Xanthomonas* species and mediate protein secretion from the bacterial periplasm into the extracellular milieu. We previously reported that the Xps-T2S system from the tomato and pepper pathogen *Xanthomonas euvesicatoria* contributes to bacterial pathogenicity by secreting xylanases, a protease and a lipase. T2S substrates likely manipulate plant cell wall components to promote bacterial survival and proliferation however, their precise functions and plant targets are largely unknown. Here, we show that the T2S system from *X. euvesicatoria* and related pathogens is required for bacterial *in vitro* growth in minimal medium containing plant cell wall extracts or highly abundant cell wall-derived carbohydrates such as cellulose or the hemicellulose xylan as sole carbon source. The T2S system is also required for extracellular protease activity. Together, this suggests that T2S substrates degrade plant cell wall and proteins during the infection process. In agreement with these observations, mass spectrometry analyses of apoplastic fluid from infected tomato leaves identified xylanases, polygalacturonases, cellulases and numerous proteases as T2S substrates from *X. euvesicatoria*. Additional *in vitro* assays confirmed type II-dependent secretion and enzyme activity of all identified substrates. Our studies provide insight into potential roles of T2S substrates from *Xanthomonas* species in nutrient acquisition and bacterial colonization of the plant apoplast.

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Allelopathic interactions of *Parthenium hysterophorus*: Implications for the use of *Parthenium hysterophorus* as soil amendment to combat *Macrophomina phaseolina* causing charcoal rot in maize

M. Akbar<sup>1</sup>, N. Aslam<sup>1</sup>, T. Khalil<sup>1</sup>

<sup>1</sup>University of Gujrat, Department of Botany, Gujrat, Pakistan

Allelopathy plays a pivotal role in agriculture. Allelochemicals from numerous plants have been reported to exert their effects in combating the fungal pathogens. Moreover, these allelochemicals have also been reported to affect the growth of recipient plants. Allelopathic activity of *Parthenium hysterophorus* have been well recognized as having effects on fungal pathogens like *Macrophomina phaseolina* as well as on plants but there are no reports that describe the allelopathic effects of *P. hysterophorus* about *in vivo* control efficacy against *M. phaseolina* as well as its effects on the recipient plant. Herein, we reported that *P. hysterophorus* has allelopathic effects on the pathogen as well as on the crop. Treatments were included to estimate the effects of *P. hysterophorus* amendment into the soil on the growth, biochemical and physiological functions with or without pathogen at 3 concentrations. *P. hysterophorus* amended soil @ 0.5% (w/w) reduced the disease incidence, disease severity index, area under disease incidence progress curve and area under disease incidence progress curve to 40, 23, 38 and 35%, respectively over infested control. Moreover, this concentration increased shoot length, shoot dry mass, root dry mass, chlorophyll a, b and carotenoids by 15.9, 18.6, 29.4, 7.5, 19.3, and 10.1%, over infested control. This concentration also exerted minimum inhibitory effects on the defense related antioxidant enzymes (super oxide dismutase, peroxidase, and catalase), and physiological [Carbon assimilation rate (*A*), stomatal conductance (*g<sub>s</sub>*), transpiration (*E*), and internal carbon dioxide concentration (*C<sub>i</sub>*)] functions of maize. Although, the higher concentrations (1 and 1.5%) of *P. hysterophorus* were more inhibitory on the pathogen, but these concentrations also caused significantly more deleterious effects on the maize plants. These results suggest to test the donor plants for having allelopathic effects on the recipient plants before testing the donor plants meant to combat the pathogens in the field crops.

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**A new level of RNA-based plant protection - dsRNAs designed from functionally characterized siRNAs highly effective against variable pathogens**

M. Knoblich<sup>1</sup>, T. Gursinsky<sup>1</sup>, S. Gago-Zachert<sup>1</sup>, C. Weinholdt<sup>2</sup>, J. Grau<sup>2</sup>, S. E. Behrens<sup>1</sup>

<sup>1</sup>Institute of Biochemistry and Biotechnology, Martin Luther University Halle-Wittenberg, NFI, Halle a. d. Saale, Germany

<sup>2</sup>Institute of Computer Science, Martin Luther University Halle-Wittenberg, NFIII, Halle a. d. Saale, Germany

RNA-mediated crop protection increasingly becomes a viable alternative to agrochemicals that threaten biodiversity and human health. Pathogen-derived double-stranded dsRNAs are processed into small interfering RNAs (siRNAs), which can then induce silencing of target RNAs, e.g. viral genomes. However, with currently used dsRNAs, which largely consist of undefined regions of the target RNAs, silencing is often ineffective: processing generates siRNA pools that contain only a few functionally effective siRNAs (here called “esiRNAs”). Using a recently developed *in vitro* screen that reliably identifies esiRNAs from siRNA pools<sup>(1-4)</sup>, we identified esiRNAs against Cucumber Mosaic Virus (CMV), a devastating plant pathogen. Topical application of esiRNAs to plants resulted in highly effective protection against massive CMV infection. However, optimal protection was achieved with newly designed multivalent “effective dsRNAs” (edsRNAs), which contain the sequences of several esiRNAs and are preferentially processed into precisely these esiRNAs. The esiRNA components can attack one or more target RNAs at different sites, be active in different silencing complexes and provide cross-protection against different viral variants, important properties for combating rapidly mutating pathogens such as CMV. esiRNAs and edsRNAs have thus been established as a new class of “RNA actives” that significantly increase the efficacy and specificity of RNA-mediated plant protection<sup>5</sup>.

References

<sup>1</sup>Schuck,J., Gursinsky,T., Pantaleo,V., Burgyán,J. and Behrens,S.-E. (2013) AGO/RISC-mediated antiviral RNA silencing in a plant *in vitro* system. *Nucleic Acids Res.*, **41**, 5090–5103.

<sup>2</sup>Gago-Zachert,S., Schuck,J., Weinholdt,C., Knoblich,M., Pantaleo,V., Grosse,I., Gursinsky,T. and Behrens,S.-E. (2019) Highly efficacious antiviral protection of plants by small interfering RNAs identified *in vitro*. *Nucleic Acids Res.*, **47**, 9343–9357.

<sup>3</sup><https://www.sciencemag.org/news/2019/08/new-medicine-could-vaccinate-plants-against-devastating-viruses>

<sup>4</sup>Gruber,C., Gursinsky,T., Gago-Zachert,S., Pantaleo,V. and Behrens,S.-E. (2023) Effective antiviral application of antisense in plants by exploiting accessible sites in the target RNA. *Int. J. Mol. Sci.*, **24**, 17153.

<sup>5</sup>bioRxiv preprint doi: <https://doi.org/10.1101/2024.06.03.597145>

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**Enhancing viticulture resilience against trunk diseases by harnessing Interkingdom crosstalks among grapevine, pathogenic endophytic fungi, and soil prokaryotes**

I. Khattab<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Institute for biological Interfaces 5 & Joseph Gottlieb Kölreuter Institut für Pflanzenwissenschaften, Eggenstein-Leopoldshafen, Germany

With the ongoing climate change, grapevine trunk diseases (GTDs) have become a threatening challenge for viticulture worldwide. The disease outbreak occurs under dry, hot summers and is often controlled by chemical communication between the host and pathogenic endophytes. Under severe abiotic stress, plants accumulate more lignin precursor, ferulic acid, which is perceived by the fungus *Neofusicocum parvum* as an alert signal, so-called “plant surrender signal”, that the host might die from other environmental stresses. The fungus herein switches to necrotrophic behavior to kill the host, release spores outside damaged trunks, and infect new plants (Khattab et al., 2022). In the absence of ferulic acid, the fungus could act as latent endophyte secreting 4-hydroxyphenylacetic acid (4-HPA), which mimics auxin signaling and inhibits phytoalexins biosynthesis, e.g. piceatannol (Flubacher et al., 2023).

However, genotypes from wild grapes population could control the infection progress by allocating phenylpropanoid pathway towards bioactive stilbenes, e.g., viniferins, rather than lignin (Khattab et al., 2021). Integrating resistant genotypes in resistance breeding is a very long-term approach. As an immediate strategy, soil microbiome has been investigated using shotgun metagenome sequencing to sort out candidate taxa promoting viticulture resilience against GTDs. In this context, the disease outbreak in 10 vineyards across Southwest Germany was linked to shifts in rhizosphere microbiota, especially in beneficial taxa of Arbuscular mycorrhiza (Unpublished data). Additionally, enriching the soil with compost and biochar significantly promoted plant resilience, and shifted rhizobiome composition and function. As an upstream for grapevine defense, the microbiome of soil mixed with compost and biochar showed a higher abundance of chorismate pathway gene families due to the high enrichment of some prokaryotic taxa, e.g., *Methanosarcina* from Archaea and *g\_Chryseolinea* from bacteria (unpublished). Many prokaryotic taxa also showed potential for degrading 4-HPA, the fungal signal suppressing plant defense for successful infection. Harnessing interkingdom crosstalks among such taxa with grapevine could be a sustainable therapy for resilient viticulture against GTDs.

References

Flubacher et al. (PCE, 2023) <https://doi.org/10.1111/pce.14670>

Khattab et al. (PCE, 2022) <https://doi.org/10.1111/pce.14468>

Khattab et al. (New Phytologist, 2021) <https://doi.org/10.1111/nph.16919>



P 256

Cell wall integrity and elicitor peptide signaling modulate phytoalexin-mediated pathogen defense in Arabidopsis

R. N. Morton<sup>1</sup>, A. Rajendran<sup>1</sup>, L. Fleischberger<sup>1</sup>, T. Engelsdorf<sup>1</sup>

<sup>1</sup>Philipps-Universität Marburg, Marburg, Germany

The plant cell wall provides mechanical support to plant cells and plays an important role in responses to abiotic and biotic stress. This is achieved by innate mechanisms which monitor cell wall integrity (CWI) and trigger compensatory responses should this integrity be impaired. Available knowledge suggests that several plasma membrane-localized proteins are associated with CWI surveillance, of which the receptor kinase THESEUS1 (THE1) has been identified as a key CWI monitor in Arabidopsis (1,2).

We investigated defense-related responses initiated by THE1-dependent CWI signaling and found an accumulation of the phytoalexin camalexin upon CWI impairment caused by cellulose biosynthesis inhibition (CBI). Camalexin contributes to resistance against fungal pathogens and its biosynthesis is induced by the transcription factor WRKY33, MAP kinases and the phytohormones ethylene and jasmonic acid (JA)(3). We show that upon CBI treatment, both THE1 and WRKY33 are required for camalexin accumulation, while THE1 is not required for WRKY33 expression. RNAseq analysis indicated upregulation of several genes involved in JA biosynthesis and signaling. Most of these genes were suppressed after co-treatment with the plant elicitor peptide Pep3, which is consistent with Pep3-dependent suppression of CBI-induced JA accumulation. Furthermore, camalexin accumulation depended on intact JA biosynthesis and Pep3-induced suppression was lifted after exogenous JA treatment, indicating a central role for JA in regulating camalexin accumulation after CWI impairment. Similar to Pep3, pathogen-derived elicitors such as flg22 and chitohehexose were able to suppress CBI-induced camalexin accumulation, indicating the presence of a general regulatory mechanism balancing CWI with pattern-triggered immunity. In agreement with a role of CWI surveillance in pathogen defense, THE1 loss-of-function mutants showed increased susceptibility to the cell wall-penetrating fungal pathogen *Colletotrichum higginsianum*, while accumulation of camalexin and of the defense-related phytohormone salicylic acid was reduced.

References

- 1 Engelsdorf, T. *et al. Science Signaling* 11 (2018).
- 2 Bacete, L. *et al. PNAS* 119, e2119258119 (2022).
- 3 Zhou, J. *et al. The Plant Cell* 34, 3066-3087 (2022).

P 257

Beneficial interaction between plant secondary indole metabolites and soil-microbiome

S. Jeong<sup>1</sup>, V. Schütz<sup>2</sup>, M. Schulz<sup>1</sup>, P. Dörmann<sup>1</sup>

<sup>1</sup>University of Bonn, Institute of Molecular Physiology and Biotechnology of Plants (IMBIO), Bonn, Germany

<sup>2</sup>Seoul National University, Research Center of Plant Plasticity, Seoul, South Korea

Shaping of the soil microbiome by plant secondary metabolites covers an important domain in plant-microbe interactions. The addition of the two indole metabolites 2-benzoxazolinone (BOA) and gramine revealed metabolite-dependent shifts in the bacterial composition [1]. Bacterial strains were isolated from the treated soil to study the impact and degradation pathways of secondary metabolites. Several bacterial strains capable of degrading either BOA and gramine and their degradation products were identified by liquid chromatography-mass spectrometry. The functions of the degradation products for plant growth and physiology are investigated in following studies. One *Arthrobacter* strain was able to survive from the exposure to BOA-derived 2-acetoaminophenol (AAP), and it could nitrate AAP in subsequent reactions. Exposure to nitro-AAP derivatives resulted in the up-regulation of terpene synthase (*TPS04*) expression in *A. thaliana* and increased the production of geranylinalool that inhibits sphingolipid metabolism in herbivores and plants. Another *Arthrobacter sp.* was able to degrade gramine via indole-3-carboxaldehyde (I3A) and indole-3-carboxylic acid (I3C). Proteomic analysis revealed that peroxidases in *Arthrobacter sp.* are involved in early-stage gramine degradation. I3A and I3C are related to auxins and provide plant growth-promoting properties. I3A application resulted in an increased fresh weight and root development of *A. thaliana*. Expression of auxin-related genes was upregulated as shown by transcriptomic analysis and real-time qPCR. Taken together, our data highlight the beneficial effects of plant secondary indole metabolites and their interaction with bacterial strains in soil.

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**Bacteriophage based plant biocontrol strategies, understanding the mode of 'phage to seed' binding and the role of the seed-coat mucilage**

S. Erdrich<sup>1,2,3</sup>, U. Schurr<sup>1</sup>, J. Frunzke<sup>2,3</sup>, B. Arsova<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, IBG-2 – Plant Sciences, Root Dynamics Group, Jülich, Germany

<sup>2</sup>Heinrich-Heine University, Düsseldorf, Germany

<sup>3</sup>Forschungszentrum Jülich, IBG 1- Biotechnology, Jülich, Germany

Plant pathogenic bacteria are estimated to account for more than 10% of annual yield losses and are gaining resistance against classical control strategies. Bacteriophages - viruses of bacteria may point the way to sustainable biocontrol.

We isolated 9 novel phages against *Agrobacterium tumefaciens* (1), *Pseudomonas syringae* pv. *tomato* (1), *Ps* pv. *lapsa* (1), *Xanthomonas campestris* (1) and *X. translucens* (6). All viruses were morphologically (TEM) and genetically characterized. Activity was proven by testing ability of the phages to suppress bacterial growth at various multiplicity of infection. The 7 *Xanthomonas* phages were specific to their isolation hosts and did not infect closely related beneficial bacteria (Erdrich *et al.*, 2022 *Viruses*).

As many bacterial pathogens are soil or seed transmitted, we tested phage binding on seeds, particularly the interaction with the seed-coat mucilage (SCM). Comparing the phage binding on Arabidopsis seeds with and without SCM we found that 2 of 3 tested phages were dependent on the SCM for binding. Podophage Athelas, against *P.s.*, showed the highest dependency on SCM. This characteristic of podoviruses was confirmed among the *Autographiviridae* of the systematic *E. coli* (BASEL) phage collection. To understand the phage-SCM binding, we checked the physical matrix characteristic of the extracted SCM using TEM. More interestingly we used Arabidopsis SCM mutants and identified the diffusible cellulose as important component for phage binding. Stability tests showed that phages can be coated onto seeds and will remain active up to 28 days - relevant for further application (Erdrich *et al.*, 2024 *Microb Biotechnol*).

Finally, focusing on the early stages of infection, qPCR on Arabidopsis seedlings found upregulation of salicylic acid signaling pathways in the presence of the pathogen but downregulation in the combination of pathogen and phage. RNASeq on days 2, 5, and 7, post infection, using high sequence coverage, deepened the understanding of the plant molecular response. At the same time, we could also find differential expression of bacterial genes in the different treatment combinations. The phage expression is rather asynchronous due to their short generation lifetime – but we can also confirm presence of phage transcripts throughout the time series (*unpublished*). In conclusion, we show that natural phage diversity can be harnessed for phage-based plant biocontrol and elucidate the mechanisms behind the interaction

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**Characterization of Plant Growth Promoting Abilities of *Pseudomonas argentinensis* SA190 Mutants Under Drought Stress**

B. Elkatmis<sup>1</sup>, M. Saad<sup>2</sup>, S. Kopriva<sup>1</sup>, H. Hirt<sup>2</sup>

<sup>1</sup>University of Cologne, Institute for Plant Sciences, Köln, Germany

<sup>2</sup>King Abdullah University of Science and Technology, The Center for Desert Agriculture, Thuwal, Saudi Arabia

Drought is one of the most obvious consequences of climate change and significantly affects the biomass of crops. Finding a sustainable approach to mitigating the consequences of drought stress will help increase crop yields in agriculture. Plant-growth-promoting bacteria are a sustainable option in agriculture to enhance crop yields, particularly in arid and semi-arid regions. *Pseudomonas argentinensis* SA190, discovered in Saudi Arabia, has been shown to improve plant performance under drought stress. However, more information is needed about the genes and mechanisms underlying the plant-growth-promoting effects of SA190. Transposon mutagenesis, an efficient method for studying gene functions, was used to identify genes that promote plant growth in SA190. The optimized random amplification of transposon ends (RATE-PCR) was performed to pinpoint the disrupted genes in the mutants. The identified mutants associated with the carotenoid pathway, swarming motility, and biofilm formation were tested in a plant assay to characterize their growth-promoting effect under drought stress. Preliminary data revealed that the mutations in the carotenoid pathway and the biofilm formation led to a decrease in the plant growth-promoting effect of SA190 on biomass. Notably, weak biofilm-producing mutants affected *Arabidopsis* root architecture differently than SA190. This study suggests that *CrtI* and *GGPS* genes in the carotenoid pathway and the GNAT family N-acetyltransferase, terminase ATPase and, *RpoE* genes from the biofilm formation may have a significant role in establishing a beneficial interaction between SA190 and *Arabidopsis* under drought stress.

P 260

The emerging role of TARGET OF RAPAMYCIN in the regulation of the growth-defense tradeoff in plants

S. Courbier<sup>1,2</sup>, A. Hiltbrunner<sup>1,2</sup>

<sup>1</sup>Albert-Ludwigs-Universität Freiburg, Faculty of Biology 2, Freiburg i. Br., Germany

<sup>2</sup>Centre for Integrative Biological Signalling Studies (CIBSS), Freiburg i. Br., Germany

Plants have developed advanced strategies to react and adapt to environmental changes, including competition with other organisms, even within their own species. Neighbor proximity in plants is perceived via a reduction of the red to far-red ratio (R:FR) in the light environment. As a result of low R:FR perception by phytochromes photoreceptors, plants trigger a set of molecular and physiological responses referred to as the “shade avoidance syndrome”; a process meant to outgrow neighboring vegetation. In addition to exhibiting strong growth responses, plants experiencing low R:FR display lower resistance capacities towards pathogens. However, the exact molecular components, triggered by low R:FR, leading to the onset of shade avoidance and associated with the reduced defense capacities remain to be found. Based on previous RNA-sequencing data, we identified TARGET OF RAPAMYCIN (TOR) as a potential regulator of the growth-defense tradeoff in plants. By coupling biochemistry, omics and physiology, we are currently investigating if and how low R:FR perception regulates TOR activity and signaling thereby promoting growth at the expense of defense in *Arabidopsis*. So far, we show that TOR activity is quickly increased in response to low R:FR and that TOR inhibition by AZD8055 leads to a dampening of the shade-mediated growth responses. Given the conserved nature of the TOR pathway throughout green lineages, this work ultimately aims to unravel TOR-(in)dependent molecular pathways regulating the growth-defense tradeoff in plants, which could help develop more climate and pest resilient crops in the future.

P 261

Did barley-associated fungi evolve core effectors with antimicrobial activity to target barley keystone microbes?

M. Bauer<sup>1</sup>, F. Mesny<sup>1</sup>, B. Thomma<sup>1</sup>

<sup>1</sup>University of Cologne, Plant Sciences, Köln, Germany

Plants associate with various microbes which form their microbiota. The microbiota is important for plant growth and fitness but also protects the plant from disease. For successful infection, pathogenic microbes need to overcome the plant immune system as well as the plant-associated microbiota. Pathogens secrete effectors in order to manipulate host physiology, including immunity[MOU1]. For several fungi it has been described that they additionally secrete effectors with selective antimicrobial activities, manipulating the host microbiota. Some of these effectors are reported to be important for host colonization and contribute to pathogen virulence. Based on a newly developed tool to predict antimicrobial effectors, we show that antimicrobial effectors occur across the fungal kingdom in fungi with diverse lifestyles. This suggests that they are ancient proteins which evolved to support fungal niche establishment. We hypothesize that plant-associated fungi with diverse lifestyles evolved to exploit effector proteins with antimicrobial activity to foster host colonization by antagonizing resident microbial competitors. Assuming that fungi that associate with the same host plant encounter similar microbiota, it can be hypothesized that diverse fungi that colonize the same host plant evolved shared effectors with antimicrobial activity to target keystone microbiota members of that host and facilitate tissue colonization. To address this hypothesis, we study a wide variety of fungi with different lifestyles that associate with the same host; the monocot model plant barley (*Hordeum vulgare*). First, antimicrobial effectors conserved in diverse barley-associated fungi are predicted. Next, effector candidates will be functionally validated and their contribution to barley colonization will be analysed. Additionally, possible targets of antimicrobial effectors will be identified by studying microbiota changes upon infection. Thus, we aim to enhance our understanding of the role of antimicrobial effectors in niche establishment.

P 262

## Root-derived N-hydroxypipelic acid manipulates shoot defense

P. Xu<sup>1</sup>, S. Fundneider<sup>1</sup>, B. Lange<sup>1</sup>, A. Schäffner<sup>1</sup><sup>1</sup>Helmholtz Zentrum München, Molecular Plant Physiology, Institute of Biochemical Plant Pathology, Neuherberg, Germany

Upon pathogen attack, *Arabidopsis* flavin-dependent monooxygenase1 (FMO1) is induced at the site of infection, catalyzing the final step of the biosynthesis of N-hydroxypipelic acid (NHP). NHP moves from the infected leaf to systemic leaves, promoting the biosynthesis of salicylic acid (SA) and establishing broad-spectrum immunity to pathogen infection, a phenomenon known as systemic acquired resistance (SAR). As a negative feedback mechanism, the glucosyltransferase UGT76B1 is induced during this scenario; it conjugates and inactivates both NHP and SA into NHP-O-glucoside and SA-O-glucoside, respectively.

Interestingly, both FMO1 and UGT76B1 are rarely expressed in the shoot but are constitutively present in the root of naive plants, suggesting constant biosynthesis, yet potential, parallel inactivation of NHP in the root. Consistent with this expression pattern, both free NHP and its glucoside were detected in the root of naive plants, whereas they were undetectable in the shoot. Grafting experiments combining *fmo1* shoots with *ugt76b1* roots and *vice versa* demonstrate that NHP is bidirectionally mobile between root and shoot, whereas NHP-O-glucoside is immobile. Further reciprocal root-shoot grafting experiments revealed that the loss of UGT76B1 in the root enhances wild-type shoot defense, an effect not observed in the converse combination. This finding was further corroborated by CRISPR/Cas9-mediated, tissue-specific knockout of *UGT76B1* in root cortex and endodermis cells. To identify which UGT76B1-controlled root signal promotes shoot defense, wild-type shoots were grafted onto genetically SA- or NHP-depleted *ugt76b1* rootstocks. The expression of SA marker genes was enhanced, and increased resistance against *Pseudomonas syringae* DC3000 was observed in wild-type rosettes when UGT76B1 was absent in the root, irrespective of the presence of SA in the root. However, these phenotypes are abolished when root NHP biosynthesis is lacking.

Thus, NHP is constitutively synthesized in the root by FMO1 and able to move to the shoot to manipulate its defense state. The co-expressed UGT76B1 will suppress this effect. This mechanism broadens the roles of NHP beyond being a *de novo* signal compound of SAR. Here, it mediates root-shoot communication with respect to the shoot defense status. The NHP in/activation may serve as a sensor for the root environment and soil-borne microbes (see Xu et al., same conference topic).

P 263

Establishing a molecular toolkit for the ectomycorrhizal symbiosis between *Paxillus involutus* and poplarV. Kreszies<sup>1</sup>, H. Reichelt<sup>1</sup>, S. Tasnim<sup>1</sup>, H. Wege<sup>1</sup>, I. Teichert<sup>1</sup><sup>1</sup>University of Goettingen, Forest Botany and Tree Physiology, Göttingen, Germany

Mycorrhizal symbiosis is a mutualistic interaction between plants and fungi. Most trees in temperate forests show colonization of fine roots by ectomycorrhizal fungi (ECM). The trees benefit from this symbiosis by easier access to water and mineral nutrients, and the fungi acquire carbohydrates from the trees. Many studies have focused on ectomycorrhizal interaction, and for example transcriptomic analysis has identified a huge number of genes as differentially regulated during the onset and / or maintenance of symbiosis. Yet, molecular analysis of mycorrhizal interaction partners in laboratory model systems as well as functional analysis of genes remains scarce.

To establish such system, we work on the interaction of the basidiomycete *Paxillus involutus* with the genetically tractable poplar tree species. Besides establishing conditions that promote robust growth and interaction capacity of the fungal partner, we study the mycorrhization of poplar roots with different *P. involutus* strains in different physiological environments. Further, we aim to establish a transformation method for *Paxillus involutus*, working on both, protoplast-mediated and *Agrobacterium*-mediated transformation. We will show results on the applicability of selection markers, including different antibiotics, and different basidiomycete promoters to express the resistance genes. Our methodology will enable us to analyze the basis for initiation and maintenance of the ECM symbiosis on a molecular level.

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N-hydroxyphenylacetic acid mediates root-shoot communication in response to root microbes

P. Xu<sup>1</sup>, S. Fundneider<sup>1</sup>, B. Lange<sup>1</sup>, A. Schäffner<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München, Molecular Plant Physiology, Institute of Biochemical Plant Pathology, Neuherberg, Germany

Pathogen attack induces the biosynthesis of N-hydroxyphenylacetic acid (NHP) via FLAVIN-DEPENDENT MONOOXYGENASE1 (FMO1) at the infection site of *Arabidopsis* leaves. NHP then migrates from the infection site to systemic tissues, triggering salicylic acid (SA) production and establishing systemic acquired resistance (SAR). As a negative mechanism, the glucosyltransferase UGT76B1 is induced to attenuate the immune response by glucosylating and inactivating both NHP and SA. However, in contrast to leaves, FMO1 and UGT76B1 are constitutively expressed in the roots of naïve plants. Thus, this counteracting pair of NHP biosynthesis and inactivation controls the root biosynthesis and translocation of unconjugated NHP to the shoot where it would activate SA-dependent pathogen defense (see Xu *et al.*, Abstract in same conference topic). We wondered whether this system is involved in root-shoot communication in response to soil-borne microorganisms. We inoculated roots of mTFP-labeled UGT76B1 and YFP-labeled FMO1 transgenic lines with a variety of microbes thereby assessing UGT76B1 and FMO1 at the protein level. Notably, UGT76B1 was rapidly degraded upon interaction with endophytic fungi, including several *Trichoderma* species, as well as with biotrophic pathogens like *Fusarium* species, and necrotrophs such as *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Conversely, FMO1 expression moderately increased in response to endophytic fungi but was strongly induced by many biotrophic pathogens. These varying expression patterns may result in different quantities of free and translocation-competent NHP. Therefore, we investigated the dosage effect of NHP and found that low levels of NHP promote growth, whereas high levels suppress it. Correspondingly, inoculation with endophytic fungi promoted plant growth, whereas most of biotrophic pathogens suppressed it. Different root microbial inoculations also altered shoot defense against *Pseudomonas syringae* DC3000. Furthermore, both effects on growth and defense were abolished or mitigated when FMO1 was absent. Thus, the root-shoot communication in response to soil microbes is dependent in these cases on NHP that is provided by the differential and spatially distinct expression of FMO1 and UGT76B1 in response to various root microbes. Unlike regular SAR, where signaling compounds like NHP are synthesized *de novo*, this turnover system allows for rapid and differential responses to the soil environment.

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The role of the APETALA2 transcription factor ERIK in regulating arbuscular mycorrhiza in *Lotus japonicus*

H. Zhang<sup>1,2</sup>, T. Zeng<sup>1,2</sup>, J. Pan<sup>1,2</sup>, A. Khayer<sup>1</sup>, K. Varshney<sup>1,2</sup>, R. Hüttl<sup>2</sup>, C. Gutjahr<sup>1,2</sup>

<sup>1</sup>Max Planck Institute of Molecular Plant Physiology, Root Biology and Symbiosis, Potsdam, Germany

<sup>2</sup>Technical University of Munich (TUM), TUM School of Life Sciences, Freising, Germany

80% of land plants form arbuscular mycorrhiza (AM), a mutualistic association between plant roots and Glomeromycota fungi, which benefits both partners by facilitating nutrient exchange. Karrikins, a group of butenolide compounds found in smoke, are perceived by the  $\alpha/\beta$ -hydrolase KARRIKIN INSENSITIVE2 (KAI2), operate through the F-box protein MORE AXILLARY GROWTH (MAX2) which targets Suppressor of MAX2 1 (SMAX1) for ubiquitination and subsequent proteasome degradation. The karrikin signaling pathway influences various aspects of plant growth and development. Although the influences of karrikins on AM symbiosis are recognized by karrikin defective mutant phenotype, the downstream regulatory mechanisms remain to be elucidated.

Here, we identify a transcription factor named *ERIK*, which shows significantly increased expression in the *smax1* mutant and is induced by exogenous karrikin treatment in the wild type but not in the *kai2* mutant. *ERIK* belongs to the *APETALA 2* (*AP2*) gene family and it is phylogenetically conserved in AM host species. It exhibits basal expression in non-colonized roots and is strongly induced during AM symbiosis. The transcriptional response of *ERIK* to AM is dependent on vital common symbiosis signaling pathway genes *CCaMK* and *CYCLOPS*, but its response to the karrikin pathway is independent of them. Loss-of-function *erik* mutants exhibit significantly reduced root length colonization and decreased expression of genes crucial for arbuscular development, highlighting *ERIK*'s positive regulatory role in AM symbiosis. Transcriptome sequencing of non-colonized and colonized wild-type and *erik* mutant roots, along with dexamethasone-induced ectopic *ERIK* expression in hairy roots, identified 185 potential downstream targets of *ERIK*. Among them, several genes are known to be related to AM symbiosis, including two *AP2* transcription factors *CBX1* and *WRI3*, which are involved in arbuscular development and lipid biosynthesis. Thus, we reveal an *AP2* transcription factor that regulates AM symbiosis potentially downstream of karrikin signaling.

P 267

**Inositol pyrophosphates - Master regulators of plant root endosymbioses**

K. Raj<sup>1</sup>, V. Gaugler<sup>2</sup>, M. Schädel<sup>1</sup>, H. J. Jessen<sup>3</sup>, G. Schaa<sup>2</sup>, M. K. Ried-Lasi<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry (IPB), Molecular Signal Processing, Halle a. d. Saale, Germany

<sup>2</sup>University of Bonn, Bonn, Germany

<sup>3</sup>Albert-Ludwigs-University of Freiburg, Freiburg i. Br., Germany

Tight regulation of nutrient homeostasis is vital for every cell. Plants have evolved elaborate systems to sense and signal extracellular and intracellular e.g. phosphate levels and to regulate cellular nutrient concentrations. Most land plants establish Arbuscular Mycorrhiza with phosphate-acquiring fungi, and selected members of the Fabales, Fagales, Cucurbitales and Rosales engage in root nodule symbiosis with diazotrophic bacteria. Plant phosphate starvation responses are regulated by inositol pyrophosphates and there is a direct link between phosphate and nitrogen homeostasis. Here, we show that mutations in genes potentially involved in the generation and breakdown of inositol pyrophosphates influence inositol phosphate pools in *Lotus japonicus* and affects the formation of Arbuscular Mycorrhiza as well as root nodule symbiosis. It is our goal to scrutinize the role of inositol pyrophosphate ligands and putative precursors during plant root endosymbioses and nutrient homeostasis in *Lotus japonicus* and thus to illuminate the interplay of these different plant strategies to overcome nutrient limitations.

P 268

**Effect of bacterial priming on different growth and yield parameters in wheat under field condition**

B. Soleimani<sup>1</sup>, J. Thielmann<sup>2</sup>, M. Wiegmann<sup>3</sup>, J. Schacht<sup>4</sup>, P. Schäfer<sup>2</sup>, A. Matros<sup>1</sup>, G. Wehner<sup>1</sup>

<sup>1</sup>Julius Kühn-Institute (JKI), Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany

<sup>2</sup>Justus Liebig University, Interdisciplinary Research Center for Biosystems, Land Use and Nutrition (iFZ),

Institute for Phytopathology, Gießen, Germany

<sup>3</sup>RAGT GmbH, Silstedt, Germany

<sup>4</sup>Limagrain GmbH, Rosenthal, Peine, Germany

The use of beneficial microorganisms to enhance plant defense responses and overall fortification could represent an alternative to the use of chemical fungicides and fertilizers. Interactions between plant roots and beneficial bacteria in the rhizosphere through quorum sensing (QS) such as N-acyl-homoserine lactones (AHLs) can lead to an induced systemic response (ISR) in plants against biotic stress factors such as pathogens. Several studies reported the impact of AHL on plant growth and defense in different species.

In the first phase of our study, a set of 175 winter wheat genotypes were screened for induced resistance against leaf rust (*Puccinia triticina*) by using *Ensifer meliloti* as a priming inducer under greenhouse conditions which resulted in the identification of 21 primable genotypes with increased resistance ( $p < 0.05$ ) and several quantitative trait loci (QTL). In total, 15 QTLs were identified for relative infection under control and primed conditions, as well as for priming efficiency on chromosomes 1A, 1B, 2A, 3A, 3B, 3D, 6A and 6B. Based on obtained results, twelve out of 21 identified genotypes (including Borenos and Tabasco as a susceptible and resistance genotype, respectively) and 18 genotypes (were provided by RAGT Saaten Deutschland GmbH and Limagrain) were selected for field experiments at three locations (Quedlinburg, Silstedt, and Peine). These 12 Genotypes were evaluated under three different treatments (coated with either *Bacillus amyloliquefaciens* (Starcover) or *Piriformospora indica* (*P. indica*) and uncoated (control)) in all three locations in 2022 and 2023.

Several traits such as BBCH stage, early growth, soil coverage, plant height, number of ears per plant, number of ears/1m<sup>2</sup>, thousand grain weight, number of grain per ear were measured and statistically evaluated. The genotypes showed different responses within the treatments between the locations. For instance, higher mean values were obtained for biomass under *P. indica* treatment in Peine compared to other locations. ANOVA was calculated for each trait across all treatments and showed significant effects for genotype (in all traits) and treatment (with the exception of biomass). Different treatment effect was observed between the locations in the first year of field evaluation. The same field evaluation will be repeated in all locations in 2023 and 2024 and phenotypic data will be collected and analyzed, too.

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Interacting proteins of master regulators of arbuscular mycorrhiza developmen

Y. Rizzi<sup>1,2</sup>, M. Abele<sup>3</sup>, C. Ludwig<sup>3</sup>, C. Gutjahr<sup>1,2</sup>

<sup>1</sup>Max-Planck-Institute of Molecular Plant Physiology, Department Root Biology and Symbiosis, Potsdam-Golm, Germany

<sup>2</sup>Plant Genetics, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

<sup>3</sup>Bavarian Center for Biomolecular Mass Spectrometry, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

Arbuscular mycorrhiza (AM) is an ancient and important plant symbiosis, established among about 80% of the land plant species and fungi of the subphylum *Glomeromycotina*. The fungal symbiont receives carbohydrates and lipids from the plant and, in turn, provides the plant with mineral nutrients that are collected by an extraradical hyphal network. The colonization by AM fungi culminates in the formation of highly branched structures called arbuscules, where the exchange of nutrient between both symbionts occurs. During the establishment of the symbiosis, the perception of fungal signals triggers nuclear calcium spiking in the plant cells, which is proposed to be decoded by a nuclear localized calcium and calmodulin dependent kinase (CCaMK). CCaMK interacts with and phosphorylates the transcription factor CYCLOPS. These two regulators as well as the GA-signalling repressor DELLA, are required for arbuscule development as they control the activation of transcriptional changes that are important for fungus accommodation, arbuscule formation, nutrient exchange and finally the collapse of the arbuscule. Using different techniques, it was shown that CCaMK, CYCLOPS and DELLA form a complex. Roots lacking any of these proteins show different levels of impairment in the colonization by AM fungi, but all are incapable of developing arbuscules. For this reason, we hypothesize that these master regulators might have specific as well as shared interacting partners that allow the fine-tuning of the establishment of AM symbiosis. In order to identify additional components of the complex CCaMK-CYCLOPS-DELLA as well as specific interactors of these three proteins we perform proximity labelling and enrichment of labelled proteins followed by mass spectrometry for each of these three proteins in presence or absence of AM fungi. Our study will help to reveal new molecular players involved in the AM symbiosis signalling.

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The role of microRNAs in shaping pathogenic vs symbiotic plant-fungal interactions

M. Pradhan<sup>1</sup>, N. Requena<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Molecular Phytopathology, Karlsruhe, Germany

Plants are constantly challenged by fungi that may be pathogenic or beneficial in nature. Therefore, plants need to mount appropriate responses that could discriminate between them. The interaction with fungi invoke a complex molecular machinery in the host that involve sophisticated signal exchange, and a large-scale reprogramming of gene expression. How do plants attain specificity in reprogramming of gene expression in terms of the nature of fungal infection remains poorly understood<sup>1,2</sup>. Using wild solanaceous plants, we have shown that microRNAs (miRNAs) play an important role in plant-fungal interaction in their natural habitats<sup>3,4</sup>. Using tomato as host, we here demonstrate that miRNAs help the host to discriminate between its friends (beneficial/symbiotic fungi) and foes (pathogenic fungi) by helping the plants in mounting highly specific gene expression responses to the two categories of invading fungi. For instance, infection of tomato roots by *Fusarium* reprograms the expression of 32 known miRNAs. But when beneficial fungus, *R. irregularis* colonizes the tomato roots, it entails a reprogramming of 52 known plant miRNAs. On the other hand, when the pathogenic and beneficial fungi co-colonize the roots, 46 miRNAs are differentially regulated. We further show that these miRNAs may act at multiple stages of signal transduction and response of host to beneficial and pathogenic fungi. Indeed, like miRNAs, the expression of several hundred genes of the host (tomato) transcriptome are also uniquely reprogrammed in the context of the nature of colonizing fungi – pathogenic or beneficial. miRNA-mediated regulation of pathways, such as auxin signalling, ethylene signalling and regulation of stress response, in manners specific to the nature of the fungi appear to be crucial during the response of host against friends vs foes. We believe that miRNA functions are equally important in native and agricultural settings, and hence they could be used as communication as well as regulatory tools in shaping plant-microbe interactions.

References:

1. Dodds NP, et al. (2024) *The Plant Cell*, 36, 5, 1465–1481.
2. Pradhan M & Requena N (2022) *Curr. Opin. Plant Biol.*, 69, 102259, ISSN 1369-5266.
3. Pradhan M, et al. (2020) *Plant Physiol.*, 184, 1128-1152.
4. Pradhan M, et al. (2023) *New Phytol.*, 240: 382-398.

P 271

**Interactive Effects of Maize Roots and Rhizosphere Microorganisms Under Drought**

H. Würsig<sup>1</sup>, R. Hartwig<sup>2</sup>, M. Santangeli<sup>3</sup>, D. Vetterlein<sup>1,4</sup>, E. Oburger<sup>3</sup>, M. Wimmer<sup>2</sup>, M. T. Tarkka<sup>1,5</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research, Halle a. d. Saale, Germany

<sup>2</sup>University of Hohenheim, Stuttgart, Germany

<sup>3</sup>Boku University, Vienna, Austria

<sup>4</sup>Martin-Luther-University Halle-Wittenberg, Halle a. d. Saale, Germany

<sup>5</sup>German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

Improved understanding of plant-microbe interactions is mandatory for tackling the problems in crop production that are caused by increased frequency and intensity of drought events and heat waves. Due to drought triggered alterations in root growth as well as the quantity and composition of root exudates, plants are able to shape the rhizosphere microbiome community composition, which in turn can play out in the plant's drought tolerance. To better understand drought affected plant-microbe interactions, we joined a column experiment as part of the Priority Program 2089 "Rhizosphere Spatiotemporal Organization", investigating wild-type (WT) and the *roothairless 3* mutant maize plants after 7 days without watering. Maize root gene expression, root stress enzyme activities and rhizosphere community composition of ACC deaminase carrying (*acdS*+) bacteria, which can improve crop growth and productivity under drought were analyzed. We expected that drought treatment lead to changes in gene expression levels related to drought and heat stress, plant immunity, root exudation, cell wall crosslinking and high affinity nutrient transporters. We furthermore expected higher stress enzyme activities in roots, as well as higher relative abundances of *acdS*+ Actinobacteria under drought. Our results show that watering level led to differentially expressed *heat shock proteins*, *aquaporins*, *pathogenesis related proteins*, *secondary metabolite related genes*, *expansins*, as well as genes related to mineral element uptake. Increased levels of malondialdehyde and superoxide dismutase activity were found under drought, confirming a stress response. Surprisingly, these results were accompanied by not only an impact of watering level, but also an impact of genotype on *acdS*+ community composition. In addition to increased levels of *acdS*+ Actinobacteria under drought, we also detected different relative abundances on genera level between watering levels and genotypes. Our results show how maize roots and rhizosphere microorganisms react to drought and highlight interactive effects between the presence of maize root hairs and water deficit conditioning.

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**Treated wastewater irrigation effect on *Olea europaea*: is mycorrhization a game changer?**

A. Ben Hassena<sup>1,2</sup>, L. Trabelsi<sup>2</sup>, M. Zouari<sup>2</sup>, N. Zouari<sup>3</sup>, P. Labrousse<sup>1</sup>

<sup>1</sup>E2Lim UR 24133, University of Limoges, Limoges, France

<sup>2</sup>Olive Tree Institute, University of Sfax, Sfax, Tunisia

<sup>3</sup>Higher Institute of Applied Biology of Medenine, University of Gabes, Medenine, Tunisia

Tunisia is considered as one of the Mediterranean countries the most exposed to climate change. Therefore, treated wastewater (TWW) reuse for agricultural activities, such as olive growing, appears as a promising management strategy of water scarcity. Although TWW is rich in nutrients, it may contain high amounts of salts (Na and Cl) which have deleterious effects on plant growth and development. To cope with the adverse effect of salinity, plant inoculation with arbuscular mycorrhizal fungi (AMF), could be an effective solution.

The aim of this study is to evaluate the effect of inoculation with different AMF inoculums (*Glomus deserticola*, *Gigaspora margarita* or both fungi in combination) on potted *Olea europaea* L. biomarkers after one year of saline TWW irrigation (EC=5.84 dS m<sup>-1</sup>). Two questions arise: Did the response of biomarkers of inoculated olive plants improved and did the mycorrhization constituted a game changer under saline conditions? Our results indicated that TWW irrigation decreased leaf intercellular carbon dioxide (CO<sub>2</sub>) concentration and net photosynthesis, in comparison with control treatment (tap water). These physiological alterations were observed in combination with morphological and anatomical modifications in olive plants, particularly at the leaf level. These adaptations well-known to be induced in plants under stress to cope with these harmful conditions include: 1/ an increase in trichome density and in thickness of both upper and lower epidermis and of palisade parenchyma; 2/ a decrease in thickness of spongy parenchyma and in number of stomata per mm<sup>2</sup> of leaf surface. In addition, the leaf growth rate and the leaf area were reduced under TWW irrigation, as compared to control. Interestingly, all these alterations were attenuated in mycorrhizal olive plants. Indeed, mycorrhizal symbiosis promoted the CO<sub>2</sub> diffusion which enhanced the photosynthetic capacity of olive plants. Furthermore, AMF increased trichomes and stomatal density and improved all studied biomarkers (anatomical and morphological).

This study highlighted the beneficial role of AMF in the mitigation of salt stress under TWW irrigation. These game changers reduced the deleterious effect of TWW and improved the physiological, anatomical and morphological response of potted young olive plants. The synergetic effect of the two AMF species used was also demonstrated.

Keywords: Olive plant; Mycorrhiza; Treated wastewater; Photosynthesis; Morphology; Anatomy



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**LysM receptor function in successful ectomycorrhiza formation between poplar and *Laccaria bicolor***

S. Werner<sup>1</sup>, K. Omenge<sup>1</sup>, K. Deecke<sup>2</sup>, T. Irving<sup>3</sup>, T. Rush<sup>4</sup>, C. Cristobal<sup>3</sup>, F. Sébastien<sup>5</sup>, C. Hallmann<sup>6</sup>, M. Fladung<sup>2</sup>, J. M. Ané<sup>3</sup>

<sup>1</sup>Julius Kühn-Institute, Institute for Biosafety in Plant Biotechnology, Quedlinburg, Germany

<sup>2</sup>Thünen Institute of Forest Genetics, Forest Genetics, Großhansdorf, Germany

<sup>3</sup>University of Wisconsin–Madison, Plant - microbe symbioses, Madison, WI, United States

<sup>4</sup>Oak Ridge National Laboratory, Biosciences Division, Oak Ridge, TN, United States

<sup>5</sup>CERMAV-CNRS, Grenoble, France

<sup>6</sup>Leibniz Institute of Plant Biochemistry, Department of Cell and Metabolic Biology, Halle a. d. Saale, Germany

The colonization of plants by symbiotic fungi is highly regulated by both partners. For successful symbiosis, interspecies communication depends on the interaction of specific receptors on the plant side and signaling molecules of the fungus to induce the common symbiosis signaling pathway (CSP) in the host to induce an array of genes needed for symbiosis initiation. In this context, the plant must distinguish between a symbiotic and a pathogenic fungus. This is mediated by the specificity of the receptors of the host and the fungal molecules activating them. Membrane-associated chitin binding LysM receptor proteins initiate intracellular signaling events that either induce defense against pathogens or allow symbiosis. For many pathogens, symbiotic bacteria, and arbuscular mycorrhizal fungi, receptor candidates of this class are described to be crucial for signal transmission. For the formation of ectomycorrhizal (EM) associations of symbiotic soil fungi with tree hosts, receptors are unknown. In this cooperative project, we are characterizing three LysM receptor candidates of poplar (*Populus spec.*) that might be crucial for EM formation with the fungus *Laccaria bicolor*. To understand their function for symbiosis initiation, we produced knockout mutants of all receptors (single-, double- and triple mutants). We investigated the activation of the CSP, downstream gene expression, and mycorrhiza formation. Visualization of nuclear calcium spiking as readout for CSP induction demonstrated that at least two of these three receptors seem to be required for signal transmission since spiking was highly reduced in triple and double mutants compared to wild type plants. Furthermore, induction of gene expression downstream in the CSP was impaired. Microscopic evaluation of wild type and mutant mycorrhized root tips also showed significant differences. Especially in double and triple mutants, we observed reduced mycorrhiza formation and partially deformed root tip structures than in wild type co-cultivations demonstrating the importance of these receptor candidates for EM formation. This is also supported by the reduced lateral root formation in the receptor mutants that is enhanced in wild type plants in contact with *L. bicolor*. In summary, our findings support the hypothesis that EM fungi use LysM receptor-mediated signaling pathways to establish a symbiotic association with the roots of woody plants.

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## Unravelling the ectomycorrhizal transportome contributing to plant nutrition and adaptation

S. D. Zimmermann<sup>1</sup>, T. Ho-Plágaro<sup>1,2</sup>, M. Usman<sup>1</sup>, C. Guerrero-Galán<sup>1,3</sup>, G. Houdinet<sup>1</sup>, M. Calvo-Polanco<sup>1,4</sup>, J. Ruytinx<sup>5</sup>K. Garcia<sup>1,6</sup>, C. Plassard<sup>7</sup>, I. Gaillard<sup>1</sup><sup>1</sup>CNRS, IPSiM, Montpellier, France<sup>2</sup>Estación Experimental del Zaidín (EEZ), CSIC, Department of Soil and Plant Microbiology, Granada, Spain<sup>3</sup>School of Pharmacy, Universidad San Pablo-CEU Universities, Plant Physiology, Department of Pharmaceutical and Health Sciences, Boadilla del Monte, Spain<sup>4</sup>Universidad de Salamanca, Excellence Unit AgroEnvironment, Institute of Agrobiotechnology (CIALE), Salamanca, Spain<sup>5</sup>Vrije Universiteit Brussel, <sup>6</sup>Research Groups Microbiology and Plant Genetics, Department of Bioengineering Science, Brussels, Belgium<sup>6</sup>North Carolina State University, Department of Crop and Soil Sciences, Raleigh, NC, United States<sup>7</sup>INRAE, Eco&Sols, Montpellier, France

Ectomycorrhizal (EcM) symbiosis improves plant nutrition and water absorption, due to a better exploration of the soil and an efficient translocation of poorly available nutrients in forest ecosystems. Among other major nutrients as nitrogen and phosphate (Pi), potassium (K<sup>+</sup>) as the most abundant cation in plant cells is involved in various physiological processes and in plant salt tolerance. We have shown<sup>1</sup> improvement of K<sup>+</sup> nutrition by EcM symbiosis under K<sup>+</sup> shortage using the model couple *Pinus pinaster* and *Hebeloma cylindrosporum*. Regarding the symbiotic transportome<sup>2</sup>, we contributed to identify and characterize fungal transport systems involved in uptake of nutrients from the soil and in their transfer towards the plant root at the symbiotic fungus-plant interface, the Hartig net. Fungal K<sup>+</sup> transporters and channels<sup>1-5</sup> are suggested to absorb K<sup>+</sup> from the soil (Trk & HAK) and to release K<sup>+</sup> in the Hartig net via plant root cells (*Shaker*-like & TOK). Presence of three HcTOK<sup>6</sup> (Two-pore Outward K<sup>+</sup>) members of a fungi-specific channel family indicates different roles in K<sup>+</sup> nutrition and in symbiosis. Interestingly, one of these TOK members was induced by symbiotic association with the pine. Moreover, fungal Pi transporter have been characterized and found to be regulated on the transcriptional and proteomic level (MYCOTRANS project). Remarkably, transcriptomic data<sup>7</sup> from *H. cylindrosporum* cultured alone or in symbiosis with mycorrhizal *P. pinaster* revealed, that a CDF (Cation Diffusion Facilitator) member, *HcZnT2*, was the most highly induced fungal membrane transporter. Mycorrhizal fungi modulate the transfer of macro- and micronutrients, among them Zn<sup>8</sup>, to the host plant. We demonstrated the ability of *HcZnT2* to transport Zn by functional yeast complementation. Excitingly, we showed that *HcZnT2* is upregulated in symbiotic interaction, even by a short presence of the host plant without the need of physical contact, indicating gene induction during pre-symbiotic signalling. Thus, *HcZnT2* is suggested to play a key role in Zn homeostasis and regulation during the establishment and functioning of the EcM symbiosis.

## References

<sup>1</sup>*New Phytol* 2014 10.1111/nph.12603, <sup>2</sup>*TIPS* 2016 10.1016/j.tplants.2016.07.010, <sup>3</sup>*Front Plant Sci* 2014 10.3389/fpls.2014.00337, <sup>4</sup>*PlosOne* 2020 10.1371/journal.pone.0242739, <sup>5</sup>*Env Microbiol* 2018 10.1111/1462-2920.14122, <sup>6</sup>*Trends Microbiol* 2023 10.1016/j.tim.2022.11.007, <sup>7</sup>*New Phytol* 2015 10.1111/nph.13546, <sup>8</sup>*Fungal Biol Rev* 2019 10.1016/j.fbr.2019.09.001

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Molecular mechanism of PHO2 regulating arbuscular mycorrhiza development

Z. Ma<sup>1</sup>, C. Gutjahr<sup>1</sup>, I. F. Acosta<sup>1</sup>, D. Das<sup>2</sup>, J. Alpers<sup>1</sup>, R. Hüttl<sup>3</sup>

<sup>1</sup>Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

<sup>2</sup>LMU Munich, München, Germany

<sup>3</sup>TUM Munich, München, Germany

Arbuscular mycorrhizal fungi (AMF) can establish a symbiotic relationship with most terrestrial plants. They transfer nutrients to the plant in exchange for carbohydrates and lipids. Plants majorly benefit from symbiotic transfer of the poorly accessible phosphate, which is important for plant growth and development. There are two main ways for plants to obtain nutrients: Roots can directly absorb nutrients from the soil, which is called the direct nutrient absorption pathway. In most natural situations plants obtain nutrients from the external environment through arbuscular mycorrhizal fungi, which is called the indirect (or mycorrhizal) nutrient uptake pathway. AM formation is inhibited at high phosphate status, and this is regulated by the PHR-SPX phosphate starvation response system [1-2]. However, the involvement of other components of the phosphate signalling pathway in AM symbiosis remains unclear. Here we show that the E2-ubiquitin conjugating enzyme PHOSPHATE 2 (PHO2), which attenuates phosphate uptake responses at high plant phosphate status by mediating the degradation of crucial phosphate transporters, participates in regulating AM development. We identified two *PHO2* genes in the *Lotus japonicus* genome, called respectively *PHO2A* and *PHO2B*. Root colonization of *pho2* mutants is increased under low (20 $\mu$ M) as well as high phosphate (500 $\mu$ M) fertilization, when compared to wild type (Gifu). Interestingly, the colonization of single *pho2* mutants does differ from *pho2a,b* double mutants, suggesting that both *PHO2A* and *PHO2B* are important in the regulation of AM and not simply redundant. We are currently investigating the molecular role of PHO2 in modulating root colonization by AMF according to the plant phosphate status.

References

[1]Das D, Paries M, Hobecker K, Gigl M, Dawid C, Lam HM, Zhang J, Chen M, Gutjahr C. PHOSPHATE STARVATION RESPONSE transcription factors enable arbuscular mycorrhiza symbiosis. *Nat Commun.* 2022 Jan 25;13(1):477.

[2]Paries M, Gutjahr C. The good, the bad, and the phosphate: regulation of beneficial and detrimental plant-microbe interactions by the plant phosphate status. *New Phytol.* 2023 Jul;239(1):29-46.

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Undermining the “cry for help”: how a phytopathogenic fungus undermines host recruitment of antagonistic bacteria

W. Punt<sup>1</sup>, A. Kraege<sup>1</sup>, A. Doddi<sup>1</sup>, J. Zhu<sup>1</sup>, K. Wippel<sup>2</sup>, N. Schmitz<sup>1</sup>, N. Snelders<sup>1</sup>, B. Thomma<sup>1</sup>

<sup>1</sup>University of Cologne, Institute for Plant Sciences, Köln, Germany

<sup>2</sup>University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, Netherlands

Plant pathogens secrete small cysteine-rich proteins called effectors during infection to promote disease development through various mechanisms. While effectors were typically thought to target host physiology, including host immune responses, we discovered that some effectors target the microbes that live on and in the plant to promote host colonization. More specifically, the soil-borne fungal plant pathogen *Verticillium dahliae* secretes several effectors with selective antimicrobial activity that target microbial antagonists in the microbiota of its plant hosts. We now present the functional characterization of a novel effector that displays antimicrobial activity, named Av2. Deletion of Av2 compromises the virulence of *V. dahliae* in planta, as tomato plants inoculated with the wild-type strain developed stronger symptoms of disease than plants inoculated with the deletion strain. Intriguingly, inoculation experiments using a gnotobiotic plant cultivation system revealed a microbiota-dependent virulence contribution of Av2. To investigate the effect of Av2 during host colonisation we performed amplicon sequencing and showed that Av2 modulates the host microbiota by specifically suppressing the recruitment of Pseudomonadales. Co-cultivation experiments revealed that Av2 deletion leads to reduced *V. dahliae* growth in the presence of particular plant-associated *Pseudomonas* spp. Furthermore, the same antagonistic *Pseudomonas* spp. bacteria display sensitivity to Av2 *in vitro*. Altogether, we propose that *V. dahliae* secretes Av2 to manipulate the microbiota of its host plants by undermining the “cry for help” recruitment of beneficial *Pseudomonas* spp. to ultimately promote disease development.

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**Effective of different doses of bacterial insecticide against *Trogoderma granarium* (Everts)**

F. H. Hallak<sup>1</sup>

<sup>1</sup>University of Aleppo, Agriculture Faculty - Plant Protection Dept., Aleppo, Syrian Arab Republic

This study was carried out to evaluate the effectiveness of bacterial insecticide (Vertimic) of the fourth star larve of *Trogoderma granarium* (Everts) by four treatments (A, B, C, D) at seven concentrations 100, 10, 1, 0.1, 0.01, 0.001 PPM in the treatment A, B and at four concentrations 1, 0.1, 0.01, 0.001 PPM in the treatment C, D. Mortality rate of larve was 100% at concentration 1000, 100 PPM in the treatment A and B after 24 hours, and after 48 hours in the treatment D at 1 PPM. The efficiency of the treatment concentrations and all exposure times. The efficiency of treatment D was greater as compared to the treatment C in all concentrations and all exposure times for example at 0.1, 1 PPM and after 120 hours the Mortality rate of larve was 43.33, 100% respectively in the treatment D and 0.1, 1 PPM respectively in the treatment C.

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**Plant immunity and leaf bacterial recruitment are parallel processes whose link shapes sensitivity to temperature stress**

J. Jose<sup>1</sup>, E. Teutloff<sup>2</sup>, S. Naseem<sup>2</sup>, R. Halitschke<sup>1</sup>, E. Barth<sup>3</sup>, M. Agler<sup>2</sup>

<sup>1</sup>Max-Planck Institute for Chemical Ecology, Mass Spectrometry and Metabolomics, Jena, Germany

<sup>2</sup>Friedrich Schiller University, Institute of Microbiology, Jena, Germany

<sup>3</sup>Friedrich Schiller University, Bioinformatics Core Facility Jena, Jena, Germany

Developing climate-resilient crops will be crucial due to the rising global temperatures. Some plants downregulate defenses at higher temperatures making them more prone to foliar pathogens. One strategy for minimizing this negative impact is by altering defense hormones. However, it is not yet clear how defense hormone levels affect plant microbiome assembly and how this interacts with temperature to affect overall plant health. We used chemical mutagenesis to identify a phenotypically healthy genotype of *A. thaliana*, "CLLF", that naturally recruits unusually high loads of diverse non-pathogenic leaf bacteria. CLLF hyperaccumulates salicylic acid (SA) and jasmonates and has constitutively upregulated defense responses. Accordingly, compared to the wild-type, CLLF has increased resistance to pathogens, suggesting that pathogen defense and non-pathogen recruitment can be uncoupled. Some non-pathogenic bacteria can directly grow on SA as a carbon source, which may help explain this uncoupling. CLLF also showed a high tolerance to heat stress compared to the wild-type, and it is likely linked to its heat-insensitive defense gene expression. Synthetic community experiments showed that this resilience to heat stress is compromised without a complete bacteriome, leading to dysbiosis. Thus, a full bacteriome taxonomic diversity is an important factor to be considered while developing climate-resilient crops.

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**Identifying the genetic components controlling root nodule symbiosis in *Phaseolus vulgaris* under phosphate-deficient conditions**

O. Valdés-López<sup>1</sup>, M. C. Isidra-Arellano<sup>2</sup>, M. K. Ried-Lasi<sup>3</sup>

<sup>1</sup>UNAM, Biology, Tlalneapantla, Mexico

<sup>2</sup>Royal Botanic Gardens, Kew, Crops and Global Change, London, United Kingdom

<sup>3</sup>Leibniz-Institut für Pflanzenbiochemie, Leipzig, Germany

Phosphate (Pi) deficiency is a significant challenge that impacts plant growth and crop yield worldwide. In legumes, Pi deficiency reduces nodule formation, hindering nitrogen fixation and overall plant productivity. Despite considerable progress in understanding how legumes cope with Pi deficiency while symbiotically interacting with rhizobia, the genetic mechanisms controlling this symbiosis under Pi-limiting conditions remain elusive. To tackle this knowledge gap, we have generated experimental evidence showing that plant host Pi levels modulate the root nodule symbiosis in *Phaseolus vulgaris*. Interestingly, *PHR1*, a master regulator of plant responses to Pi deficiency, is an essential genetic component in controlling root nodule symbiosis under Pi scarcity conditions. Modulating the gene expression of *PHR1* by RNA interference (RNAi) and overexpression revealed that *PHR1* can act as a negative regulator of nodule formation in *P. vulgaris* under low Pi conditions. Further whole genome transcriptional analyses on *PHR1*-RNAi and *PHR1*-overexpressing nodule primordia revealed that *PHR1* modulates the expression of crucial symbiotic genes participating in nodule formation. Our data shed light on the genetic regulation of root nodule symbiosis under Pi-deficient conditions and encourages us to investigate the participation of *PHR1* in this symbiosis further.

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The presence of arbuscular mycorrhizal fungi in plant rhizospheres drives changes in plant and herbivore microbiome composition

A. Bennett<sup>1</sup>, A. Malacrino<sup>2</sup>, A. Karley<sup>3</sup>

<sup>1</sup>Ohio State University, Evolution, Ecology, and Organismal Biology, Ohio, OH, United States

<sup>2</sup>University of Reggio Calabria, Reggio Calabria, Italy

<sup>3</sup>James Hutton Institute, Ecological Sciences, Dundee, United Kingdom

Arbuscular mycorrhizal (AM) fungi are often considered keystone rhizosphere taxa due to their associations with host plants and soil. In two systems (potato and tomato) we explored whether AM fungi influence plant microbiome composition, and the mechanism by which such an influence might occur. We grew tomatoes and potatoes with microbial communities containing AM fungi or not containing AM fungi, and exposed them to common herbivores. *Macrosiphum euphorbiae* (potato aphid) fed on potatoes, and *Manduca sexta* (tobacco hornworm), a chewing herbivore, fed on tomatoes. *M. euphorbiae* is a sap sucking aphid with a defined microbiome hosting primary and secondary symbionts, while *M. sexta* is a lepidopteran chewing herbivore with no known core microbiome. Thus, we expected these herbivores to respond to changes in the rhizosphere and host plants differently. However, in both systems we found that AM fungi in the rhizosphere altered the root microbiome and the herbivore microbiome, but not the leaf microbiome. This was surprising as the herbivore microbiome of *M. euphorbiae* and *M. sexta* are known to be assembled differently. In the tomato system we also explored whether the starting microbial community composition played a role in our results, and found that the starting microbial community composition drove larger effect sizes for shifts in microbiome composition in roots and herbivores. We then explored the mechanism for how AM fungi in the rhizosphere altered the microbiome composition of the herbivore. A previous study suggested that soil splashed on leaves, and herbivores consumed soil microbes. We tested this hypothesis by covering soil in the tomato system, and found no influence of soil coverage on herbivore microbiome composition. Thus, the influence of the rhizosphere microbiome on the herbivore microbiome occurs via the host plant. These results provide multiple opportunities for manipulating rhizosphere microbiomes in ways that could alter plant-herbivore interactions.

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A survey of beneficial rhizobacteria for grain legumes from sub-saharan africa

D. B. Kagambo<sup>1</sup>

<sup>1</sup>University of Bremen, Biology, Bremen, Germany

Plant growth promoting rhizobacteria (PGPR) offer a low cost and affordable agricultural solution to the majority of smallholder farmers in Sub-Saharan Africa (SSA). Legumes such as white cowpea/bean (*Vigna unguiculata* L. Walp) and Bambara groundnut (*Vigna subterranean* L. Verdc) are among the most important legumes in SSA providing both a source of food and income for many living in the region.

Indigenous soils and legumes were used in this study thus providing information about the local diversity of rhizobacteria populations that is an important initial step in selecting possible candidates for use as bio-fertilizers. In the first project involving soils and legumes from Tibati, Cameroon, a higher number of nodules were observed and isolated from B. groundnut plants compared to white bean plants indicating the high propensity of B. groundnut for nodulation. Phylogenetic analysis of 49 isolated *Bradyrhizobium* strains revealed over eight clusters in which isolated *Bradyrhizobium* strains shared high ITS region sequence similarity with reference type strains. Factors such as soil origin and plant host were also depicted to play a role in the cluster diversity as evidenced by presence of over 21 of 26 *Bradyrhizobium* isolates from white bean belonging to one cluster, which was a far cry from the even distribution of B. groundnut *Bradyrhizobium* isolates throughout all the clusters. Interestingly, the presence of possible novel strains was also evidenced by the relatively low ITS similarity of isolates CGN1-7 and CGC1-2 to the reference type strains.

For the 2nd project, roots from white cowpea (*Vigna unguiculata* L. Walp) were sterilised crushed and analyzed for rhizobacteria that were grown on R2A media. 21 isolates were obtained based on colony appearance and 16S rDNA sequencing was done to determine the species isolated in relation to type strains used. These were further grouped phylogenetically with a large number of isolates belonging the Proteobacteria phylum of bacteria. This was followed by tests for plant growth promoting traits; ACC-deaminase activity, phosphate solubilization, Nitrogen fixation potential and IAA production. From the analysis, 16 of the 21 isolates showed at least presence of one trait. 10 isolates tested positive for IAA production and 12 isolates tested positive for phosphate solubilization. Tests for nitrogen fixation potential were not possible due to time constraints.

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**Dynamic Adaptation of Rhizosphere Microbiomes to Mineral Nitrogen Forms**N. Maywald<sup>1</sup>, A. Hernandez-Pridybalo<sup>1</sup>, D. Francioli<sup>2</sup>, U. Ludewig<sup>1</sup><sup>1</sup>Universität Hohenheim, Ernährungsphysiologie der Kulturpflanze, Stuttgart, Germany<sup>2</sup>Hochschule Geisenheim University, Geisenheim, Germany

Soil microbiomes, which are essential for maintaining agricultural ecosystems and supporting crop vigour, have a significant influence on plant health and productivity. However, the complex responses of soil microbiomes to different forms of nitrogen (N), such as ammonium or nitrate, are influenced by a variety of factors, including initial soil pH and site-specific conditions. This variability makes it difficult to generalise the effect of N form on complex plant-microbe interactions and to draw precise conclusions about the specific effects of a particular N form on interactions between cereal crops and potentially beneficial and pathogenic microbes in agroecosystems. In this study, we investigated how initial soil pH and site variation affect the ability of ammonium and nitrate to influence microbial richness and diversity within the rhizosphere of maize plants. Soils were collected from two different field sites. One site was of particular interest due to the presence of a natural pH gradient ranging from 5.98 to 7.45, while other soil characteristics remained relatively constant. The soil from the second site had a similarly low pH of 5.89, which was then artificially adjusted to 7.39 using potassium hydroxide. We found that while initial soil pH remains the primary factor influencing microbial populations, the form of nitrogen has a remarkable ability to reshape bacterial and fungal communities within the rhizosphere in as little as three weeks, regardless of initial soil pH. Although the N source affected the composition of the bacterial community at both low and high initial soil pH, the composition of the fungal community was particularly affected at low pH conditions. To gain a thorough understanding of these shifts and their implications for crop production, we conducted in-depth analyses of plant performance, subsequent pH changes, root exudates and plant transcriptome. In particular, we focused on identifying differences in nitrogen-dependent genes, as well as plant defence-related mechanisms. This approach aims to improve the current understanding of plant and rhizosphere microbiome adaptations to nitrogen dynamics.

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**Cryptic diversity in the Prasiolaceae (Prasiolales, Trebouxiophyceae, Chlorophyta) revealed by classic isolation and cultivation**S. Heesch<sup>1</sup><sup>1</sup>University of Rostock, Institute for Biosciences, Applied Ecology & Phycology, Rostock, Germany

The Prasiolaceae is a small family of mainly aero-terrestrial green algae that is well known for the preference of its members for high-nutrient environments. Some species are found at coastal sites in and near seabird colonies, while others can also be observed in nutrient-enriched urban habitats. In central Europe, for example, Prasiolacean algae commonly occur, where male mammals urinate at the base of trees or behind pubs. At such sites, various species can grow together in close proximity.

In this study, material of Prasiolaceae collected from temperate and arctic regions (central and northern Europe, Svalbard and New Zealand) were isolated and cultivated. Microscopic entities discovered in cultures of larger species revealed a so far unknown genetic diversity within the family. These results emphasize the importance of an integrated approach combining classic isolation and cultivation techniques with molecular genetics for unravelling the taxonomy of these algae. Moreover, this study suggests that terrestrial habitats remain a major repository of unknown algal diversity.

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Improved plant abiotic stress tolerance via use of beneficial microbes and alternative fertilizers, dissected via phenomics approaches

S. Sanow<sup>1,2</sup>, O. Kapitanska<sup>3</sup>, L. Mau<sup>1</sup>, J. Kelm<sup>1</sup>, J. Kant<sup>1</sup>, D. Reinecke-Levi<sup>1</sup>, P. Huesgen<sup>4</sup>, G. Schaaf<sup>5</sup>, M. Watt<sup>6</sup>, U. Roessner<sup>7</sup>, B. Arsova<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, IBG-2 – Plant Sciences, Root Dynamics Group, Jülich, Germany

<sup>2</sup>University of California, Davis, Germany

<sup>3</sup>RPC Kvadrat, Kiev, Ukraine

<sup>4</sup>University of Freiburg, Freiburg i. Br., Germany

<sup>5</sup>University of Bonn, Bonn, Germany

<sup>6</sup>University of Melbourne, Melbourne, Australia

<sup>7</sup>Australian National University, Canberra, Australia

Current agricultural challenges arise from climatic changes and policies (e.g. fertilizer limitations) aimed at environmental preservation. Since nutrients are absorbed by roots, root functional analysis and phenotyping are crucial for improving plant resilience (Arsova *et al.*, 2020). To address fertilizer limitations and resource recycling, we work on plant-microbe interactions and using algae to recycle nutrients from water.

To combat nitrogen (N) limitation we use the crop model *Brachypodium* inoculated with *Pseudomonas koreensis* (Pk). Inoculated plants at low N showed distinct root system architecture (RSA) changes, increased N content in roots and shoots, and unique proteomic and lipidomic profiles. Phenotyping revealed N-related changes in plant leaf area early in the experiment, while bacterial responses were evident 3 weeks post-inoculation. The plant proteome at low N, when inoculated with Pk, expressed central N metabolic enzymes similar to non-inoculated plants with ten times higher N availability, the exceptions highlight regulatory hotspots influenced by Pk. Lipidome profiling at 3 timepoints showed responses mainly to N availability, with few lipids responding to Pk. These lipid changes preceded significant increases in leaf area, suggesting early biochemical adjustments. Investigations into N-fixation included growing the bacterium in N-free medium and measuring isotopic distributions of <sup>14</sup>N and <sup>15</sup>N in the plant (Sanow *et al.* 2023).

Excessive N fertilization leads to eutrophication of freshwater. We showed that wheat and *Brachypodium* can absorb Phosphorus (P) from algae, with root phenotyping revealing altered RSA but maintained functionality in algae-fed plants (Mau *et al.*, 2022). To replace mineral N, we addressed the N < P imbalance in algae fertilizer. Wheat grown in soil-filled rhizotrons, supplemented with mineral fertilizer, wastewater algae, or a combination of both showed that algae could supply P in high concentrations without causing plant P toxicity, due to P containing compounds. Phenotyping under 7 nutrient regimes indicated that the optimal algae-mineral blend was comparable to pure mineral fertilizer in projected leaf area, while also revealing RSA changes. Elemental analyses examined plant nutrient accumulation across fertilization regimes, and qPCR on transcripts from N and P metabolism showed no nutrient deficiency in optimal algae fertilization. This elucidates the potential of wastewater algae as fertilization strategy.

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Diversity atlas of root system dynamics *Inter- and intraspecific diversity of root growth and development dynamics*

M. Kuhlmann<sup>1</sup>, C. Seiler<sup>1,2</sup>, S. Shaaf<sup>1</sup>, N. Narisetti<sup>1</sup>, R. Shi<sup>1</sup>, E. Gladilin<sup>1</sup>, A. Börner<sup>3</sup>, G. Bienert<sup>1,4</sup>, B. Fraust<sup>5</sup>, R. Giehl<sup>5</sup>, A. Junker<sup>1,6</sup>, D. Knoch<sup>1</sup>, R. Meyer<sup>1</sup>, L. G. Otto<sup>7</sup>, D. Psaroudakis<sup>1,7</sup>, M. Nagel<sup>3</sup>, K. Neumann<sup>1</sup>, E. Willner<sup>3</sup>, N. von Wirén<sup>5</sup>, J. Reif<sup>7</sup>, T. Altmann<sup>1</sup>

<sup>1</sup>IPK Gatersleben, Molecular Genetics, Stadt Seeland, Germany

<sup>2</sup>JKI, Quedlinburg, Germany

<sup>3</sup>IPK Gatersleben, Genbank, Stadt Seeland, Germany

<sup>4</sup>Technische Universität München, Crop Physiology, München, Germany

<sup>5</sup>IPK Gatersleben, Physiology and Cell Biology, Stadt Seeland, Germany

<sup>6</sup>Syngenta, Plant Phenotyping, Halle a. d. Saale, Germany

<sup>7</sup>IPK Gatersleben, Breeding Research, Stadt Seeland, Germany

Roots fulfill vital functions for plants such as anchoring them in the ground, foraging the soil for water and nutrients and interacting with the edaphic environment. Although the root architecture is adapted to the necessary functionality, the diversity of the root system architecture is amazingly large. The root system architecture is genetically determined, but the expression of the root features varies substantially among individuals and in response to various environmental influences, the expression of root traits is highly plastic. Major recent advances in automated plant cultivation, root imaging, and image analysis techniques enable the detailed non-invasive assessment of root growth and development over time for large populations of individuals. They pave the way for the investigation of inter- and intra-specific variation as well as for detailed studies on the genetics of root system architecture and the reactions to environmental influences. The Rhizotron system in the IPK-PhenoSphere was utilized for a time-resolved investigation of root system diversity in seven monocotyledon and ten dicotyledon species including important crop and model plants. Plants were analysed over their juvenile growth phase covering the main period of root system growth and development. Wide inter- and intraspecific variation in the expression of 35 architectural traits including root system width and root system depth that are associated with the core functions of roots i.e. acquisition of water and mobile or immobile nutrients as well as anchoring the plant in the soil was observed and quantified with daily resolution. This developmental analysis revealed an early difference between fibrous and tap root systems, indicating a fast growth of monocotyledon plants in the vertical dimension and an early extension of root system diameter from dicotyledon plants. Derived growth models give an insight into the large diversity of inter- and intraspecific differences in the dynamics of root growth and development and root traits are identified for further discrimination.

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## Diversity of Hessian orchids, their artificial cultivation, culture and replanting - first results

J. Metzsch<sup>1</sup>, K. Cilgin<sup>1</sup>, V. Wissemann<sup>1</sup>, C. M. Müller<sup>1</sup><sup>1</sup>Justus-Liebig-Universität Gießen, Spezielle Botanik, Gießen, Germany

Hessian orchids are facing a significant risk of decline due to factors, including climate change, land use alterations and habitat fragmentation. To mitigate the potential extinction, it is crucial to implement effective conservation strategies.

For this reason, the genetic structure of six native orchids (*Dactylorhiza majalis*, *Anacamptis morio*, *Neotinea ustulata*, *Cypripedium calceolus*, *Herminium monorchis* and *Spiranthes spiralis*) are investigated by Inter Simple Sequence Repeat (ISSR) and Start Codon Targeted (SCoT) and carried out breeding experiments and strategies for successful replanting.

Preliminary results from the genetic analyses performed via ISSR primers showed that *A. morio* and *D. majalis* exhibited a moderate level of genetic differentiation, between populations. Furthermore, the first successes were achieved in the artificial cultivation of *C. calceolus* and *S. spiralis*, so the plants can possibly be reintroduced in their native habitat.

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Osmo primed Melatonin Treatment and PGPR Inoculation Regulates Key Physio-biochemical and Molecular Pathway in *Brassica juncea* under Cd stressT. Bhardwaj<sup>1</sup>, R. Bhardwaj<sup>1</sup><sup>1</sup>Guru Nanak Dev University, Department of Botanical and Environmental Sciences, Amritsar, India

Heavy metal (HM) contamination in agricultural field has become a matter of grave concern. Cadmium (Cd) is a mobile, non-essential trace element that impacts human health and crops physiological attributes. Plants are the primary entry point for heavy metals into the food chain. Hence, there is a dire need for effective management practices for ensuring Cd resilience and agroecosystems restoration. Melatonin (Mlt) is a pleiotropic molecule with many diverse actions in plants. Its antioxidant potential plays significant role in heavy metal remediation. Heavy metal-resistant plant growth-promoting rhizobacteria (HMR-PGPR) are well known for resisting heavy metal toxicities and enhancing plant growth and yield. The present study was designed to investigate the stress extenuation role of melatonin (Mlt) supplemented with HMR-PGPR (*Pseudomonas putida* (Pp) and *Pseudomonas fluorescens* (Pf)) in 10-day-old Cd-stressed (0.3 mM) *Brassica juncea* L. seedlings. The present work explored morphological traits, photosynthetic efficiency and phenol metabolism in melatonin-PGPR inoculated *B. juncea* seedlings. Also, in depth molecular studies such as RT-PCR and transcriptomic analyses were performed. A significant increase in photosynthetic pigments and secondary metabolites after treatment with melatonin, *P. putida*, and *P. fluorescens* in Cd stressed *B. juncea* seedlings was observed, which was further validated with transcriptome analysis. Comparative transcriptome analyses identified the following differentially expressed genes (DEGs): 455 upregulated and 4921 downregulated in Cn-vs-Cd, 5953 upregulated and 430 downregulated in Cd-vs-Mlt, 3368 upregulated and 137 downregulated in Cd-vs-Mlt-Pp-Pf, and 2238 upregulated and 27 downregulated in Cd-vs-Mlt-Pp-Pf-Cd comparative groups. The Gene ontology and Kyoto encyclopaedia of genes articulated the impact of stress and ameliorators in *B. juncea* seedlings. Expression profiling of key photosynthetic genes (*psb A*, *psb B*, *CHS*, *PAL*, and *PSY*) showed greater expression in melatonin-rhizobacteria-treated Cd stressed *B. juncea* seedlings. This study provides valuable information as it suggests the potential mechanisms by which Mlt-PGPR confers protection to photosynthesis in Cd-stressed *B. juncea* plants.

**Keywords:** Cadmium; melatonin; PGPR; transcriptome; photosynthesis; gene expression



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DNA markers targeting three cellular genomes for the discrimination between *Taxus baccata*, *T. cuspidata* and *T. × media*

D. Bross<sup>1</sup>, H. Schröder<sup>2</sup>, E. Pers-Kamczyc<sup>3</sup>, B. Kersten<sup>1</sup>

<sup>1</sup>Thuenen-Institute of Forest Genetics, Genome Research, Großhansdorf, Germany

<sup>2</sup>Thuenen-Institute of Forest Genetics, Species and origin identification, Großhansdorf, Germany

<sup>3</sup>Institute of Dendrology PAS, Plant Genetic and Environmental Interactions, Kórnik, Poland

Ever since the discovery of the anticancer medication Paclitaxel, the genus *Taxus* L. has received growing attention within the scientific community. As the species identification in *Taxus* based on morphological differences has historically been difficult, molecular markers are important tools to consistently identify *Taxus* species. The increasing availability of public sequencing data supports efforts to find and exploit such markers. We identified DNA sequence variants for the differentiation of the three most abundantly occurring *Taxus* species in Europe, *T. baccata* L., *T. cuspidata* Sieb. & Zucc., and their hybrid, *T. × media* Rehder in the three cellular genomes. Differentiating SNPs were detected in the ITS region of the nuclear genome and in the *cox1* region of the mitochondrial genome. InDels located in the *psbB\_psal* intergenic linker and *chlN* region of the chloroplast genome were also selected for the differentiation of *T. baccata* and *T. cuspidata*. Based on the identified variants, robust PCR-based markers were developed for genotyping. In combination, these markers can also identify the crossing direction of *T. × media* samples. Markers were tested against a set of over 120 *Taxus* individuals with pre-existing phenotypical species declarations. The crossing directions of the *T. × media* cultivars could be genetically identified (or confirmed, in the case of previously documented crosses) in this study. Our results of the tests on *T. × media* samples underline the predominantly paternal inheritance of the mitochondrial genome in *T. × media*. A minimal marker set was defined for rapid and cost-efficient identification of species and of crossing direction in hybrids in future studies.

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Modeling metabolic dependencies in resource competition and cross-feeding among host-microbe communities

M. Feierabend<sup>1</sup>, N. Töpfer<sup>2</sup>

<sup>1</sup>University of Cologne, Institute for Plant Sciences, Metabolic Reconstruction and Flux Modelling, Köln, Germany

<sup>2</sup>University of Cologne, Cluster of Excellence on Plant Sciences, Institute for Plant Sciences, Köln, Germany

In natural environments, plants share their habitat with a myriad of microorganisms which include bacteria, fungi, oomycetes, archaea and viruses. Plants and their associated microbiome form complex, multilateral and highly dynamic interactions. The plant-associated microbiome conveys fitness advantages to the plant host as such promotion of plant health, improved acquisition of nutrients, and resilience against pathogen attacks and abiotic stress conditions. Metabolic dependencies and metabolic cross-feeding are a driving force of these interactions.

One way to understand the complexity of biological systems is by using mechanistic computational models. Among these, genome-scale metabolic modeling is particularly suited as it is based on the biochemical network topologies and requires only minimal input. It allows for the integration and interpretation of metabolic data and prediction of metabolic fluxes on a large scale. Here, we use genome-scale metabolic models to understand metabolic processes within plants and their associated microbiota. We develop computational frameworks which integrate microbial genome-scale metabolic models with plant metabolic models. Connecting these models allows for the analysis of metabolic fluxes of individual and combined organisms. The integrated model enables the investigation of the effects of perturbations, such as gene deletions or mutations, on the host-microbiome interactions. To reduce topological uncertainties, we will also reconstruct multi-strain microbial models and ensemble models, which provide alternative metabolic pathway representations.

One of our test cases focuses on understanding *Sphingomonas* depletion after pathogen attack. *Sphingomonas* is a member of the core microbiota in various plant species. Meta-analyses have shown that irrespective of plant species or type of pathogen, *Sphingomonas* often became statistically significantly depleted following biotic stress conditions. By using genome-scale metabolic models, we work on elucidating potential metabolic factors for this depletion using the combined knowledge about the plant's metabolism, its effects on bacterial metabolism and interaction effects. This framework represents an important step towards a mechanistic understanding of plant-microbial interactions, aiming to develop its application in sustainable agricultural practices.

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Exploring the genetic adaptation of *SnRK1* and sugar-signalling for the high light-induced stress response in streptophyte algae and early land plants.

C. Kühn<sup>1</sup>, A. S. Richter<sup>1</sup>

<sup>1</sup>University of Rostock, Department of Plant Metabolism, Rostock, Germany

Plants, being immobile organisms, must acclimate to dynamic environmental conditions. Through evolutionary processes, plants have evolved mechanisms enabling growth, reproduction, and survival when facing stressful conditions. Employing various approaches, we aim to understand the genetic adaptations permitting the acclimation to abiotic factors, such as variations in light intensity. The presence of intense light, which can detrimentally affect plant growth, is sensed by chloroplasts and subsequently relayed to the nucleus through signaling pathways. Within flowering plants, the *sucrose non-fermenting related kinase 1* (*SnRK1*) has a pivotal role, mediating responses to intense light depending on cellular sugar levels.

The transition from water to land marks the evolutionary journey of previously aquatic algae, which ventured onto land, adapting to terrestrial conditions and evolving from non-vascular to vascular plants, eventually leading to the emergence of flowering plants. However, signaling cascades orchestrating acclimation to intense light in basal (land) plants, such as streptophytic algae and mosses, remain largely unexplored. Streptophytic algae like *Zygnema circumcarinatum*, and the moss *Physcomitrium patens* are closely related to land plants, both possessing *SnRK1* homologues. Yet, the functional significance of this protein kinase and its role in the evolution of land plants remains obscure.

This ambiguity forms the foundation of our studies. Within this project, I aim to elucidate the genetic adaptations of *SnRK1* and its involvement in responding to high light stress in *Zygnema* and *Physcomitrium*.

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Genetic information for the conservation of segetal plants

P. Tran<sup>1</sup>, V. Herklotz<sup>1</sup>, C. M. Ritz<sup>1,2</sup>, B. Usadel<sup>3</sup>, H. Schmied<sup>4</sup>, L. Fortmann<sup>4</sup>, S. Meyer<sup>1,5</sup>, D. Harpke<sup>6</sup>, K. Wesche<sup>1,2</sup>

<sup>1</sup>Senckenberg Museum of Natural History, Department of Botany, Görlitz, Germany

<sup>2</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

<sup>3</sup>Forschungszentrum Jülich GmbH, IBG-4, Jülich, Germany

<sup>4</sup>Stiftung Rheinische Kulturlandschaft, Bonn, Germany

<sup>5</sup>Albrecht-von-Haller Institute for Plant Sciences, University of Goettingen, Plant Ecology and Ecosystems Research, Göttingen, Germany

<sup>6</sup>Leibniz Institute of Plant Genetics and Plant Crop Research (IPK), Gatersleben, Germany

Arable land is Germany's most important and dominant habitat with a landcover >1/3. Segetal plants, which occur in these habitats, contribute substantially to plant biodiversity and thus to diversity of higher trophic levels since they provide food sources for insect pollinators or herbivores. Segetal species are, however in massive decline over the last decades and restauration schemes need to be developed. According to current legislation, diaspores for restauration actions can be transferred within each of 22 local provenance regions, but data on genetic diversity and differentiation of segetal plants that reflect these, are still missing.

Our project aims to investigate population genomics of seven common and 15 rare segetal species across Germany using both: ddRADseq- and PoolSeq methods. ddRADseq of the widespread corn flower (*Centaurea cyanus*) revealed no patterns of geographical differentiation. Genetic variation within populations was much higher than between populations, suggesting that the local provenances do not capture a large share of the population structure.

Genetic structures in plants are often species-specific, depending on functional traits, habitat preferences and on populations size since rare species are stronger genetically differentiated than common ones. We will subsequently analyze genetic structures for the larger set (seven common and 15 rare species) of segetal plants differing in these traits. In addition, we will analyze cultivated *Centaurea cyanus* accessions using PoolSeq, aiming to detect possible domestication traits, which may play a major role for plant fitness.

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Pollen and anther morphological variation was shaped by domestication in rye (*Secale cereale* L.)

C. Waesch<sup>1</sup>, Y. Gao<sup>1</sup>, N. Koch<sup>1</sup>, C. S. Gaede<sup>1</sup>, T. Hornick<sup>2,3</sup>, C. Dusny<sup>3</sup>, J. Fuchs<sup>4</sup>, A. Börner<sup>4</sup>, A. Himmelbach<sup>4</sup>, M. Mascher<sup>2,4</sup>, K. Pillen<sup>1</sup>, S. Dunker<sup>2,3</sup>, S. Dreissig<sup>1,2</sup>

<sup>1</sup>Martin-Luther University Halle-Wittenberg, Agricultural and nutritional sciences, Halle a. d. Saale, Germany

<sup>2</sup>German Centre for Integrative Biodiversity Research iDiv Halle-Jena-Leipzig, Leipzig, Germany

<sup>3</sup>Helmholtz-Centre for Environmental Research – UFZ Leipzig, Leipzig, Germany

<sup>4</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland / OT Gatersleben, Germany

In plants and animals, pollen and sperm morphology are incredibly diverse across species. Cross-pollination provides a mechanism to recombine genetic variants in a population which, among other evolutionary forces, may facilitate adaptation. Across plant species, pollen morphological diversity is broadly linked to different pollination systems. However, the extent of within-species diversity is less well understood. Further on, modulating pollination mechanisms in crops presents an opportunity to improve hybrid breeding programs.

Our study aims to investigate quantitative variations in pollen and anther morphology in rye (*Secale cereale* L.), a wind-pollinating grass species. For this purpose, we analysed 339 rye individuals derived from a diverse set of 64 prior classified rye accessions ranging from domesticated (221), wild-like (91) and wild (4) individuals.

A PCA using reduced representation sequencing data (GBS) based on 56,713 SNPs revealed a clustering based on the degree of domestication. We quantified pollen morphology in 286 individuals using multispectral imaging flow cytometry, and measured anther length via light microscopy in 314 individuals, which revealed pronounced within-species diversity. We conducted genome-wide association scans and found five and eight genomic regions associated with pollen length and anther length, respectively. A subset of these loci overlapped with previously identified domestication loci for which the underlying trait was unknown. Our  $P_{ST}-F_{ST}$  analysis, suggests that pollen and anther traits were under selection throughout rye domestication. A population genomic analysis revealed signatures of selection at one of five loci associated with pollen length, as well as at three out of eight loci associated with anther length. Underlining that, we found significantly higher pollen and anther length in domesticated rye.

In conclusion, this suggests that selection for larger pollen grains and longer anthers occurred throughout rye domestication. Our study extends our knowledge of the genetic architecture underlying within-species pollen and anther morphological diversity and further unravels domestication traits in rye.

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Global genomic diversity of a South American oil-palm species: *Acrocomia totai* (Arecaceae)

F. A. Vergara<sup>1</sup>, F. He<sup>1</sup>, J. Morales M.<sup>2</sup>, M. Moraes<sup>3</sup>, D. Wassner<sup>4</sup>, M. Zucchi<sup>5</sup>, A. Mason<sup>1</sup>

<sup>1</sup>INRES-Universität Bonn, Bonn, Germany

<sup>2</sup>Instituto de Biologia, UNICAMP, Campinas, Brazil

<sup>3</sup>Instituto de Ecología, UMSA, La Paz, Bolivia

<sup>4</sup>Facultad de Agronomía, UBA, Buenos Aires, Argentina

<sup>5</sup>APTA, Polo Centro Sul, Piracicaba, Brazil

*Acrocomia* palms are native neotropical inhabitants of the Americas. Among these palms, the species *Acrocomia totai* is the most common in the central region of South America. Although the distribution and diversity of this species has been studied previously, most work to date has looked at only specific locations, or has mixed this species with relative species *A. aculeata* due to taxonomic controversy. Comprehensive biodiversity studies of native species are important because they can inform fields such as phylogenetics, ecology, taxonomy, and agronomy, as well as support local strategies for management and conservation. The objective of the present work was to assess the genomic diversity of *A. totai* across the total area of occurrence. For this aim, genomic DNA extracted from dried leaf material was sequenced after GBS library preparation and subsequently analyzed with 93,224 recovered SNP variants to make phylogeographic inferences. Through hierarchical clustering it was possible to determine that the most distinct group corresponds to the western groups of palms (west Bolivia and northwest Argentina), while the remaining palms could be subdivided into two groups: the north (parts of west Brazil) and a larger group that includes palms located in the south (northeast Argentina and south Paraguay) and center (north Paraguay, east Bolivia and parts of west Brazil) of the area of occurrence. To a large extent, the hierarchical clusters did not overlap in these regions, showing a parapatric distribution, and there was a good correspondence between geographic distribution and genetic distance between individuals. The admixture analyses showed that the populations were not strictly delineated and suggested gene flow between most sub-populations. Our results suggest that *A. totai* has a relatively panmictic species distribution, with more genetic diversity within than between populations, but nevertheless highlights geographic location as a significant factor in subpopulation structure within this species.

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Deciphering the nectar secretion physiology of evolutionarily specialized trichomatous extrafloral nectaries in *Clerodendrum chinense* (Osbeck). Mabb

S. Paul<sup>1</sup>, A. Mitra<sup>1</sup>

<sup>1</sup>Indian Institute of Technology Kharagpur, Agricultural and Food Engineering Department, Kharagpur, India

Extrafloral nectaries are specialized nectar-secreting secretory structures present on both vegetative as well as reproductive parts of many plants, mainly provide indirect defense against herbivore attacks by recruiting ants, predators, and parasitoids, but not involved in pollination. Thus, these structures maintain mutual relationships between arthropods and plants. *Clerodendrum chinense* is an ant-guarded herb belonging to the family Lamiaceae with a patelliform-shaped nectary gland clustered at the lamina base and the calyx outer surface. These patelliform nectaries have evolved from simpler secretory trichomes in the course of evolution. Functionally, aggregation of many secretory trichomes confined to a small region forming patelliform nectary can be as cost-effective as big glands with complicated architecture. In this study, we used histochemical, ultrastructural, and metabolomics approaches to check and compare the complete metabolic machinery necessary for nectar production in leaf and calyx nectaries. *Both the nectaries* secrete nectar in a developmentally programmed manner, with younger nectaries secreting more than the older senescent ones. Light microscopic study of both the nectary glands revealed four distinct tissue regions: the outer secretory layer, an intermediate layer, middle nectariferous tissue, and lower sub-nectariferous tissue. Histochemical staining and ultrastructural study showed the presence of a large number of mitochondria in nectariferous tissue indicating their activeness in nectar synthesis and secretion while the intermediate layer was found to be lipid-rich, which regulates the water flow in the nectar by acting as a hydrophobic barrier. Presence of vascular patches with prominent phloem elements and rudimentary xylem tissue confers more sweeter nectar production in calyx nectaries as compared to leaf nectaries, attracting more ants. Metabolomic analysis showed the presence of higher metabolites in the nectary tissues and phloem sap during the active secretion phase. Additionally, an *in-situ* enzyme histolocalization study showed increased invertase activity in the nectary tissue confers high metabolic activity in the nectariferous tissue region, which corresponds to higher nectar secretion. In conclusion, this study illuminates the cell physiological machinery involved in the nectar secretion which advances our understanding of both its physiological and ecological purposes.

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Investigating Phenolics in Response to UV Exposure in the Zygnematophycean Alga *Mesotaenium endlicherianum*

C. Kunz<sup>1</sup>, J. Fürst-Jansen<sup>1</sup>, T. Darienko<sup>1,2</sup>, I. N Abreu<sup>1,3</sup>, M. Lorenz<sup>2</sup>, J. de Vries<sup>1,4,5</sup>

<sup>1</sup>Institute of Microbiology and Genetics, Department of Applied Bioinformatics, Göttingen, Germany

<sup>2</sup>Albrecht-von-Haller Institute for Plant Science, Department of Experimental Phycology and SAG Culture Collection of Algae, Göttingen, Germany

<sup>3</sup>Albrecht-von-Haller Institute for Plant Sciences, Department of Plant Biochemistry, Göttingen, Germany

<sup>4</sup>Campus Institute Data Science (CIDAS), Göttingen, Germany

<sup>5</sup>Göttingen Center for Molecular Biosciences (GZMB), Göttingen, Germany

For successful plant survival, organisms must be able to respond dynamically to rapidly changing environmental conditions. The response to abiotic challenges was crucial in the process of terrestrialisation that led to embryophytes. Based on phylogenetic analyses, their closest streptophyte algal relatives are Zygnematophyceae, on which this contribution focuses. We exposed *Mesotaenium endlicherianum* to UV-B irradiance to elucidate its response to this major abiotic stressor. Contextualised with data from embryophytes, we aim to infer the underlying molecular stress-response toolkit that was used during plant terrestrialisation. We designed two UV-B stress setups, one focussing on the early metabolic response during an UV-B exposure of three hours followed by three hours of recovery with hourly sampling. The second setup investigates the late response after repeated UV-B exposure. For this, *Mesotaenium endlicherianum* was exposed to four hours of UV-B irradiance for three days followed by a day of recovery and sampling after 96 hours. Additional (photo)-physiological measurements were taken and revealed an expected drop in average quantum yield. Untargeted metabolite fingerprinting analysis was performed using an Ultra High Pressure Liquid Chromatography Quadruple Time Of Flight Mass Spectrometer (UHPLC Q-TOF MS) system. UV-induced metabolites such as phenylpropanoid derived compounds and purpurogallin have been identified in preliminary analyses. Additionally, transcriptomic analyses will reveal underlying gene regulatory mechanisms responsible for UV-B tolerance.

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Chronological transcriptome reveals the common program for gynoecium development in eudicots

D. Kong<sup>1</sup>, L. H. Nguyen<sup>1</sup>, A. Becker<sup>1</sup>

<sup>1</sup>Justus-Liebig-University Giessen, Botanical Institute, Gießen, Germany

The growth and development of gynoecium are crucial components of the reproductive process in flowering plants. The diversity of gynoecium and floral architecture among different plant species is a significant aspect of plant evolution. Although many of the regulatory genes involved in gynoecium have been characterized, we know little about the changes in GRNs that are involved in the dynamic development of gynoecium at high resolution. California poppy (*Eschscholzia californica*), a member of Ranunculales, which is a sister lineage to all other eudicots. Here, we present high-resolution profiles of a range of expression dynamics from the floral meristem (FM) to the gynoecium in California poppy obtained by laser microdissection and analyzed a large set of regulatory factors by gene co-expression clustering. This includes, but is not limited to, YABBYs, MADS transcription factors, and ARFs that are differentially expressed at early and late stages of morphogenesis, and some of them have been shown to play important roles in floral meristem determinacy, such as *EscaLFY*. We further compared the dynamic transcriptome clustering of gynoecium in California poppy (dried dehiscent fruits) with Arabidopsis (dried dehiscent fruits) and tomato (fleshy fruits). We characterized the expression trends of a range of homeotic genes during gynoecium development and described the conservation of GRNs in fruit morphogenesis after 127 Mya of independent evolution.

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Phylo- and cytogenetics unravel the complex evolutionary history of autumn-flowering Iberian crocuses

R. An<sup>1</sup>, D. Harpke<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genebank, Gatersleben, Germany

The Iberian autumn-flowering *Crocus serotinus* group, comprising *C. clusii*, *C. cobbii*, *C. nudiflorus*, and *C. salzmannii*, harbours several populations with unclear taxonomic affiliation. Variable genome sizes and chromosome numbers indicate the presence of hybridization, which might be one of the main reasons for the taxonomic uncertainties. Therefore, we aimed to unravel phylogenetic relationship, identify hybrids, and analyze the karyotype evolution of this group. We employed a comprehensive approach including phylogenetic analyses of two chloroplast regions, genotyping-by-sequencing (GBS) of 279 individuals representing 110 populations, rDNA spacers of 49 populations, comparative analysis of repetitive DNA proportion in 26 individuals, genome size measurements and chromosome counting. This approach enabled the identification of 8 diploid taxonomical units and at least three different allopolyploidizations, one autopolyploidization, one homoploid hybridization. The polyploids exhibit genome size from  $2C = 6.7$  to  $9.2$  pg with chromosome counts of  $2n = 36, 44, 48$ . In contrast, diploids are characterized by chromosome counts of  $2n = 20, 22, 24$  genome size ranging from  $2C = 3.3$  to  $4.6$  pg with the exception of *C. salzmannii* ( $2n = 22, 24, 2C = 4.8$  to  $7.2$  pg). Our comparative analyses of repetitive DNA, along with GBS and nuclear single-copy markers data, suggest that the larger genome of *C. salzmannii* did not originate by recent polyploidization or repetitive DNA amplification, but is likely caused by an earlier polyploidization event followed by diploidization. Chromosome numbers in the diploids are likely caused by descending dysploidy. In several cases, telomeres are located not only at the terminal regions of chromosomes but also along the chromosome arms, probably as a result of chromosomal rearrangements. Through phylogenetic analysis of whole chloroplast genomes from this group and outgroups, our findings indicate that diversification in this group began approximately 6 million years ago, following their migration from the western Balkans and Italy to the Iberian Peninsula. Furthermore, the chloroplast *ycf1* haplotype network reveals distinct biogeographic patterns within the Iberian Peninsula.

Keywords: crocus, genotyping-by-sequencing (GBS), phylogeny, hybridization, polyploidization, chromosome rearrangement

P 300

Application of B-class genes of flowering on species-rich genus *Salvia* – Duplications and variabilities as potentials drivers for species diversity

S. Wetters<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Joseph Gottlieb Kölreuter Institute of Plant Science (JKIP), Karlsruhe, Germany

The genus *Salvia* sensu lato, comprising around 1000 species, half of which are found in the Americas, belongs to the taxonomically most challenging genera within the Lamiaceae. A part of the genus' diversity can be ascribed to the staminal lever mechanism as changes in geometry of the sexual organs may establish propagation barriers. However, the structural, functional, and evolutionary context of the underlying genes has not yet been elaborated. While B-class genes of flowering, *GLOBOSA* and *DEFICIENS*, are known as factors defining the identity of petals and stamen, they maintain their expression in petals and stamen throughout anthesis for both, *Salvia pratensis* L. as species from Europe, and *Salvia elegans* Vahl from the New World [1]. In-depth analysis of B-class flowering genes *GLOBOSA* and *DEFICIENS* revealed the typical MADS-MIKK type transcription factor domains. Phylogenies for *GLOBOSA* and its binding partner *DEFICIENS* demonstrates a genus-wide duplication of *DEFICIENS* and a specific duplication of *GLOBOSA* in all *Salvia* species of the New-World biodiversity hotspot. Hence, a duplication of *GLOBOSA* might have enabled the intense radiation of New-World *Salvia* by neo-functionalisation of a flower identity gene for morphogenetic control of corolla and stamen geometry [1][2]. For *DEFICIENS*, two paralogs have been shown that can be phylogenetically strictly delineated from their closely related heterodimeric interaction partner *GLOBOSA* and simultaneously varied remarkably among themselves in their intronic structure and coding sequences. Within these paralogs of *DEFICIENS*, striking differences in the second and third subdomain of the K-box domain (responsible for dimerization and tetramerization) were uncovered. Further analysis of the variable intronic regions of B-class genes revealed an extreme intraspecific divergence of the intron 4 of *DEFICIENS* paralog 1 within *Salvia pratensis*. The biological reason for the striking divergence of intron 4 remains enigmatic, but its application for population studies could function as proxy for genetic variability of the species.

References

[1] Wetters, S. (2020). Evo-devo of flowers and flowering genes: *Salvia* as a case study. PhD thesis, [publikationen.bibliothek.kit.edu](http://publikationen.bibliothek.kit.edu)

[2] Wetters, S. and Nick, P. (2024). B-class gene *GLOBOSA* – A facilitator for enriched species diversity of *Salvia* in the New World? *Horticulture Research*, under revision

P 302

The *Crocus* panrepeatome: dynamics and dysploid

N. Waminal<sup>1</sup>, F. Blattner<sup>1</sup>, D. Harpke<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Experimental Taxonomy, Gatersleben, Germany

Among the ~240 *Crocus* species, chromosome numbers vary considerably ( $2n = 6, 8, 10, 12, 14, 16, 18, 20, 22, 23, 24, 26, 28, 30, 32, 34, 36, 44, 48, 64$ ) making *Crocus* one of the most diverse genera in terms of chromosome number among those with monocentric chromosomes. We hypothesize that repeat dynamics influenced chromosomal rearrangements and descending dysploidy contributing to chromosome number diversification in *Crocus*. To explore this hypothesis, we analyzed the *Crocus* panrepeatome from 215 samples representing the entire genus and performed repeat and single-copy chromosome-specific FISH to visualize descending dysploidy. Genome proportions of repeats ranged from 35–73% significantly influencing genome size differences. For example, genome size was significantly negatively correlated with chromosome number among diploids in the series *Verni* indicating dysploidy. Out of 41,084 repeats including transposable elements and satellite DNA 2,210 were significantly amplified in either of the *Crocus* subgroups. While some repeats preferentially amplified in specific clades, they could be detected in low copies in other clades supporting the library hypothesis that shared repeats are differently amplified between related taxa as well as their potential contribution to cladogenesis. Some microsatellites (2–20 nt) also showed clade-specific amplification and co-amplification with certain repeats. One example is the vertebrate-type telomeric repeat (TTAGGG)<sub>n</sub> which did not only replace the Arabidopsis-type telomeric repeat (TTTAGGG)<sub>n</sub> at the ends of chromosomes but also co-amplified by insertion in some *Ty1/Copia* LTR retrotransposons. FISH analysis of an abundant satellite repeat (Crsat042) in series *Verni* showed incremental amplification along with descending dysploidy. Moreover, separate chromosomes of *C. longiflorus* ( $2n = 28, 2C = 3.2$  pg) formed fused chromosome blocks in *C. vernus* ( $2n = 8, 2C = 5.7$  pg) flanked by Crsat042, confirming descending dysploidy by chromosome fusion that seems facilitated by amplification of repetitive elements. This work shows the relevance of panrepeatome analysis in obtaining a comprehensive picture of the interplay between repeats and karyotype changes enhancing our understanding of the descending dysploidy in *Crocus* which may be similar in other plant groups.

P 303

Population genetics of *Crassula helmsii* (T. KIRK) COCKAYNE (Crassulaceae) within two regions of German

B. Feller<sup>1</sup>, C. M. Müller<sup>1</sup>, V. Wissemann<sup>1</sup>

<sup>1</sup>Justus-Liebig-Universität Gießen, Spezielle Botanik, Gießen, Germany

The Project examines the diversity of invasive and potential invasive aquatic alien species in Hesse. *Crassula helmsii* (New Zealand pygmyweed) is an invasive alien species to many countries in the northern hemisphere. Its distribution began in the early 20th century and it is widespread since the 1980's. Besides other alien species, *Crassula helmsii* is one, that has already a great appearance in coastal Germany's ponds and lakesides. It is affecting species growth and species richness by occupying resources and space in the locality and gives a significant impact on the ecology. But only little is known about the genetic composition within the populations and among the regions. Our results show only a small molecular variance between populations or regions (Hesse and Schleswig Holstein) but a high molecular variance within those populations. The populations of *Crassula helmsii* have a middle to low genetic diversity. So far, we state multiple import events from one locality with few but different genotypes.

P 304

Importance of Soil Silicon Availability in Drought Stress Tolerance of Annual Species in Arid and Semi-arid Rangelands

J. Ouyang<sup>1</sup>

<sup>1</sup>Leibniz-Zentrum für Agrarlandschaftsforschung (ZALF), Landscape Functioning, Müncheberg, Germany

Silicon (Si) in soil is mobilized from amorphous silicon (ASi) and absorbed by plants as silicic acid. In addition to that, Si also serves other purposes, such as boosting plant nutrient absorption. The improvement of soil water holding capacity and plant available water can be improved by increasing the amount of ASi in the soil. Drought, being one of the most important abiotic stresses, influences ecosystem function and causes dramatic yield reductions, particularly in drylands. Previous studies mentioned that Si concentration can influence drought resilience in several plant species. However, the interactions between Si on ecosystem functioning and plant community to drought responses are poorly understood for drylands. To get a comprehensive knowledge of plant development and drought resistance, we exposed the 9 selected annual species from 3 functional groups (grasses, forbs, and legumes) in Si-rich and Si-poor soil. These plants experienced a moderate drought period during the vegetative phase in a greenhouse experiment. Soil nutrient stoichiometry, plant nutrient concentration, plant growth, and relevant functional traits were analyzed further.

Obtained results demonstrated that the addition of silicon availability resulted in a surprising alleviation in drought stress tolerance of plants, enhanced Si absorption, and translocation to shoots to enhance plant development, especially among grass species. Interestingly, legumes are recognized as poor Si accumulation species due to a lack of Si-transporters, while Si availability still has a great effect on their relevant ecological processes. In this study, slightly negative effects were detected in legumes under higher Si availability, suggesting the potential competition of water between the rhizosphere and ASi. The differences in response to soil Si availability observed and evaluated among the 9 different species in this study were attributed to the capacity to improve Si availability in the rhizosphere, uptake, and potential allocation to shoots. Theoretically, this aims to understand the mechanisms underlying community assembly; practically, it focuses on managing and conserving drylands under global change.

P 305

## Revolver flowers – from morphology to pollinator behaviour

J. Jeiter<sup>1</sup><sup>1</sup>TU Dresden, Chair of Botany, Dresden, Germany

Revolver flowers represent a specific type of floral architecture in which the nectar reward is divided into several equal portions, evenly distributed around the centre of a radial flower. In the literature, revolver flowers are associated with separate compartments, each containing a portion of the reward. Well known examples are *Aquilegia* (Ranunculaceae) with five separate spurs or *Geranium robertianum* (Geraniaceae) with five chambers formed by the sepals, petals and stamens. Other examples include the genus *Codon* (Codonaceae), with 10-12 chambers separated by septa, and many species of Loasaceae, where five staminodial complexes between the petals contain the nectar. These few examples already demonstrate that revolver flowers are found throughout angiosperms, raising the question of why this type of floral architecture has evolved so commonly and what are the evolutionary drivers behind it?

Preliminary data suggest that revolver flowers are even more widespread than described in studies of radial flowers with separate nectary compartments. In numerous flowers, nectar is secreted from separate glands, and in many large-flowered species, the secreted nectar does not form a large volume but is deposited as a thin film on the surface of the nectary. In both cases, the typical revolver-flower behaviour can be induced in the pollinator, i.e. the pollinator positions itself on the flower and moves around the central axis of the flower, probing each of the compartments. Despite this apparent abundance of revolver flowers, there is surprisingly little data on their morphology, evolution and function, especially basic data such as nectar characteristics and observations of pollinator behaviour.

In this poster I will describe a way to study revolver flowers and their functionality from a morphological and ecological perspective. Hydrophyllaceae will be used as an example to show the complex nature of revolver flower evolution. Furthermore, I will give an overview of where revolver flowers can be found and what aspects need to be considered when defining, which flower acts as a revolver flower under which conditions.

P 306

## Genetic basis and environmental plasticity of meiotic recombination rates

S. Dreissig<sup>1</sup>, C. Waesch<sup>1</sup>, M. S. Seidel<sup>1</sup>, Y. Gao<sup>1</sup><sup>1</sup>Martin-Luther-University Halle-Wittenberg, Halle a. d. Saale, Germany

Meiotic recombination is fundamental to eukaryotic reproduction, as it ensures the formation of balanced gametes, and because it generates genetic diversity upon which natural selection can act. Patterns of recombination vary between species, populations, individuals, sexes, and along chromosomes, and, are influenced by environmental factors. While the general meiotic machinery is highly conserved across species, there is considerable genetic diversity in meiotic genes even within a species, suggesting an evolutionary potential. Studies in plants and animals are beginning to shed light on the genetic basis of meiotic recombination rate variation, often via genome-wide association scans. In our work, we use rye (*Secale cereale* L.), a self-incompatible, wind-pollinating grass species as a model system. We used genome sequence data of 1650 rye individuals, performed population-scale single-pollen sequencing across 1320 pollen of 252 plants, and used quantitative cytogenetics methods to explore the genetic basis and environmental plasticity of meiotic recombination rate variation. Through these approaches, we uncovered heritable variation in recombination landscapes, with several loci of large effect size, and a significant effect of temperature and nutrient availability on recombination rates. Furthermore, we found differences in recombination landscapes when measured before and after effective fertilization (i.e. in male gametes vs. offspring), suggesting that recombination in peri-centromeric regions might have negative consequences and be selected against at population-level.



P 307

Genetic diversity and structure of natural *Shorea robusta* populations in India as revealed by microsatellites markers

G. Mishra<sup>1</sup>, S. Pandey<sup>2</sup>, R. Kant<sup>1</sup>, R. K. Meena<sup>1</sup>, M. S. Bhandari<sup>1</sup>

<sup>1</sup>Forest Research Institute, Dehradun, Uttarakhand, India, Division of Genetics and Tree Improvement, Dehradun, India

<sup>2</sup>Forest Research Institute, Department of Forest Protection, Dehradun, India

The foundation for effective selection practices, breeding, and conservation of forest trees lies in understanding genetic variations, which can be evaluated through the use of molecular markers. *Shorea robusta*, a wind-pollinated timber species of significant commercial value in southern Asia, has experienced a sharp decline in stocks. It is attributed to factors such as excessive logging, inadequate natural regeneration, overexploitation, and habitat fragmentation. Consequently, there is an urgent requirement to develop robust genetic conservation approaches.

In this study, we investigated the genetic diversity and structure of fifteen populations using microsatellite-based marker in the state of Uttarakhand (India). Out of 60 primers, 24 showed polymorphisms. Notably, ten primers were used omitting 14 null alleles. Estimates of genetic diversity (NA=3.69, HO=0.377, HE=0.555) were similar when compared to other tropical tree species. The polymorphism information content (PIC) was noted as 0.252. Also, the gene flow estimate was  $Nm=0.728$  migrant per generation that suggested a very limited gene flow with very low value of genetic differentiation ( $F_{ST}=0.281$ ). In the structural analysis, bar plot for estimated Q-matrix at K=2 for different sampled populations revealed two distinct clusters. Moreover, the AMOVA revealed that most of the genetic variation (76%) was confined within population.

The accessibility of sequence information and novel SSR markers through our study, potentially enriches the current knowledge of the genomic background for *S. robusta* and for implying conservation programme when extrapolated on a large scale. Based on results, protection of populations is recommended for the sustainable conservation of genetic resources and rare alleles.

P 309

The role of LEUNIG and SEUSS transcriptional regulators during land plant evolution

J. V. Garrecht<sup>1</sup>, J. Ingelfinger<sup>2</sup>, A. Becker<sup>1</sup>

<sup>1</sup>Justus-Liebig-Universität Gießen, Institut für Botanik, Gießen, Germany

<sup>2</sup>Technical University of Kaiserslautern, Kaiserslautern, Germany

The adaptation of reproductive strategies to the terrestrial environment was one of the crucial steps of the transition from water to land that plants had to overcome. During land plant evolution, various adaptations emerged, from the simple, water-dependent fertilization mechanism of bryophytes to the morphologically highly diverse flowers of angiosperms. This wide range of reproductive strategies makes it especially noteworthy that some of the essential regulators of flower development in angiosperms are present in all major land plant lineages.

In *Arabidopsis thaliana*, the transcriptional regulators LEUNIG (LUG) and SEUSS (SEU) play an important role in flower development: LUG and SEU first form a heterodimer, which then can interact with floral organ identity proteins such as APETALA1, and other developmental regulators such as AINTEGUMENTA.

The moss *Physcomitrium patens* encodes, like *Arabidopsis*, several homologs of LUG and SEU, raising the questions of (1) how exactly did these regulators co-evolve with each other and other transcriptional regulators to become such important floral regulators, and (2) if their ancestral function was also related to sexual development.

We use Yeast-Two Hybrid and Bifluorescence Complementation assays to study protein interactions and the CRISPR-Cas system for multiplex *Physcomitrium* mutagenesis. We present our data on (1) the protein interaction of the *Physcomitrium* and other land plant LUG and SEU homologs and the protein domains important in the co-evolution between LUG and SEU and their interactors, heterodimer formation and for target protein interaction. Further, we show that removing LUG and SEU function from the *Physcomitrium* genomes results in growth retardation and will present detailed phenotypic analysis of the LUG and SEU mutants of *Physcomitrium*.

P 310

Status, changing landscape and farmers criteria for the continuity of paddy (*Oryza sativa*) landraces in the upland ecosystem of Jharkhand, India: A conservation perspective

N. Kumari<sup>1</sup>, S. Choudhary<sup>2</sup>, B. Jha<sup>1</sup>

<sup>1</sup>Birsa Agricultural University, Department of Extension Education, Kanke, India

<sup>2</sup>National Bureau of Plant Genetic Resources, Ranchi, India

The present study involved paddy landraces naturalized widely across Jharkhand, India, at an altitude range of 6 (Sahibganj) to 768 (Garhwa) metres above sea level. They naturalized across diverse niche areas, including fat to sloped uplands, lowlands, rivulet edges, pond/dam basins, swampy regions with varying altitudes, and broad edaphic ranges. The high number of paddy landraces collected from the Central and North-Eastern Plateau (CNEP) sub-zone was well supported by the complementarity analysis. Landraces from iron- and aluminium- (West and East Singhbhum) and mica- (Giridih and Koderma) rich regions are a potential source of extraordinary mineral tolerance and bioaccumulation. These ecologically stressful habitats owing to limitations in soil pH, soil texture, and other factors are considered potential sites for the evolution of unique traditional varieties. However, the resource base was critically genetically eroded from 2005 to 2021, particularly in Ranchi, Hazaribagh, Ramgarh, Chatra, Bokaro, Dhanbad, Koderma, Deoghar, and Palamu. The CNEP sub-zone witnessed the most genetic erosion of landraces. At the same time, the continued cultivation of landraces in the steeper landscape of both the Western Plateau and South Eastern Plateau sub-zones underscored their value in risk aversion under challenging environmental conditions. Selection criteria analysis of continued paddy landraces demonstrated farmers' preference for functional traits that influence livelihood security in the local context. These traits are vital for mainstreaming registered landraces into the production chain under changed climatic conditions

P 311

Engineering the Wheat and Barley Spike Architecture by Targeted Mutagenesis of the Transcription Factors Branched Head and Squamosa-promotor Binding Protein-like 14 and 17

C. Hertig<sup>1</sup>, C. Marthe<sup>1</sup>, A. Junker<sup>2,3</sup>, R. Koppolu<sup>4</sup>, T. Schnurbusch<sup>4</sup>, J. Kumlehn<sup>1</sup>

<sup>1</sup>IPK Gatersleben, Physiology and Cell Biology, Gatersleben, Germany

<sup>2</sup>Syngenta Seeds GmbH, Bad Salzuffen, Germany

<sup>3</sup>IPK Gatersleben, Molecular Genetics, Gatersleben, Germany

<sup>4</sup>IPK Gatersleben, Breeding Research, Gatersleben, Germany

The use of RNA-guided Cas9 endonucleases in genome engineering represents a significant advancement in the breeding of cereal species, including wheat (*Triticum aestivum*, L.) and barley (*Hordeum vulgare*, L.). This technology facilitates the targeted induction of specific mutations in trait-relevant genes, thereby accelerating breeding processes.

Target motifs were selected in the wheat gene *BRANCHED HEAD* (*BH*) and in the barley genes *SQUAMOSA-PROMOTOR BINDING PROTEIN-like 14* and *17* (*SPL14/SPL17*). The suitable guide RNA (gRNA)/*cas9* constructs were then cloned. Stable genetic transformation was carried out by ballistic gene transfer (wheat) or *Agrobacterium*-mediated introduction of T-DNA (barley) to immature embryos. The subsequently regenerated plants were examined for targeted mutations and the presence of T-DNAs.

In wheat, a collection of individuals carrying various mutated alleles and their combinations was deployed. Plants with one or two mutant *bh* homoeoalleles showed phenotypic modifications in ear phenotype, grain size, grain number, and partially in roots. The presence of three mutated *bh* homoeoalleles typically led to drastic changes in the ears, forming supernumerary spikelets and branched spikes, associated with a significant loss in fertility. The growth of pre-selected mutants under field-like conditions revealed a potential for higher yield. Overall, the investigation further elucidated the importance of the transcription factor BH for the development of the spike and the grains as well as of the roots.

The mutations in barley *spl14* led to a delayed generative development and, in some cases, to the reactivation of lateral spikelet formation. In addition, the ears were shortened and formed fewer grains. The mutations produced in *spl17* did not affect generative development, but did affect ear length and grain formation. Plants carrying double mutations in *spl14* and *spl17* showed a drastic aggravation of the *spl14* phenotype, with their generative development being severely disrupted so that no ears were formed. This suggests that SPL14 exerts a major function in early generative development, while SPL17 is acting as a co-factor.

References

Hertig, C. W. 2023. Leibniz Universität Hannover, Dissertation, XX, 217 S., DOI: <https://doi.org/10.15488/13269>

Hertig, C. W. 2023. *Vorträge für Pflanzenzüchtung* 89: 11-15

Thiel, J., R. Koppolu, C. Trautewig, C. Hertig et al. 2021. *Science Advances*, 7: eabf0832., DOI: <https://doi.org/10.1126/sciadv.abf0832>

P 312

Can you identify Canadian Arabidopsis? You can now! – A comprehensive, reliable synoptic reference collection of *Arabidopsis thaliana* in Vancouver, BC

I. Kontos<sup>1</sup>, L. P. J. Lipsen<sup>1</sup>, M. Todesco<sup>2</sup>

<sup>1</sup>University of British Columbia (UBC), Beaty Biodiversity Museum, Vancouver, Canada

<sup>2</sup>University of British Columbia (UBC), Botany (Michael Smith Laboratories), Vancouver, Canada

*Arabidopsis thaliana* was the first plant to have its genome fully sequenced and has become a model species for many plant biology fields. Its short life cycle and relatively small genome size allow plant scientists to quickly answer outstanding questions.

While *A.thaliana* populations harbor huge research potential for studies, being able to properly identify *A.thaliana* in the field is crucial to the scientific community. As it becomes rarer for plant scientists to have the traditional skill set of field taxonomy, it is more important than ever to have a comprehensive, curated collection of *A.thaliana* to ensure accurate identification, including full documentation of its variation, available to all researchers worldwide.

This synoptic specimen reference collection of *A. thaliana* was collected from 50 wild populations located in Vancouver, British Columbia, Canada. It documents the range of variation found within and among populations in the area and is available on-line at the UBC Herbarium. This collection represents a reliable genetic resource and reference for researchers to become familiar with the most common morphological of this important research species.

P 313

Investigating chloroplast development in barley via functional characterization of *HvLST* and *HvCMF* genes

S. Jhingan<sup>1</sup>, R. E. Hoffie<sup>2</sup>, A. Knosp<sup>2</sup>, S. Sommerfeld<sup>2</sup>, J. Kumlehn<sup>2</sup>, N. Stein<sup>1,3</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genomics of Genetic Resources (GGR), Gatersleben, Germany

<sup>2</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Plant Reproductive Biology (PRB), Gatersleben, Germany

<sup>3</sup>Martin Luther University Halle-Wittenberg, Halle a. d. Saale, Germany

Although genes encoded in the plastome are responsible for plastid gene transcription, translation, and photosynthesis, chloroplast biogenesis is mainly governed by genes encoded in the nucleus through the interaction of the nuclear and chloroplast genomes. Loss of function in these underlying genes can compromise normal chloroplast development, resulting in plants with reduced photosynthetic efficiency, variegated leaves, albino seedlings, or even lethal effects. Previously, mutant collections were screened using low resolution genetic mapping, whole genome re-sequencing and comparative functional analyses to identify candidate genes controlling chloroplast development and thylakoid structure in barley. Consequently, the CCT Motif Family genes *HvCMF3* and *HvCMF7* were identified and functionally characterised via random mutagenesis (EMS TILLING) and CRISPR-Cas9 mediated targeted genome editing. Using a similar approach, the *ATP-Dependent Clp Protease Subunit C1* (*HvClpC1*) was later proposed as a candidate for the variegated barley mutant termed *luteostrians* (*LST*). In this project we aim to further elucidate chloroplast development in barley by inducing CRISPR-Cas9 mediated loss-of-function mutations in three and nine homologs of the *HvLST* and *HvCMF* gene families, respectively. Functional mutations in *HvClpC1* and its homologs in barley can validate their putative role in normal maturation and development of chloroplasts. On the other hand, induced functional mutations in the *HvCMF* homologs and the subsequent pyramiding of resulting mutant alleles will shed light on their possible dosage-dependent role in this context.

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The Evolutionary History of *Ranunculus* section *Euromontani*E. S. Lorberg<sup>1</sup>, S. Tomasello<sup>1</sup>, E. Hörandl<sup>1</sup><sup>1</sup>University of Göttingen, Department of Systematics, Biodiversity and Evolution of Plants, Göttingen, Germany

Questions: The *Ranunculus montanus* group, or section *Euromontani*, comprises ca. 15 diploid and polyploid species and is widespread across mountain ranges of Central, Eastern, Southern Europe, and Northern Africa. Its monophyly within the genus is well-supported, yet relationships and speciation processes within the section are largely unclear (1).

Methods: Hybrid-capture-based target enrichment of 700 nuclear single-copy gene regions was utilized to gather sequence data from 34 individuals from 12 species within the complex. Whole genome sequencing was used as well to acquire plastid DNA. Phylogenies were then inferred using RAxML (2) based on both nuclear and chloroplast DNA. The divergence time of the section was estimated using BEAST 2 (3), and ancestral ranges were reconstructed with BioGeoBEARS (4) to establish whether speciation occurred through dispersal or vicariance. As ploidy levels are not known for all species of the section, they were inferred from sequence data with HMMploidy (5). Phasing was used to identify the putative parent species of polyploids with Hybphaser (6).

Results: The plastome was phylogenetically not informative but showed a geographic pattern. Nuclear DNA produced a well-supported and resolved phylogeny, with the exception of some incongruent positions of polyploids such as *R. montanus* s.str. Polyploids were of allopolyploid origin. The crown age of section *Euromontani* was estimated as 2.32 Mya. Ancestral area reconstruction indicates the European Alps as the most likely distribution range of the last common ancestor.

Conclusions: The climatic deterioration during the early Pleistocene likely permitted the expansion of the cold adapted ancestor of the *Ranunculus* section *Euromontani* to other mountain ranges. The subsequent climatic oscillations may have served to periodically isolate populations from different mountain ranges, allowing allopatric speciation and rapid radiation. Secondary contact hybridization might have resulted in allopolyploid speciation in the Alps.

## References

- (1) Hörandl & Emadzadeh 2012. *Perspect Plant Ecol*. DOI: 10.1016/j.ppees.2012.04.001
- (2) Alexandros 2014. *Bioinformatics*. DOI: 10.1093/bioinformatics/btu033
- (3) Bouckaert et al. 2019. *PLOS Comput Biol*. DOI: 10.1371/journal.pcbi.1006650
- (4) Matzke 2018. DOI: 10.5281/zenodo.1463215
- (5) Soraggi et al. 2022. *Peer Community J*. DOI: 10.24072/pcjournal.178
- (6) Nauheimer et al. 2021. *Appl Plant Sci*. DOI: 10.1002/aps3.11441

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## Genetic Engineering of Oats and Lupins

K. M. Pathi<sup>1</sup>, T. Sprink<sup>1</sup><sup>1</sup>Julius Kühn-Institut, Institut für die Sicherheit biotechnologischer Verfahren bei Pflanzen, Quedlinburg, Germany

Genetic engineering as well as modern breeding in oats and lupins is hindered by inefficient plant tissue/ cell culture protocols. Establishing robust regeneration protocols is imperative for both classical and modern breeding. Furthermore, the availability of complete genome sequences for lupin- and oat species provides a solid foundation for genome-editing studies and accelerated breeding initiatives.

Oats are a globally significant crop, yet existing protocols, based primarily on Garry and GAF lines, are limited by their agronomically poor traits. To address these limitations, extensive efforts have been made to establish an effective oat tissue culture system for commercial oat cultivars. Various explant sources, including grains, split grains, mature embryos, leaf bases, immature embryos, shoot apical meristems, and roots, have been utilized to induce callus formation and regeneration. The most successful shoot multiplication, averaging 30 shoots per explant, was achieved from embryogenic callus. *Agrobacterium*-mediated transformation and bombardment techniques have been employed, resulting in the emergence of GFP-positive callus, shoots, roots, and PCR-positive plants. Protoplast technology established, isolating viable protoplasts with over 80% transformation efficiency via PEG-based transfection. The protocol effectively addresses genotype dependency in plant tissue culture, tested on 30 commercial oat accessions, including summer, winter varieties, and the diploid accession *Avena strigosa*. To target the *A. sativa* MLO1 gene with CRISPR approach, guide RNAs were designed, cloned into vectors and transformed. The plants are now in the regeneration stage.

In the study of sweet lupins various explant sources, including hypocotyls, cotyledons, immature zygotic cotyledons, and shoot apical meristems, were investigated to establish a plant cell and tissue culture system. Callus induction from diverse explant sources yielded somatic embryogenic-like structures, particularly from cotyledons and hypocotyls. Embryogenic callus derived from immature zygotic cotyledons facilitated multiple shoot formation. Conversely, embryo epics and shoot apical meristems yielded multiple shoots without callus formation. Embryogenic callus and meristematic regions were successfully transformed using GFP-expressing constructs. Protoplasts were isolated and transfected, achieving a transformation efficiency exceeding 50%. Subsequent culture led to the development of micro-callus.

P 316

Addressing phylogeny and taxonomy of *Betula* L. genus using different approaches

A. Tarieiev<sup>1</sup>, O. Gailing<sup>1</sup>

<sup>1</sup>Georg-August-Universität Göttingen, Forstgenetik und Forstpflanzenzüchtung, Göttingen, Germany

The genus *Betula* L. is represented by common pioneer trees and shrubs of temperate and boreal zones in the Northern Hemisphere. Birches are characterized by high vegetative variability, phenotypic plasticity, a wide range of ploidies (from  $2n=2x=28$  to  $2n=12x=168$ ), and frequent hybridization (especially an introgressive one) between different species. That is why the phylogeny of this group is very complex and remains not well resolved.

In the current study, we tried to infer the phylogenetic relationships between birch species in Europe using different approaches.

1. Phylogenetic reconstruction based on traditional genetic markers: internal transcribed spacers, plastid genes.
2. Phylogenetic reconstruction based on the newly developed set of 20 COS-associated SSR markers.
3. Phylogenetic reconstruction based on complete plastid genome assemblies.

Conclusions

Individual traditional genetic markers in general lack resolution power and can therefore only distinguish major well-established taxa and/or addressing some specific problems.

A newly developed SSR marker set allowed the identification of the clusters of highly distinct taxa: *B. pendula* aggr., *B. nana*, *B. humilis* and *B. pubescens* aggr. It is not possible to resolve fine-scale phylogeny and to infer the phylogeny of polyploid complexes using these markers.

Plastome-based phylogeny in the case of *Betula* L. genus provided significantly better resolution in comparison to previously used markers and therefore is useful for resolving complex groups of closely related taxa. However, it reflects variation in the maternally inherited chloroplast genome and therefore cannot be used for addressing cases of hybridization. There is a need for more high-quality plastome assemblies for different birch taxa.

According to our estimation, the best possible approach to address birch phylogeny would be high-coverage whole-genome sequencing for a range of birch taxa including polyploid ones (preferably long-read with HiC to achieve a better assembly and also to infer epigenetic data) combined with other types of data (morphology, anatomy, “-omix” data, distribution, etc.).

P 317

Water-based solvent-casted films with UV- blocking effect produced from the Antarctic microalga

*Klebsormidium* sp. ASYA19

B. Ballik<sup>1</sup>, O. Aras<sup>2</sup>, T. Çakmak<sup>3</sup>, M. Kazancı<sup>4</sup>, K. Herburger<sup>1</sup>

<sup>1</sup>Biological Sciences, Cell Biology of Phototrophic Marine Organisms, Rostock, Germany

<sup>2</sup>Natural sciences and Engineering, Nanoscience and Nanoengineering, Istanbul, Turkey

<sup>3</sup>Natural sciences and Engineering, Molecular Biology and Genetics, Istanbul, Turkey

<sup>4</sup>Natural sciences and Engineering, Biomedical Engineering, Istanbul, Turkey

Microalgae offer great potential for designing new biomaterials with a broad range of applications. A popular topic is the use of these photosynthetic organisms as raw material for producing bioplastic, giving a sustainable alternative to petroleum-derived products. *Klebsormidium* comprises filamentous aero-terrestrial microalgae that are equipped with a water-repellent layer covering the cell wall to cope with water scarcity. At the same time, due to the harsh conditions in their natural habitat, they have the ability to synthesize secondary metabolites called mycosporine-like amino acids (MAAs) that protect the cell against high PAR and UV. This unique combination of features makes them a promising biological resource for producing sustainable materials. In this study, the solvent casting method was used to obtain bioplastic films with UV-blocking ability from a psychrotolerant epilithic *Klebsormidium* sp. strain (ASYA19) isolated from Horseshoe Island in Antarctica. MAA accumulation was induced using high PAR ( $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Then, the lyophilized microalgae biomass was mixed with distilled water and films were casted. The optimal condition for film casting was determined as a 5% microalgae suspension incubated for 36 hours at 15°C and 70% humidity. Additionally, glycerol (15%, 30% and 45% (w/w)) or polyvinylalcohol (25%, 50%, 75%, 100%(v/w)) were used to increase the thermal and mechanical strength of the film obtained from raw *Klebsormidium* biomass. Mechanical characterization parameters such as tensile stress, elongation (%) and elasticity modulus of films were determined by mechanical tests. Thermal and chemical characterization of bioactive films were measured using TGA-DSC and FT-IR analysis. Additionally, MAA-induced UV blocking effect and microalgae-based antioxidant effect were determined by spectrophotometric methods.

Keywords: Microalgae, mycosporine-like aminoacids, solvent-casting, film, green chemistry, bioplastics

P 318

## The interplay between selection and the genomic landscape during the domestication of grain amaranth

C. Graf<sup>1</sup>, M. Stetter<sup>1</sup><sup>1</sup>University of Cologne, Köln, Germany

The process of adaptation is vital for populations facing changing environments. A number of potentially adaptive traits have been studied intensively, but intrinsic traits like the genomic landscape i.e., the composition and structure of the genome, have received less attention. Multiple features of the genomic landscape influence the molecular outcome of adaptation and recent evidence indicates that some features might be under selection themselves. However, the interplay between the genomic landscape and adaptation remains unclear. To address the effect of selection on the genomic landscape, we study the domestication of the ancient pseudocereal amaranth. Grain amaranth has been domesticated three times independently from the same wild species in different regions of the Americas. Our goal is to understand how parallel selection-changed the crop's genomic landscape. We combine quantitative and comparative genomics to estimate recombination rates and chromatin structures along this adaptive gradient. We are constructing recombination maps for each of the three grain species and their wild relatives from whole genome sequencing of almost 3,000 F2 individuals. Furthermore, we employ ATAC-sequencing to assess chromatin in wild and domesticated amaranth populations. We identified common and species-specific chromatin features, showing that overall chromatin composition is conserved despite domestication selection. We find that up to 2.6% of the genome changed its chromatin state between wild and domesticated amaranth. Integrating these differentially open regions with selective sweep data linked these changes to adaptation and identified two disease resistance genes unique to the change from wild ancestor to *A. caudatus* and *A. cruentus* respectively. The combination of chromatin structure and recombination landscapes along the selection gradient from wild to domesticated amaranth can give insights into the interplay between the genomic landscape and plant adaptation. Our model of repeated adaptation provides the opportunity to understand how the genomic landscape is shaped by selection.

P 320

## Plant species composition and spatial distribution patterns of taxonomic and phylogenetic heterogeneity along an altitudinal gradient in Sham Valley, Ladakh

D. Angmo<sup>1</sup>, S. Kaur<sup>1</sup>, H. P. Singh<sup>2</sup><sup>1</sup>Panjab University, Botany, Chandigarh, India<sup>2</sup>Panjab University, Environment Studies, Chandigarh, India

Understanding plant distribution and community structure along environmental gradients is vital for comprehending the ecological mechanisms driving community assembly in response to changes in environmental factors. Both taxonomic and phylogenetic diversity can provide insights and unravel the relationships between biodiversity and ecosystem functioning. In this study, we used objective analytical methods to examine taxonomic and phylogenetic diversity trends across an elevational gradient in the Sham Valley, Ladakh, in Trans-Himalayan region. To determine species occurrence, extensive surveys were carried out from 2501 to 5500 m a.s.l. A varied group of 211 plant taxa from 153 genera and 44 families was found. The greatest plant diversity was found at 3501-4000 m a.s.l., followed by 3001-3500, 4001-4500, 2501-3000, 4501-5000, and 5001-5500 m. At all altitudes, Asteraceae was the dominating family, with herbs being the main growth type. Taxonomic and phylogenetic diversity varied from 0.29 to 0.98 and 0.17 to 0.66, respectively, when comparing beta diversity pairings from lowest to greatest elevation. Species turnover caused the differences between plant communities at lower and middle or higher altitudes, resulting in taxonomically and phylogenetically diverse assemblages at 2501–3500 m a.s.l. However, the taxonomic and phylogenetic composition of plant communities varied due to nestedness between 4001 and 5500 m a.s.l. Both of these factors affect species distribution at middle elevations (3501–4000 m a.s.l.) for taxonomic beta diversity. Apparently, this elevational zone separates subalpine and alpine ecosystems. It indicates that the trans-Himalayan ecosystem has both biotic resource diversity and spatial heterogeneity. This study significantly enhances our understanding of vegetation organisation in the unexplored trans-Himalayan region, a biodiversity hotspot. The research provides many bioresource applications, including bioresource conservation, climate change prediction, and ecological knowledge preservation.

P 321

Manipulation of flowering time in carrot by gene editing

H. Liu<sup>1</sup>, S. Jackson<sup>1</sup>

<sup>1</sup>The University of Warwick, Coventry, United Kingdom

Carrot (*Daucus carota* L.) is one of the most important cultivated vegetables. As it is usually a biennial, the breeding of carrots is usually a lengthy process unless early flowering can be induced. Commercially, however, premature bolting (early flowering) can result in a complete loss of the commercial value of a carrot crop as bolting affects the growth of the storage tap root. Therefore, being able to control flowering time is very important for carrot breeding and production.

Genetically editing genes controlling flowering in carrots can alter flowering time to create improved varieties with increased yield potential. The main aim of this project is to manipulate carrot flowering time and create two new types of carrots with altered flowering times: an annual carrot (with no vernalisation requirement), and a late-flowering (bolting resistant) carrot. To remove the vernalisation requirement, we targeted the *FLC* & *FRI* genes. To delay flowering, we targeted the *FT* and *GI* genes. To promote the commercialisation potential of the edited crops, the insertion of foreign DNA sequences will be avoided. We are using an improved non-integrative CRISPR approach involving the transformation of protoplasts with Cas9 protein-gRNA ribonucleoproteins (RNPs). The *DcFT*, *DcGI*, and *DcHd3A* have been successfully edited to date, and we have now regenerated several plant lines with different edits in the *DcFT* gene which are now being grown to analyse how their flowering time has been affected.

P 322

CRISPR/Cas9-induced loss of function of *BnHVA22c* and *BnCRT1a* boosts plant immune response and reduces the susceptibility of oilseed rape (*Brassica napus*) to fungal (*Verticillium longisporum*) infection

W. Ye<sup>1</sup>, L. Han<sup>1</sup>, D. Schenke<sup>1</sup>, S. Rietz<sup>2</sup>, D. Cai<sup>1</sup>

<sup>1</sup>Institute of Phytopathology Christian-Albrechts University of Kiel, Department Molecular Phytopathology, Kiel, Germany

<sup>2</sup>NPZ Innovation GmbH, Holtsee, Germany

*Verticillium longisporum* (VI43) is a soilborne hemi-biotrophic fungal pathogen causing stem striping on oilseed rape and severe yield losses. Breeding for resistant varieties is the most promising approach to control this disease. HVA22c and CRT1a are both endoplasmic reticulum proteins. Both genes are up-regulated by VI43 infection. To explore their role in the plant-fungus interaction, we applied CRISPR/Cas in oilseed rape. Both mutants with loss of function showed significantly reduced susceptibility to VI43 infection with impaired disease symptoms compared to the wild-type. Transcriptomic analysis revealed that the loss of function of *BnHVA22c* or *BnCRT1a* in oilseed-rape altered drastically transcriptomic landscapes with many genes (904 DEGs in *Bnhva22c* and 430 DEGs in *Bncrt1a*) downregulated. GO and KEGG analyses identified distinct items and pathways enriched in both mutants, including impaired ER and Golgi apparatus function and enhanced ethylene (ET)-signaling. Although a large number of genes were up-regulated in both wild-type (1105 DEGs) and mutants of *Bnhva22c* (1098 DEGs) and *Bncrt1a* (2260 DEGs) by VI43 infection, we found that a large number of genes related to plant immune responses were highly upregulated in both mutants compared with the infected wild-type, e.g. in the phenylpropanoid biosynthesis, glutathione metabolism, and plant-pathogen interactions, respectively. Noticeably, *MPK3* and its substrates *ACS2* in *Bncrt1a* and *ACS6* in *Bnhva22c* and its downstream gene *WRKY33* were all significantly upregulated, suggesting an enhanced MAPK signaling. In addition, five calcium-dependent serine/threonine kinase genes were highly upregulated in the *Bncrt1a* mutant. Genes known to be involved in plant defence response were highly upregulated by VI43 infection in both mutants compared with the wild type, which include several PR- and PDF proteins, oxidative bursts (*RBOHD*) and strengthening plant cell walls (*CCoAOMT1*, *EXT3*), and reducing the viability of pathogens (*CHI*, *OSM34*). These results demonstrate that HVA22c and CRT1a are functionally required for a fully compatible plant-fungus interaction. Dysfunction of the ER and Golgi network maybe attenuates VI43 virulence and boosts the plant's immune response by e.g. activating MPK3 signaling. This study provides an excellent example for the potential of CRISPR/Cas9 in improving plant resistance to diseases. A possible mode of action is being discussed.

P 323

Inducible CRISPR Cas9 System to study lethal knockouts in *Arabidopsis thaliana*A. Struß<sup>1</sup><sup>1</sup>Ruhr-University Bochum, Molecular and cellular Botany, Dortmund, Germany

Recent studies drew our attention to the predominantly expressed PI4P phosphatases SAC7 and SAC8. To investigate their relevance on a cellular level and their impact on other related proteins, knockouts are required to observe potential changes. In preliminary work we verified a redundancy of those lipid phosphatases which raises the necessity to create double knockouts to perform *in vivo* experiments. While the knockout of SAC8 does not show a phenotype, the knockout of SAC7 leads to impaired root hair growth. The simultaneous knockout of SAC7 and SAC8 yields a lethal phenotype. This aggravates further studies as experiments with the double knockout can only be performed in seedling stage. For this purpose, we developed an estradiol inducible CRISPR Cas9 system to generate knockouts at a desired growth stage while classic knockouts are limited to the seedling stage. To facilitate the genotyping of knockout plants, the system uses two sgRNAs to target one gene. By using two sgRNAs a large segment is removed from the gene, which can be detected by PCR using primers that flank the sgRNA binding sites. Tests of this system show that the induced expression of Cas9 leads to complete gene knockouts. However, our studies showed that not all cells are affected by estradiol which ultimately leads to the generation of chimeric plants. Nevertheless, the previously described phenotypes can be resembled. Wildtype plants that contain the inducible CRISPR system with sgRNAs for SAC7 show impaired root hair growth when grown on estradiol containing medium while plants with sgRNAs for SAC8 are unaffected in growth. The simultaneous knockout of SAC7 and SAC8 leads to the previously described lethal phenotype. This phenotype can be obtained in seedling stage when grown on estradiol medium or in later growth stages when estradiol is applied afterwards. Further test, which are still ongoing, should show the efficiency of the system by evaluating RNA and protein levels post induction.

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## The Science of Blue Flowers: Phylogenetic, Genomic and Transcriptomic Analysis of Blue Flowering Plants

C. M. Dasso<sup>1</sup>, J. I. Lüpkes<sup>1</sup>, V. Berg<sup>1</sup><sup>1</sup>Plant Biotechnology and Bioinformatics, Plant Biology & BRICS, TU Braunschweig, Braunschweig, Germany

Flavonoids, known for their antioxidant properties, are the subject of intense interest in contemporary research. While anthocyanins, particularly those yielding red hues, have received considerable attention, exploring blue flower colouration remains open for significant discoveries. The SynBio2024-Team of TU Braunschweig is dedicated to exploring the phylogenetic origins and identifying mechanisms responsible for blue flower colouration. This phylogenetic approach serves as a valuable tool for systematically studying different colouration mechanisms across various plant lineages, offering insights into their evolutionary history and genetic makeup. Through our research, we investigate various blue-flowering species, aiming to decipher the intricate mechanisms responsible for the creation of blue colour complexes. The formation of the blue cornflower complex is explored in detail. After identifying the genes responsible for the blue flower colouration of the cornflower and the dayflower, validation is performed through transformation into *Arabidopsis thaliana*. We aim to deepen our understanding of blue flower colouration in plants and to explore its potential as a biomarker or for the engineering of ornamental plants.

P 325

## Enhancing Tree Resilience to Climate Change: Biotechnological Strategies and Genome Editing

T. Bruegmann<sup>1</sup>, A. Fendel<sup>1</sup>, V. Zahn<sup>1</sup>, M. Fladung<sup>1</sup><sup>1</sup>Thünen Institute of Forest Genetics, Großhansdorf, Germany

Various breeding and silvicultural strategies are being pursued to adapt forest tree species and even entire forests to climate change. In particular, traits such as drought stress tolerance and resistance to already existing but also novel pathogens are two major tree breeding objectives. However, the velocity of climate change is a major problem that is difficult to reconcile with the long generation cycles of forest trees. Here, the implementation of biotechnology provides promising approaches. Plant biotechnological tools, such as tissue culture, genetic engineering and genome editing, as part of plant breeding, can contribute to a rapid climate change adaptation of forest trees. Advantageously, biotechnological treatments are not dependent on flowering and fruiting.

New possibilities and great potential are offered by genome editing techniques such as CRISPR/Cas, which are already being used intensively in crop research and for an increasing number of tree species. Some methods already developed for crop species have yet to be transferred to forest tree species. Even though the basic CRISPR/Cas mechanism seems to be rather universal, the insertion of the Cas nucleases needs to be further addressed when working with different tree species. In addition to classical, gene technology-based approaches to research, DNA-free approaches are also being pursued to generate nature-identical genetic modifications.

Genome editing is being used to characterize the function of genes and to unravel biochemical pathways. A better understanding of the genetic basis of morphological and physiological traits can support genotype-based breeding. Although most of the abiotic and biotic traits are encoded by numerous genes, modifying a few genes can quantitatively increase the resilience against the effects of climate change. However, at least in Europe, regulatory aspects have to be considered when dealing with genome editing.



P 326

Targeted cis-regulatory mutagenesis uncovers pleiotropic effects of the *phototropin2* gene in tomato

K. Princy<sup>1</sup>, N. R. Nizampatnam<sup>1</sup>, N. Gupta<sup>1</sup>, V. V. Satyavathi<sup>1</sup>, R. Sharma<sup>1</sup>, Y. Sreelakshmi<sup>1</sup>

<sup>1</sup>University of Hyderabad, Plant Sciences, Hyderabad, India

*Phototropin 1 & 2* are the major blue light photoreceptors in higher plants, responsible for functions such as phototropism, chloroplast photorelocation, stomatal opening, leaf flattening, and leaf positioning. Though they exhibit functional redundancy, *phototropin 2* is the crucial player under high fluence conditions. Though the intrinsic functions of these blue light receptor kinases have been studied in depth in the model plant, *Arabidopsis*, critical studies are required to reveal signaling pathways and genetic regulation of these genes in crop plants. Cis-regulatory dissection is an emerging field in reverse genetics where the functional aspects of cis elements present upstream of a gene can be studied. Here, in our study, we have manipulated the cis-regulatory elements of the *phot2* gene in tomato to uncover their functions and probe for any novel functions in an agriculturally important crop like tomato. Through CRISPR/Cas9 driven editing of the cis-elements, multiple alleles for the target promoter region were created. In the stable edited lines that are transgene-free, *phot2* function was compromised. Leaf flattening response, an essential aspect for fine tuning multiple plant physiological processes like photosynthesis and transpiration, was drastically affected. Also, chloroplast photorelocation responses were significantly affected in all plants carrying different cis-element editing patterns. In addition, significant pleiotropic phenotypes such as altered shoot, leaf and inflorescence architecture, were observed. All these, put together suggest towards the intricate regulatory network of genes acting along with *phot2* for fine tuning multiple physiological responses in tomato. The observed phenotypic alterations in the edited *phot2* lines could be of agricultural impact in tomato.

Keywords: CRISPR/Cas9, Blue-light photoreceptor, *phototropin2*, cis-regulatory elements, chloroplast photorelocation, leaf flattening, pleiotropy

P 327

An efficient CRISPR-based method for mutational analyses of redundant gene functions in winter and spring rapeseed

K. Ille<sup>1</sup>, S. Melzer<sup>1</sup>

<sup>1</sup>CAU Kiel, Plant Physiology, Botanical Institute, Kiel, Germany

Many gene functions are widely studied and understood in *Arabidopsis*. However, the lack of efficient transformation systems often limits applying and verifying this knowledge in crops. Monocot crops or crops like sugar beet are considered recalcitrant to *Agrobacterium*-mediated transformation and recovering transgenic plants from these crops is difficult or impossible. *Brassica napus*, a member of the Brassicaceae family, is often transformed by *Agrobacterium*-mediated hypocotyl transformation but not all growth types respond equally well to it. Spring-type rapeseed, which flowers and sets seed within one year, responds fairly well to transformation. However, winter-type rapeseed, which needs vernalization to start flowering, is recalcitrant to *in vitro* regeneration and transformation. The analysis of gene functions in rapeseed is further complicated by the allotetraploid nature of its genome and the genome triplication in the *Brassica* genus, which consequentially caused a high number of gene copies for each *Arabidopsis* ortholog.

We developed a transformation method that facilitates the regeneration of winter-type rapeseed and allows us to transform winter-type and spring-type rapeseed efficiently in small-scale experiments. We further demonstrated, using the example of *Bna.CLV3* and *Bna.SPL9/15*, that most gene homologs are effectively edited by CRISPR/Cas9 using this transformation protocol. We already observed mutant phenotypes for *Bna.CLV3* and *Bna.SPL9/15* in primary transformants, meaning that up to 8 genes, were edited in both alleles and were consequently knocked out. Therefore, we can now analyze complex gene networks involving many gene homologs that mostly act redundantly in winter and spring rapeseed.

P 328

Painting with your own colors: Using Cas9 fused exonucleases to engineer cis-genic purple tomatoes

A. Brand<sup>1</sup>, T. Schreiber<sup>1</sup>, A. Tissier<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry, Cell and Metabolic Biology, Halle a. d. Saale, Germany

Public perception and the extensive regulation of GMOs are still major obstacles to the molecular engineering of crop plants. However, distinct legislation from several countries on novel genomic techniques (NGTs), specifically on gene editing using CRISPR, represents new opportunities to overcome challenges of conventional plant breeding and transgenic technologies. Current applications of CRISPR/Cas endonucleases include generating indels for knockouts and editing of single bases or short DNA sequences. A recent approach demonstrated that the use of viral 5'-exonucleases fused to the Cas9 endonuclease significantly enhance homology-directed repair (HDR) in plants, facilitating the scar-free integration of several kilobases in transient and stable assays (Schreiber et al. 2024). We aim to exploit this innovative tool to engineer cis-genic purple tomatoes by inserting fruit-specific regulatory sequences, occurring naturally in the tomato genome, in the promoter region of genes regulating anthocyanin biosynthesis. The R2R3-MYB transcription factor *ANTHOCYANIN 2* (*SIAN2*) has been shown to promote accumulation of anthocyanins when overexpressed with a constitutive promoter (Jian et al. 2019) making it an interesting candidate for promoter editing. Additionally, by examining the promoter sequence of the *AN2* ortholog in the wild relative of tomato, *Solanum nigrum*, novel regulatory sequences can be identified to manipulate the expression of this gene in cultivated tomato varieties. Combining HDR with targeted CRISPR mutagenesis could potentially replicate agronomic traits created through transgenesis, bypassing the stringent regulations of GMOs.

Schreiber et al. (2024) *Molecular Plant*, 17: 824-837; Jian et al. (2019) *Horticulture Research*, 6: 22.

P 329

**A genome-wide 5-methyl cytosine reference atlas of a sugar beet genotype**

M. Wulfhorst<sup>1</sup>, K. Sielemann<sup>1</sup>, N. Schmidt<sup>2</sup>, L. Mann<sup>2</sup>, V. Vilperte<sup>3</sup>, P. Viehöver<sup>1</sup>, B. Weisshaar<sup>1</sup>, B. Schulz<sup>3</sup>, T. Heitkam<sup>2,4</sup>, D. Holtgräwe<sup>1</sup>

<sup>1</sup>Universität Bielefeld, Genetics and Genomics of Plants, Bielefeld, Germany

<sup>2</sup>Technische Universität Dresden, Faculty of Biology, Dresden, Germany

<sup>3</sup>KWS SAAT SE, Einbeck, Germany

<sup>4</sup>University of Graz, Institute of Biology, Graz, Austria

Epigenetic modifications, such as DNA methylation, play a major role in phenotypic plasticity and also affect an enormous variety of plant traits [1]. Nonetheless, both, the genome-wide pattern itself and the regulation of heritability of DNA methylation patterns remain widely unexplored in the crop sugar beet (*Beta vulgaris* subsp. *vulgaris*). Consequently, it is not yet possible to benefit from epigenetic breeding approaches in sugar beet.

The crop is characterized by its narrow genetic background that originates from its relatively young breeding history. Therefore, epigenetic modifications could be expedient to widen the domestication bottleneck. We aim to investigate the occurrence, stability and heritability of DNA methylation patterns in sugar beet in methylation deficient mutant lines. To generate a methylome that can serve as a reference, cytosine methylation (5mC) patterns were detected and analysed genome-wide in the reference genotype KWS2320.

To detect 5mC in CG, CHH and CHG contexts and localize it at base-pair position resolution within the genome, high molecular weight DNA was isolated from leaf tissue and subjected to Oxford Nanopore Technologies (ONT) sequencing. The data sets were mapped to a corresponding high-continuous genome sequence assembly [2]. For interpretation of the 5mC signals, DeepSignal Plant was used [3]. Initial results show that the pipeline is capable to detect 99.7 % of the 204.8 Mio cytosines in a 34.8 x coverage read set in the sugar beet genome. Genome-wide 14.74 % of those cytosines were classified as methylated in leaves.

To comprehend methylation patterns in sugar beet, a detailed 5mC reference atlas will be generated for KWS2320. Including additional gene/region specific analysis (e.g. bolting/sweet genes and (retro-)transposon families) it forms a reliable basis for future scientific research and economic applications like epigenetic breeding.

References

[1] Rajpal et al. (2022). Epigenetic variation: A major player in facilitating plant fitness under changing environmental conditions. *Frontiers in Cell and Development Biology* 10:1020958

[2] Sielemann et al. (2023). Genome assembly, structural and functional annotation, and mRNA coverage/length files for KWS2320ONT\_v1.0 and Strube U2BvONT\_v1.0. <https://pub.uni-bielefeld.de/record/2966927>

[3] Ni et al. (2021). Genome-wide detection of cytosine methylations in plant from Nanopore data using deep learning. *Nature Communications* 12:5976

P 330

**A rocky road to CRISPR - Dealing with *in vitro* recalcitrance in European beech (*Fagus sylvatica* L.)**

V. Zahn<sup>1</sup>, A. J. Sievers<sup>1</sup>, M. Fladung<sup>1</sup>, T. Brueggemann<sup>1</sup>

<sup>1</sup>Thünen Institute of Forest Genetics, Großhansdorf, Germany

As the most common deciduous tree species in Central Europe, European beech (*Fagus sylvatica*) is an important component of European forest ecosystems and an important economic factor. Due to increasing drought, especially since 2018, *F. sylvatica* is suffering in European forests. As a result, numerous beech stands are threatened ("beech dieback"). Modern biotechnological methods such as genome editing with CRISPR/Cas can be used to investigate the genetic basis of tolerances to abiotic stress and thus support the selection of drought-tolerant beech.

To date, however, neither genome editing nor the previously required genetic transformation of *F. sylvatica* has been reported. This is mainly because *F. sylvatica* has been known to be recalcitrant to *in vitro* propagation and regeneration since the 1980s. Consequently, the road to CRISPR (*in vitro* regeneration, transformation, CRISPR/Cas components) must be followed from the very beginning.

To obtain a stable *in vitro* line for propagation, regeneration, and transformation, seedlings of *F. sylvatica* were transferred to *in vitro* culture using shoots and shoot tips. Protocols were adapted for an *in planta* transformation approach to circumvent *in vitro* regeneration. For this purpose, *in vitro* shoot tip transfer was combined with needle perforation and vacuum infiltration with *Agrobacterium tumefaciens* GV30101-pMP90RK and EHA105 transferring a *RUBY* marker. Seedling leaves were used for protoplast isolation. Initial experiments on PEG-mediated transformation of protoplasts with plasmid-DNA were performed using a 35S::*mEGFP* reporter construct. Both transformation systems will be used for proof-of-principle CRISPR/Cas9 and *tCas12a* gene editing tests in *F. sylvatica*.

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Elucidating gene regulatory networks in crops using DAP-seq

I. Mora-Ramirez<sup>1</sup>, K. Liu<sup>1</sup>, K. Nagesh<sup>1</sup>, A. Himmelbach<sup>1</sup>, T. Schnurbusch<sup>1</sup>, J. Schippers<sup>1</sup>  
<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Plant domestication started over 10.000 years ago, representing a fast and guided evolution of plant body plan to serve human needs. Only with the advent of the genomics era it became apparent that humans unknowingly selected specific genetic traits to improve their crops. Plant organs grow to characteristic sizes and shapes that are genetically controlled. Interestingly, the cause for the improved plant traits is often due to altered transcription factor activity. Transcription factors are key determinants in regulating plant growth by modulating the activity of tens to hundreds of genes. In contrast to model plants, we have a poor understanding of the gene networks in crops.

Within the seed development lab at the IPK Gatersleben we aim to uncover transcription factor networks in crops. To this end, the target genes of transcription factors that are differentially expressed during growth and development or upon stress are determined by using a DNA affinity purification sequencing approach. The gene networks that we uncover are being validated by using different approaches, including protoplast-based transactivation assays, RNA-seq, functional genomics through transgenic models, and EMSA assays for analysing the DNA-binding properties. Here an overview is provided of our DAP-seq pipeline that has been successfully applied in barley, maize, sugar beet, and wheat. Examples of transcription factors controlling spike and/or seed development and stress adaptation are highlighted. By revealing the molecular networks of key transcription factors we expect that the obtained information can impact directly on targeted breeding strategies that are necessary to keep up with the increasing yield demands for crop production and the generation of climate-resilient crops.

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Elucidating the effect of single gene modifications on drought stress tolerance of poplars (*Populus*)

A. Fendel<sup>1</sup>, F. Wiedemann<sup>1,2</sup>, M. Fladung<sup>1</sup>, T. Bruegmann<sup>1</sup>  
<sup>1</sup>Thünen Institute of Forest Genetics, Großhansdorf, Germany  
<sup>2</sup>Leibniz University, Hannover, Germany

Climate change and associated drought scenarios threaten the worldwide growth and wood production of forest trees. The adaptability of plant species to such environments is strongly dependent on genetic information and regulations, controlling morphological, physiological, and biochemical adaptive mechanisms. However, the single impact of genes regarding tolerances to such as drought in forest trees remains elusive. Here, we apply genetic engineering and genome editing mechanisms (constitutive overexpression and CRISPR/Cas9 knockout, respectively) in the model tree genus poplar (*Populus*) to accurately classify and characterize genes involved in drought stress tolerance. Single genes, especially those potentially involved in plant hormone or amino acid accumulation, were identified in the European poplar species *Populus × canescens*. Accordingly, the candidate gene *Delta1-pyrroline-5-carboxylate synthase 1 (PcP5CS1)* was constitutively overexpressed. Several in vitro-propagated overexpression lines were analyzed on their altered drought stress tolerance under controlled stress conditions in the greenhouse, utilizing invasive and non-invasive phenotyping methods. Amongst others, the improvement of survivability rates of more than 40 % could be observed in constitutively overexpressed *PcP5CS1* trees compared to wild types. Moreover, in strong overexpression lines, phenotypic observations suggest an influence of *PcP5CS1* on chlorophyll accumulation and movement, as well as overall leaf morphology. This approach contributes to the understanding of single gene contributions on the complex network of drought stress tolerance. In addition, the identified key genes can be considered in targeted forest plant breeding approaches to obtain stress-tolerant trees, either by molecular selection from natural forests or via biotechnological approaches.

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Establishment of cost-efficient and sustainable cultivation for yam - Characterization of primary metabolites and identification of genetic markers for breeding-relevant properties

J. Drotleff<sup>1</sup>, J. Epping<sup>1</sup>

<sup>1</sup>Universität Münster, Institute of Plant Biology and Biotechnology, Münster, Germany

The Chinese yam (*Dioscorea polystachya*) variety Lichtyam® has been cultivated at the Andreashof e.V. over several years. *Dioscorea polystachya* is used in traditional Chinese medicine for its health promoting properties. Studies with rodents have demonstrated an anti-diabetic effect. The tubers therefore have the potential to have protective effects against diabetes and related cardiovascular diseases. In order to make these benefits of yam available to more people, innovations are needed in the field of yam cultivation.

As the tubers can reach a length of up to one meter, cultivation is performed in raised beds built on pallets at the Andreashof. Due to a new EU Organic Farming regulation, this cultivation method can no longer be used, as organic certification requires soil-based cultivation throughout. Therefore, a new cost-efficient and sustainable production system, complying with the new EU regulation needs to be established. Four different cultivation systems are tested by the Andreashof.

The development of new cultivation systems is scientifically monitored to maintain nutritional quality. Therefore, a profile of primary metabolites like starch, resistant starch, amylose, amylopectin, sugars, proteins and fatty acids will be created to compare the different cultivation systems regarding their effects on nutritional quality.

In addition, crosses are made between the male-flowering variety Lichtyam® and the female-flowering variety Igname, which differ in some traits like tuber shape, bulbil size and starch composition, to generate yam cultivars with improved traits. The offspring will be analyzed by Genotyping-by-sequencing (GBS) to develop genetic markers for phenotypic and biochemical traits. This is particularly relevant for yam, as they only flower in the second year of cultivation after crossing, and genetic markers would offer the opportunity to select for certain traits already in the first year of cultivation. The markers developed are intended to provide the basis for breeding approaches to generate cultivars with health-promoting properties and phenotypic characteristics that simplify cultivation, e.g. improved tuber shape.

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Functional Profiling and Regulatory Element Analysis of the Male Gametophyte-Specific Promoter Fragment in the *Arabidopsis PIRL6* Gene

A. T. G<sup>1</sup>, J. M. Shah<sup>1</sup>

<sup>1</sup>Central University of Kerala, Plant Science, Kasaragod, India

Plant gametophyte-specific promoters are vital for genome editing, crop breeding, and hybrid generation. However, there is limited understanding of the regulatory mechanisms of gametophyte-specific promoters. While there are a handful of studies characterizing female gametophyte-specific promoters, regulatory elements responsible for pollen-specific gene expression are not well characterized. *Plant Intracellular Ras-group Leucine-Rich-Repeat 6 (PIRL6)* is one of the genes known to express during male/female gametogenesis in *Arabidopsis thaliana*. We reasoned that its promoter harbors regulatory elements for gametophyte-specific expression. Thus, we fused differing lengths of promoter deletions of *Arabidopsis PIRL6* to the *GUS* gene and based on *GUS* expression in transgenic *A. thaliana* lines we identified a key region for pollen-specific gene expression. In addition, we identified two putative phytohormone-responsive elements in the same promoter by transient expression assay of the promoter-*GUS* fusion constructs in *Nicotiana benthamiana* leaves. We observed *GUS* gene overexpression in response to abscisic acid and gibberellin, likely due to the presence of ABRE and P-box elements in the promoter. To identify the remaining cis-regulatory elements, we compared *PIRL6* promoter to 50 other gametophyte-specific co-expressed genes. Using various bioinformatic tools we predicted several other motifs that are likely involved in gametophyte-specific gene regulation. Therefore, the pollen-specific promoter region we characterized could be directly used for targeted gene editing and a better understanding of plant reproductive regularity.

P 335

Exploring the role of H4 acetylation in shaping patterns of recombination

M. S. Seidel<sup>1</sup>, S. Dreissig<sup>1</sup>

<sup>1</sup>Martin-Luther-University Halle-Wittenberg, Institute of Agriculture, Halle a. d. Saale, Germany

Meiosis is a specialised form of cell division that originated in the early history of eukaryotes. This sexual life cycle is of evolutionary value because it increases genetic variation. Meiotic recombination events are initiated by Spo11-mediated double-strand breaks (DSBs). This can lead to the formation of crossing over (CO) and hence to the reciprocal exchange of DNA fragments, resulting in allele reshuffling. The recombination landscape is shaped by the interplay of axial proteins, chromatin state and post-translational modifications such as acetylation of N-terminal histone tails.

This study aims to understand how histone H4 acetylation affects the rate and distribution of meiotic recombination in rye (*Secale cereale* L.) and *Arabidopsis thaliana*.

In our previous work, we found that recombination landscapes differ between wild and domestication populations of rye. Presumably, genes influencing the recombination landscape were indirectly co-selected throughout the domestication of rye. Genome-wide association scans for variation in the size of low recombining regions in wild and domesticated rye accessions identified a major QTL, with a histone H4 acetyltransferase as the most likely candidate gene (ScESA1, an ortholog of yeast ESA1 and *Arabidopsis* HAM1). Based on the high similarity to the histone H4 acetyltransferases in *Arabidopsis* (HAM1) as well as yeast (ESA1), ScESA1 is likely to have H4 acetyltransferase activity. Histone H3 acetylation, on the other hand, is known to promote COs in *Arabidopsis*, but our knowledge of the role of H4 acetylation in regulating meiotic recombination in plants is scarce. To analyse the influence of ScESA1 on meiotic recombination, a rye knockout mutant will be generated using CRISPR/Cas9. In addition, a highly conserved 410 bp transposon-like insertion has been identified in the putative ScESA1 promoter region of predominantly domesticated accessions. In addition to the ScESA1 knockout, this factor will be subjected to relative transcriptional and protein expression analysis. In addition, cytological analysis of meiocytes will be undertaken with respect to chromatin modifications and axial proteins that regulate meiotic recombination events.

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The mechanisms of heavy metal resistance in Zygnematophyceae and liverworts: first steps towards the phycoremediation of contaminated freshwater

J. Woide<sup>1</sup>, A. Degenhardt<sup>1</sup>, H. Buschmann<sup>1</sup>

<sup>1</sup>Hochschule Mittweida, Molecular Biotechnology, Mittweida, Germany

Bioremediation is a process in which selected organisms are used to clean up the environment. While climate change worsens heavy metal pollution of our planet's biosphere, high quality freshwater is becoming ever more precious. The main hypothesis to be tested in this research is that the mode of resistance to heavy metals differs between algae and land plants. Understanding this difference can help us to select or even create the best suited organisms for freshwater clean-up. We therefore compare the land-living liverwort *Marchantia*, the amphibic liverwort *Ricia* and the aquatic alga *Zygnema* with respect to heavy metal response and heavy metal take-up and storage. *Ricia* and *Zygnema* are easy-to-harvest water-living organisms and do therefore serve as model bioremediators.

We show that plastid morphology and photosynthetic efficiency is immediately affected upon heavy metal exposure. Cell proliferation is reported to be affected by heavy metals and we clarify that this involves the cytoskeleton. Some plants and algae are known to capture heavy metal ions in their cell walls (rather than the vacuole) and we measure relative biosorption and how this relates to heavy metal resistance. We also embrace synthetic biology to improve heavy metal resistance. We therefore searched the genomes of algae and liverworts to detect putative bioremediation genes. Introducing genes from algae to liverworts (or vice versa) can potentially improve the biosorption of heavy metals.

Our research leads the way towards the clean-up of heavy metal-contaminated freshwater with easy-to-harvest plants and algae.

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**Impact of epigenetic and post-transcriptional gene regulation on rubber biosynthesis in dandelion**T. Poloczek<sup>1</sup>, J. Wiemann<sup>2</sup>, N. van Dam<sup>1</sup>, C. Schulze Gronover<sup>2</sup>, D. Prüfer<sup>1,2</sup><sup>1</sup>Institute of Plant Biology and Biotechnology, AG Prüfer, Münster, Germany<sup>2</sup>Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Münster, Germany

Natural Rubber (NR) is indispensable in many products due to its unique properties, which have not yet been successfully replicated by synthetic substitutes. The dandelion, *Taraxacum koksaghyz*, has emerged as a promising candidate for NR production because it accumulates large amounts of NR in its roots compared to its root mass (1). However, due to its small size, NR production is limited, leading to initiatives to increase biomass through crossbreeding with the common dandelion *Taraxacum officinale*. While intraspecific breeding has successfully enhanced NR content, the NR yield in interspecific F1 hybrids falls short of expectations. This has initiated additional research into the regulatory factors potentially limiting NR biosynthesis.

This work focuses on understanding the regulation of NR biosynthesis in interspecific F1 hybrids by investigating the epigenetic regulatory mechanisms which might influence gene expression and genome-wide regulation. It was found that key enzymes participating in rubber synthesis have lower expression levels in hybrids compared to *T. koksaghyz*. We hypothesize that epigenetic regulatory mechanisms such as DNA methylation and post-transcriptional regulation through microRNA (miRNA) interfere in gene expression related to NR synthesis.

Based on a sample pool comprised of *T. koksaghyz*, *T. officinale*, and their F1 hybrids, a comprehensive suite of analytical methodologies was employed to investigate the epigenetic mechanisms influencing gene expression. To profile gene methylation, whole genome bisulfite sequencing (WGBS) was conducted, followed by the establishment of a bioinformatics pipeline designed to identify differentially methylated regions (DMRs). Additionally, to explore miRNA-based regulatory mechanisms, small RNA (sRNA) sequencing was performed. This was complemented by computational approaches aimed at identifying differentially expressed miRNAs.

The outcomes of this study are expected to provide insights into the molecular hurdles of NR production in dandelion hybrids. Identifying specific epigenetic factors could guide targeted breeding and genetic engineering strategies to enhance NR yield in early dandelion hybrid generations, offering a sustainable and economically viable source for NR.

## References

1. van Beilen, J. B. & Poirier, Y. Guayule and Russian Dandelion as Alternative Sources of Natural Rubber. *Crit. Rev. Biotechnol.* 27, 217–231 (2007).

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**CRISPR-Cas Mediated Plant Immunization: A New Frontier in Climate Challenges**M. Barupal<sup>1</sup><sup>1</sup>Jai Narain Vyas University Jodhpur, Botany, Jodhpur, India

Changing climate may increase the abiotic and biotic stresses, posing a threat to crops worldwide. Pathogens strategically target plant cells, diverting resources for their own growth. In response, plants have their own defense system, however, depends on various factors. Plant pathogens are a threat to global food security. Enhancing resistance to these pathogens can not only reduce crop losses but also minimize the cost of control measures. CRISPR (clustered regularly interspaced short palindromic repeats) technology offers a powerful answer to the challenge of enhancing plant immunity. CRISPR can enhance plant defenses by enabling precise manipulation of genes involved in pathogen detection and defense molecule synthesis. Recent research demonstrates the effectiveness of CRISPR-Cas9 in strengthening plant defenses against a range of pathogens, spanning viruses, bacteria, and fungi. Acting as an adaptive immune system, CRISPR-Cas9 precisely targets and cleaves foreign DNA sequences, such as those found in the beet severe curly top virus (BSCTV), reducing virus accumulation and introducing mutations that render plants more resistant to infection or knockdown of TaMKP1 in wheat, resulting in increased resistance to rust (caused by *Puccinia striiformis* f. sp. *tritici*) and powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*) or as targeting the antimicrobial peptides or S-genes or NLR genes in various crops to improve disease resistance without growth penalty. CRISPR based editing Compared to previous gene editing tools like Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs), CRISPR-Cas9 offers heightened efficiency, precision, and versatility in modifying the plant genome, facilitating targeted adjustments to genes associated with immune responses. By engineering plants to express Cas endonuclease genes and short guide RNAs (sgRNAs) targeting specific pathogen genomes or host defense genes, researchers can augment plant defenses and reduce susceptibility to infection. Efforts to develop non-transgenic delivery methods for CRISPR-Cas9 complexes into plant cells signify a move towards reducing reliance on genetically modified organisms (GMOs) for pathogen resistance. These advancements hold promise for enhancing crop protection, fortifying global food security, and promoting sustainable agricultural practices by enhancing the intrinsic immune systems of plants.

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Phenotypic and transcriptional outputs of an ancestral wheat allele on spike morphogenesis

A. Tawale<sup>1</sup>, R. Abbai<sup>1</sup>, T. Schnurbusch<sup>2,1</sup>, G. Golan<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

<sup>2</sup>Martin Luther University Halle-Wittenberg, Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, Halle a. d. Saale, Germany

Inflorescence length is an important trait that captured the interest of biologists and breeders from the hopeful perspective of increasing the grain yield potential of cereals like wheat (*Triticum* spp.). The wheat inflorescence, termed spike, and its architecture is crucial for influencing cereal crop productivity. Here, we investigated a spike-length quantitative trait locus (QTL), i.e., *QSL.ipk-4AL*, located on the long arm of chromosome 4A in tetraploid wheat<sup>1</sup>. Fine mapping of the *QSL.ipk-4AL* using a set of introgression lines (BC4F3) narrowed down the QTL interval to 2.95 Mbp, where introgressions from the ancestral wild emmer wheat (WEW) accession Zavitan<sup>2</sup> promoted spike length in the background of an elite durum cultivar Svevo<sup>3</sup>. Investigation of the pleiotropic effects of the WEW allele on other spike and grain-related traits revealed a higher biomass allocation to the rachis, increased grain length, and a lower spike grain yield compared to Svevo. Phenotypic analysis also revealed that the increase in rachis length (and spike length) in genotypes carrying the WEW allele is due to increased internode length without affecting the spike node number. To identify candidate genes underlying the QTL, we combined the genetic mapping with a transcriptome study of the rachis in contrasting genotypic pools at four developmental stages during the significant spike-growth phase. Our analyses revealed that 25 out of 39 high-confidence genes in the ancestral *QSL.ipk-4AL* pool were expressed in the rachis, while 14 genes did not express (<0.1 TPM). Five of the differentially expressed genes exhibited expression patterns corresponding to the observed differences in spike growth between genotypes over time, making them promising candidates. Further investigation into structural and sequence-level variations could help identify the causal gene underlying the QTL. Thus, our transcript data set increases our understanding of the underlying mechanisms associated with the QTL effect on spike length, thereby providing novel insights into wheat rachis growth.

References

1. Wolde, G. M. *et al. Theor. Appl. Genet.* 132, 1661–1676 (2019).
2. Maccaferri, M. *et al. Nat. Genet.* 51, 885–895 (2019).
3. Avni, R. *et al. Science.* 357, 93–97 (2017).

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Efficient Targeted Insertion in Plant Genomes via Protoplast Regeneration

C. T. Hsu<sup>1</sup>, Y. H. Yuan<sup>1</sup>, Y. C. Lin<sup>1</sup>, S. Lin<sup>1</sup>, Q. W. Cheng<sup>1</sup>, F. H. Wu<sup>1</sup>, J. Sheen<sup>1</sup>, M. C. Shih<sup>1</sup>, C. S. Lin<sup>1</sup>

<sup>1</sup>Academia Sinica, Agricultural Biotechnology Research Center, Taipei, Taiwan

Genome editing can be achieved by inserting DNA sequences into specific locations. Our protocol eliminates the need for expensive equipment, chemical and donor DNA modifications, or plasmid construction by using polyethylene glycol-calcium to deliver single-stranded DNA oligonucleotides and CRISPR-Cas9 ribonucleoproteins into protoplasts. Plants regenerated from edited protoplasts achieved targeted insertion efficiencies of up to 50% in *Nicotiana benthamiana* and 13.6% in *Brassica oleracea* without antibiotic selection. Using a 60-nt donor containing 27-nt homologous arms, 6 out of 22 regenerated *N. benthamiana* plants showed targeted insertions, with one plant containing a precise insertion of a 6 bp HindIII site. The inserted sequences were transmitted to the next generation, providing an opportunity for future exploration of versatile genome editing through targeted DNA insertion in plants.

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Allelopathic activity of *Euphorbia hirta* against *Avena fatua* and *Rumex dentatus* and identification of potential allelochemicals

M. Akbar<sup>1</sup>, T. Taqdees<sup>1</sup>, T. Khalil<sup>1</sup>

<sup>1</sup>University of Gujrat, Department of Botany, Gujrat, Pakistan

Phenomenon of allelopathy can be utilized to develop eco-friendly herbicides and there are number of reports of allelopathic effects of many weeds against other weeds but no reports are available that show the allelopathic effects of *Euphorbia hirta* against *Avena fatua* and *Rumex dentatus*. In the present research, allelopathic activity of *E. hirta* was investigated against *R. dentatus* and *A. fatua*. *E. hirta* extract was prepared in distilled water (dH<sub>2</sub>O) (10g:100mL w/v) as 100% extract concentration. Lower concentration (50%) was prepared by adding dH<sub>2</sub>O to 100% extract. The allelopathic activity of *E. hirta* was evaluated by growing *R. dentatus* and *A. fatua* either alone or grown side by side with wheat plants in pots. The experiments were repeated twice. There were 5 treatments viz., dH<sub>2</sub>O, half dose herbicide, full dose herbicide, 50% plant extract, and 100% plant extract. In *in vivo* bioassays, the effect of 50% and 100% plant extract of *E. hirta* on shoot dry biomass of *A. fatua* and wheat was non-significant in general, while there was 50% and 67% significant decline in shoot dry biomass of *R. dentatus*, respectively, when grown alone. Moreover, when *R. dentatus* was grown side by side with wheat, there was 71% and 86% decrease in shoot dry weight of *R. dentatus* at 50% and 100% extract concentrations of *E. hirta*, respectively. The reference herbicides, Sulfosulfuron, at full dose, significantly inhibited shoot dry weight of *A. fatua* by 14%, while, Fluroxypyr Meptyl + Florasulam + MCPA Isooctyl, completely eradicated *R. dentatus*, when grown side by side with wheat. The Gas Chromatography Mass Spectrometry (GCMS) analysis of *E. hirta* extract depicted the presence of potent allelochemicals, the major ones including quercetin, hexadecanoic acid, methyl ester,  $\beta$ -sitosterol, afzelin, gallic acid, neophytadiene, stigmasterol, trans,trans-2,6- Dimethyl-2,6- Octadiene-1,8-Diol, and 2,3,5-trimethyl-1H-pyrrole. In conclusion, the aqueous extract of *E. hirta* exhibited herbicidal activity against *R. dentatus* weed, without harming the wheat, so, *E. hirta* can be utilized to discover newer herbicidal compounds.

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Update of the rye reference genome assembly, Lo7\_V3

E. Chen<sup>1</sup>, S. Jhingan<sup>1</sup>, A. Himmelbach<sup>1</sup>, M. Mascher<sup>1,2</sup>, N. Stein<sup>1,3</sup>

<sup>1</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

<sup>2</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

<sup>3</sup>Martin Luther University Halle-Wittenberg, Halle a. d. Saale, Germany

Rye (*Secale cereale* L.) is a valuable food and forage crop with stress tolerance (biotic and abiotic) and high yield potential, which plays an important role as a genetic resource for wheat and triticale improvement via introgressive hybridization. Here, we provide an updated version of the chromosome-scale reference genome sequence assembly of the rye inbred line Lo7 using long reads, hereafter referred to as Lo7\_V3. We assembled a highly contiguous Lo7 genome by integrating high-coverage PacBio HiFi and Oxford Nanopore long reads, Illumina paired-end reads, high-throughput chromatin conformation capture (Hi-C) data, full-length isoform sequencing (Iso-Seq) and a Bionano optical genome map. Compared to the previous Lo7\_V2 assembly (Rabanus-Wallace, M. T. et al., *Nat Genet* 2021), we corrected the orientation errors of contigs in each chromosome and revealed that the centromeres of all seven chromosomes were fully assembled. We observed expanded repetitive sequence clusters containing known rye satellite sequences (e.g., pSc119.2, pSc200 and pSc250) on chromosomes 2R and 3R. We are validating the localization of the repeat clusters and the centromere regions by cytological evidences (fluorescence in situ hybridization, FISH) and by chromatin immunoprecipitation sequencing (ChIP-Seq) for centromeric histone H3 (CENH3), respectively. The improved Lo7\_V3 genome assembly will underpin future genomic research and crop improvement in rye and related cereal crop species.



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**AGROFLUX: An innovative sensor platform to study high-frequency responses in water, carbon and greenhouse gas fluxes in a complex arable landscape**

M. Dubbert<sup>1</sup>, A. Dahlmann<sup>1</sup>, S. Vaidya<sup>1</sup>, M. Sommer<sup>1</sup>, M. Schmidt<sup>1</sup>, D. Dubbert<sup>1</sup>, V. Gernot<sup>1</sup>, J. Augustin<sup>1</sup>, M. Hoffmann<sup>1</sup>  
<sup>1</sup>ZALF, Landscape Processes, Müncheberg, Germany

Improved agricultural practices increasing the water use efficiency (WUE), reducing greenhouse gas emissions (GHG) and/or improving atmospheric C sequestration rates within the soil are crucial for an adaptation and/or mitigation to the global climate crisis. However, processes driving water (H<sub>2</sub>O), carbon (C) and GHG fluxes within the soil-plant-atmosphere continuum of agricultural used landscapes are complex and flux dynamics differ substantially in time and space. Hence, to upscale and evaluate the effects/benefits of any new agricultural practice aiming towards improving WUE, soil C sequestration and/or GHG emissions, accurate and precise information on the complex spatio-temporal H<sub>2</sub>O, C and GHG flux pattern, their drivers and underlying processes are required.

Current approaches to investigate flux dynamics and their underlying processes are laborious and have to choose between high spatial or temporal resolution due to methodological constraints. In an effort to overcome this, a novel, fully automated robotic field sensor platform was established and combined with an IoT network and remote sensing approaches. Here, an innovative, continuously operating automated robotic field sensor platform is presented. The platform was mounted on fixed tracks, stretching over an experimental field (size) which covers three different, distinct soil types. It carries multiple sensors to measure GHG and water vapor concentrations and isotope signatures of water vapor and CO<sub>2</sub>. Combined with two chambers which can be accurately positioned in three dimensions at the experimental field below, this system facilitates to detect small-scale spatial heterogeneity and short-term temporal variability of H<sub>2</sub>O, C and GHG flux dynamics as well as crop and soil conditions over a range of possible experimental setups. The automated, continuous estimation of d<sub>18</sub>O and dD of evapotranspiration further provides the basis to partition water fluxes alongside the flux-based partitioning of C and GHG fluxes. This particularly promotes to assess not only ecosystem but component specific water use efficiencies. Hence, this platform produces a detailed picture of H<sub>2</sub>O, C and GHG dynamics across different treatments and crop cycles, with a high-degree of accuracy and reproducibility.

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**Optimization of natural deep eutectic solvent-based extraction of carotenoids for diverse applications in food**

N. N. Kuttty<sup>1</sup>, S. Dhumane<sup>1</sup>

<sup>1</sup>Dr. Vishwanath Karad MIT World Peace University, Department of Biosciences and Technology, Pune, India

Natural deep eutectic solvents (NADES) are an emerging solvent for the extraction of various phytochemicals (specialized metabolites). They are composed of mixtures of hydrogen bond donor and hydrogen bond acceptor compounds in different stoichiometric ratios. NADES-based solvents follow principles of green chemistry; these solvents are easy to prepare, have the advantages of being eco-friendly, and are of low cost. Ultrasound-assisted extraction of metabolites such as carotenoids from marigold flowers was carried out using different hydrophobic NADES. High-performance Liquid Chromatography revealed the presence of  $\beta$ -carotene in the extracts. The optimal NADES system for the extraction was found to be a molar combination of choline chloride and tartaric acid. These extracts were also assessed for their stability under diverse conditions such as heating, storage, and exposure to light. The stability studies of NADES-based extracts indicated the potential of using them as natural colorants in food thus replacing harmful synthetic colorants. Plant protective activities of NADES-based extracts were studied against a fungal pathogen viz. *Botrytis cinerea*. NADES-based extracts also showed antifungal properties against the pathogen. Scanning electron microscopy revealed the presence of distorted mycelia and shrunken filaments indicating the inhibition of growth by these extracts. Further, the NADES-based extracts were incorporated into a biopolymer-based film made up of starch, sorbitol, and polyvinyl alcohol which could be used as a food packaging film. NADES systems have also been reported to have plasticizer effects for starch-based films. Various physical properties of the biopolymer film, biodegradability of the film and its potential to indicate the shelf life of tomatoes was also assessed over thirty days. This study indicates the potential of NADES-based extracts as food colorants, and plant protective agents, and for developing sustainable smart food packaging technology. Considering the properties of NADES-based extracts, these solvent systems could significantly contribute to food security and food safety in the times to come.

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Differential drought responses in tomato landrace and commercial cultivar: implications for enhanced horticultural production

A. Pompeiano<sup>1</sup>, T. M. Moles<sup>1</sup>, L. Mariotti<sup>1</sup>, D. Di Baccio<sup>2</sup>, A. Santaniello<sup>3</sup>, A. Scartazza<sup>2</sup>, T. Huarancca Reyes<sup>1</sup>, L. Guglielminetti<sup>1</sup>

<sup>1</sup>University of Pisa, Department of Agriculture, Food and Environment, Pisa, Italy

<sup>2</sup>National Research Council, Research Institute on Terrestrial Ecosystems, Pisa, Italy

<sup>3</sup>Scuola Superiore Sant'Anna, PlantLab, Center of Plant Sciences, Pisa, Italy

Tomato (*Solanum lycopersicum* L.) is a major crop in the Mediterranean basin, vulnerable to drought at any crop stage. Landraces are traditional, locally adapted varieties with greater resilience to water scarcity than modern cultivars. This study compares the responses of Ciettaicale (CE), a tomato landrace and Moneymaker (MM), a commercial cultivar, to controlled soil water deficit at the early vegetative stage using biometric, physiological, and molecular analyses. Our data highlights that CE copes better with prolonged and severe drought stress, activating distinct physiological, biochemical, and molecular processes. CE sustains higher water content, leading to greater root biomass and a higher root/shoot ratio under drought compared to MM. Although pigment responses to drought did not differ markedly, the main ratios revealed different defense mechanisms. CE and MM showed opposite trends in actual photon yield of PSII photochemistry ( $\Phi$ PSII) and non-photochemical quenching (NPQ) under drought stress, with CE increasing non-radiative energy dissipation as heat while decreasing PSII electron transport rate and CO<sub>2</sub> uptake capacity. However, CE maintained unaltered substomatal CO<sub>2</sub> concentration ( $C_i$ ) under drought, suggesting a balance between stomatal and non-stomatal photosynthetic limitations. In contrast, MM showed increased  $C_i$  with rising drought intensity, indicating greater biochemical limitations to photosynthesis than diffusive limitations due to stomatal closure. Drought mainly limits photosynthesis through diffusive resistances in CE and metabolic impairment in MM. Changes in antioxidant molecules and enzymes highlighted the ability of the landrace to activate cellular processes to partially control ROS production and to induce a drought acclimation. Multicanonical analysis revealed clear genotype separation along the drought gradient, except for CE, which showed complex drought response and introgression of tolerance traits, particularly under moderate stress. The landrace's differential responses to progressive stress explain its superior endurance under water-deficit conditions, which threaten plant growth and physiological status. CE's flexible and coordinated mechanism, especially evident under moderate stress, conserves water and energy efficiently. Utilizing such genotypes can significantly improve horticultural production under drought conditions.

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Cell Wall Remodeling in the Green Macroalgae *Ulva* in Response to Environmental Input

Y. Pan<sup>1</sup>, K. Herburger<sup>1</sup>

<sup>1</sup>University of Rostock, Institute of Biological Sciences, Rostock, Germany

*Ulva* is a globally occurring green macroalga capable of producing large amounts of biomass. The bulk material in this biomass are cell walls, which serve as the only physical barrier between the algal protoplasts and the environment. *Ulva* cell walls are composed of various complex polysaccharides and can alter their composition and structure dramatically in response to changed living conditions (remodeling). As an intertidal alga, *Ulva* is frequently exposed to desiccation stress and sunlight exposure, for example due to tidal and seasonal changes. Exploring the cell remodeling process during desiccation and rehydration will help us understand the survival strategy *Ulva* takes.

The experiment is designed to simulate natural desiccation-rehydration cycles in the lab under different light and temperature regimes. Cell wall polysaccharides will be probed with various specific antibodies to identify their distribution patterns. Removal of cell wall polysaccharides via digesting enzymes and evaluating the physiological consequences for cells will allow for identifying functional roles of polysaccharide during stress exposure. Click chemistry and nanoSIMS are used to label monosaccharides and then evaluate cellular polysaccharides re-allocations *in situ/in vivo*. The composition of the cell wall will be analyzed using advanced microarray profiling techniques, allowing to quantify cell wall remodeling pattern in *Ulva*.

This research will provide a systematic workflow for tracking the cell wall formation by using multiple imaging and analytical tools from glycobiology. We anticipate uncovering new mechanisms of cell wall remodeling in intertidal algae.

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A combined landscape genetic study and species distribution modeling (SDM) of the *Hordeum murinum* L. complex

M. Keshavarzi<sup>1</sup>, R. Tabaripour<sup>1</sup>

<sup>1</sup>Alzahra university, Plant Sciences, Tehran, Iran

The wild species *Hordeum murinum*, which is closely related to *H. vulgare* has its diversification center in the Mediterranean region and contains three subspecies, namely, *H. murinum* subsp. *leporinum*, *H. murinum* subsp. *glaucum*, and *H. murinum* subsp. *murinum*. These subspecies comprise a complex group due to their high morphological similarity and unresolved phylogeny. The current survey looks at the genetic diversity and landscape genetic analyses of *H. murinum* subsp. *glaucum* in Iran using ISSR molecular markers and presents data on these aspects of the three subspecies within *H. murinum* growing in various parts of the world based on the nuclear ITS sequences. We performed Species distribution models (SDM) modeling for *H. murinum* subsp. *glaucum* around the world in response to climate change. Several computational methods were used in our study, including redundancy analysis (RDA), canonical correspondence analysis (CCA), latent factor analysis (LFMM), along spatial principal components analysis (sPCA), for landscape genetic analyses. We also used two SDM models of MAXENT and random forest to predict the species' geographical distribution due to climatic changes. The results revealed moderate to low levels of genetic diversity among geographical populations of *H. murinum* subsp. *glaucum* in Iran. These populations varied significantly in their genetic content, as revealed by AMOVA. The genetic diversity of these populations was structured by spatial variables of a local geographic area, as indicated by sPCA. A similar result was obtained for ITS sequences in all three subspecies of *H. murinum* studied. RDA and LFMM identified the potentially adaptive SNPs. The SDM modeling showed the geographical distribution and occurrence of *H. murinum* subsp. *glaucum* is not negatively affected by climate change, both within Iran and around the globe.

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Establishing a click chemistry-based toolset to visualize polysaccharide deposition in green algae

Q. Wang<sup>1</sup>, K. Herburger<sup>1</sup>

<sup>1</sup>University of Rostock, Rostock, Germany

Plant cell walls are a polysaccharide-rich matrix and researching these polysaccharides is fundamental for understanding the wall's structure and dynamics, facilitating the utilization of this renewable resource for energy and materials. Green algae are excellent model systems for studying cell walls, because they grow fast under lab conditions and usually do not require invasive sample preparations or sectioning. Cell wall-specific molecular probes and optical microscopy for real-time imaging are crucial tools for studying algal cell walls. Such *in situ* studies often rely on molecular probes like antibodies, yet most of them have been developed for land plants and may not recognize targets in green algae. The lack of probing tools limits insights into cell wall architecture and responses to environmental changes.

Here we aim to develop a metabolic labelling approach based on non-invasive click chemistry, allowing to detect various algal polysaccharides *in vivo*. This toolset will help us to study cell wall structural changes and carbon allocation in green algae (Chlorophyta and Charophyta).

The rationale of this approach is feeding functionalized monosaccharide building blocks (MBBs) to living algal cells, followed by conjugating them via a "click-reaction" with an added dye. Toxicity tests have identified several MBBs that are non-toxic to algal cells and a variety of fluorescent dyes are being tested for click chemistry reactions to label the cell walls. Localizing and quantifying MBB-containing polysaccharides in both living cells and fixed, resin-embedded sections will provide insights into their localization in cell walls and subcellular compartments at both the micro- and nanoscale. To identify cell wall components with MBB-dye complexes, targeted enzymatic digestion and HPAEC-PAD analysis will be used.

This project will develop a non-invasive method allowing for tracking algal polysaccharides *in vivo*, enabling the precise localization of cell wall formation events in living cells and advancing our understanding of algal polysaccharide functions

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A machine-learning-approach to study the plasticity of flowering time in a worldwide field trial using an exotic barley population

T. Schmutzer<sup>1</sup>, C. Westhues<sup>2</sup>, R. Dass<sup>2</sup>, C. Dreischer<sup>2</sup>, A. Flavell<sup>3</sup>, K. Pillen<sup>1</sup>, A. Maurer<sup>1</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg, Plant Breeding, Halle a. d. Saale, Germany

<sup>2</sup>Computomics GmbH, Tübingen, Germany

<sup>3</sup>University of Dundee, Plant Sciences, Dundee, United Kingdom

Barley (*Hordeum vulgare ssp. vulgare*) ranks fourth in the world as most important cereal, provides food and beverages for humans and is feed for animal husbandry. Our HEB-25 population has been derived by crossing 25 wild barleys (*H. vulgare ssp. spontaneum*) with one elite barley cultivar.

Throughout several experiments the complete HEB-25 with 1420 lines was screened for flowering time changes throughout several years and locations in seven environments. We intended to use the CERIS package to perform an informed search for effects that reinstate the environmental dimension for GWAS studies. CERIS searches the sets of average values obtained for an environmental parameter (e.g. flowering time) from different periods of time and calculates the correlation of these values to environmental means.

We performed exome capture sequencing for the complete NAM population comprised by 1420 HEB lines. Variant calling detected more than half a million high quality SNPs. To reduce the complexity and dimensionality of the data we performed a linkage disequilibrium pruning and reduced the dataset to 186,237 SNPs. To be able to construct a machine learning (ML) model that is capable to incorporate multiple feature (e.g. environmental data) we selected the most informative SNPs. These were used to performed a machine learning based genome wide association mapping study (GWAS) and the CERIS package was applied to search for an environmental index. We run the model construction once without and with incorporated environmental data. By applying the shapley value concept we furthermore put emphasize on interpretation of the constructed ML models and by this revealed for each parameters its impact that it has to form the model. Hereby, the 25 most important feature in the models were ranked in their importance of the model to predict the performance. Our concept provides a valuable source of high-resolution SNP markers and shows potentials of ML to better utilize complex data for the example of flowering time prediction.

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Establishing a Minimal Endosymbiotic Metabolism in Cyanobacteria

J. Hofer<sup>1</sup>, T. Schulze<sup>2</sup>, L. Witting<sup>3</sup>, D. Kohlheyer<sup>3</sup>, M. Eisenhut<sup>2</sup>, A. Weber<sup>1</sup>

<sup>1</sup>Heinrich-Heine-Universität Düsseldorf, Biochemie der Pflanzen, Düsseldorf, Germany

<sup>2</sup>Universität Bielefeld, Computational Biology, Bielefeld, Germany

<sup>3</sup>Forschungszentrum Jülich, Microscale Bioengineering, Jülich, Germany

During primary endosymbiosis, a proto-cyanobacterium was engulfed by a eukaryotic cell and became the precursor of the plastids present in almost all recent eukaryotic photosynthetic organisms (Rodríguez-Ezpeleta *et al.* 2005). This symbiosis likely developed via linkage of the endosymbiont's and host's energy metabolisms. To recapitulate key steps in the event of primary endosymbiosis, we test the hypothesis that metabolic connectivity was initiated by the loss of the endosymbiont's ability to store carbon for energy production. Cyanobacteria in which glycogen synthesis is disrupted via deletion of the ADP-glucose pyrophosphorylase (GlgC) are unable to store carbon and energy over extended periods of time. This results in compromised growth during periods of darkness and export of excess carbon in the light (Gründel *et al.* 2012). As per the *ménage-à-trois* (MAT) hypothesis, this is a central step in enabling the development of a stable endosymbiotic relationship (Ball *et al.* 2015). By introducing hexose-phosphate transporters and ATP/ADP-antiporters similar to those found in recent plastids into the *glgC* deletion mutants of the model cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* UTEX 2973, we seek to control the metabolic overflow reactions and enable rescue of the impaired growth during dark cycles by external energy supply. IC-MS measurements and <sup>13</sup>C-labelling will be used to illumine metabolic changes during diurnal growth and will thus allow identification of metabolites involved in establishing metabolic connectivity.

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The effect of Electro Magnetic Fields (EMFs) on seed germination and seedlings Physiological changes of *Coriandrum sativum* L. to achieve the optimum frequency of Electro Magnetic Field

Z. Atghia<sup>1</sup>

<sup>1</sup>Freelancer, Plant Biotechnology, Istanbul, Turkey

**Abstract:** Recently, most scientists and researchers have been focused on Pharmacological and Medicinal Plants to consider Antioxidant components and Secondary Metabolites. *Coriandrum sativum*, known as the Coriander, is one of the most important Medicinal plants from Apiaceae family. One of the methods for increasing the amount of Secondary Metabolites such as Anti Cancer/Tumor Metabolites, is to utilize Electro Magnetic Fields (EMF) as the Stress which influences on seed germination and seedlings Physiological changes by changing intensities and times of effects. The Positive and Negative effects are influenced the features such as rate of seeds and seedlings growth as well as the Biomass. The Process is taken by changing in potential electricity of cells membrane and physiological processes while there is no engineer manipulated in cells; Valsivoski, 2003. So, this physiological method has been the most functional optimum that causes the next generous will appear without any engineered manipulation. It is considered 3 frequencies of EMF such as 3, 5, 7 Mili Tesla as the treatments in 3 different times 5 min, 10 min, 15min, for each of the treatments in 2 repeats. Based on numerical analyser, the result shows the most functional optimum in 7 Mili Telsa under the seeds germination and growth than the normal seedlings, therefor this test is done again in 3 new different EMFs including, 6.5, 7.5, 8.5 Mili Tesla in 2 repeats. The new numerical analyser data displays that the time is another important factor for the EMF effects, so that the time as 10 minutes in both 7.5 Mili Tesla and 8.5 Mili Tesla treatments implies the high rate of seeds germination and growth in normal for seedlings up to upper leaves than first step as 2 leaves seedlings, meanwhile in other treatments are going to be abnormal leaves in shapes and sizes, consequently they are going to necrosis and apoptosis. As result, the observations make the time and the frequencies as the main factors by together to get the maximum anti-cancer metabolites in defined time for the future pharmacological processes of industry. **Keywords:** Electromagnetic field, EMF, *Coriandrum sativum*, Secondary Metabolite, Anti cancer/tumour metabolite, Anti-cancer/tumour herbal drug, Coriander.

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From cyanobacteria to cell organelle - Engineering and studying a synthetic cyanobacterial endosymbiont

T. Schulze<sup>1</sup>, J. Hofer<sup>2</sup>, L. Witting<sup>3</sup>, J. Volke<sup>2</sup>, D. Kohlheyer<sup>3</sup>, A. Weber<sup>2</sup>, M. Eisenhut<sup>1</sup>

<sup>1</sup>Bielefeld University, Computational Biology, Bielefeld, Germany

<sup>2</sup>Heinrich-Heine University, Plant Biochemistry, Düsseldorf, Germany

<sup>3</sup>Forschungszentrum Jülich, Microscale Bioengineering, Jülich, Germany

Cyanobacteria are prokaryotes capable of performing oxygenic photosynthesis and responsible for the increase in the atmospheric oxygen levels. The event of primary endosymbiosis describes the internalization of a single proto-cyanobacterium by a eukaryotic cell to become the ancestor of plastids in eukaryotic photosynthetic organisms. We will recapitulate the events proposed by the “ménage à trois” (MAT) hypothesis that enabled the proto-cyanobacterium to become an endosymbiont<sup>1</sup>. The MAT-suggested main event was the inevitable linkage of carbon and energy metabolism. The endosymbiont loses its ability to efficiently store carbon as substrate for respiration and energy production and instead excretes excess carbon during the day to feed the host. In return, the endosymbiont becomes dependent on the host’s energy supply during the night. Thus, a mutually beneficial relationship was formed. To test this hypothesis, we will utilize markerless knockout mutants in the model cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* UTEX 2973. We will stepwise engineer them into synthetic organelles and cultivate them on microfluidic chips functioning as the host’s cytoplasm. Starting with ADP-glucose pyrophosphorylase (*glgC*) to impair the cyanobacterium’s ability to synthesize the carbon storage molecule glycogen, genes will be sequentially knocked out. According to our hypothesis, implementation of a hexose-phosphate transporter and an ATP/ADP antiporter will allow the compensation of energy deficiencies of the  $\Delta$ *glgC* mutant strain. Changes in the levels of metabolites will be monitored by MS-based analytics and *in vivo* by the introduction of metabolite sensors. The effects of the changes to the transcriptome will be analyzed with RNA-seq, revealing possible targets for further knockouts.

References

1. Facchinelli, F., Colleoni, C., Ball, S. G. & Weber, A. P. M. Chlamydia, cyanobiont, or host: who was on top in the ménage à trois? *Trends in Plant Science* 18, 673–679 (2013).

P 356

Liquid in vitro culture system allows gradual intensification of osmotic stress in *Solanum tuberosum* through sorbitolK. Wellpott<sup>1</sup>, M. Herde<sup>2</sup>, T. Winkelmann<sup>1</sup>, C. Bündig<sup>1</sup><sup>1</sup>Leibniz Universität Hannover, Department of Woody Plant and Propagation Physiology, Hannover, Germany<sup>2</sup>Leibniz Universität Hannover, Department of Molecular Nutrition and Biochemistry of Plants, Hannover, Germany

Drought stress is one of the main problems in potato cultivation. In order to ensure high-quality tubers and thus stable yields, it is already common practice to apply artificial irrigation. The predicted climate change will further exacerbate this problem, especially in the strong vegetative growth phase in spring and early summer. In order to cope with the dry periods, plants adapt to the reduced water availability and try to tolerate the stress through intrinsic mechanisms, which e.g. include specific changes in gene expression.

The aim of the experiment was to analyse the expression of eight candidate genes within an in vitro experiment under osmotic stress by sorbitol in order to characterise these genes for their potential as biomarkers for the early detection of drought stress (Wellpott et al. 2024). The candidate genes were derived from an earlier proteomic study on plants under drought stress in a rain-out-shelter (Wellpott et al. 2021).

For this purpose, four starch potato genotypes with divergent stress responses were sampled in two independent experiments 0 and 7 days after stress onset. Growth parameters were recorded and sorbitol content was measured.

Shoots were analysed for gene expression of the eight potential marker genes by quantitative RT-PCR. Four of the eight genes (serine transhydroxymethyltransferase (SHMT), cell wall/vacuolar inhibitor of fructosidase (INH1), peroxidase 51-like (POD) and subtilase family protein (SBT1.7)) showed consistent changes in expression across all genotypes. SHMT, POD and SBT7.1 showed decreased expression, whereas INH1 showed increased expression. The increase correlated with the proteomic data, in which INH1 was also more abundant after drought stress. Therefore, INH1 could be a suitable candidate to detect the onset of drought stress in potato plants. However, sorbitol content was found to increase in shoots.

## References

Wellpott K, Jozefowicz AM, Mock HP, Meise P, Schum A, Winkelmann T, Bündig C (2021) Identification of candidate proteins in drought stress tolerant and sensitive starch potato genotypes (*Solanum tuberosum* L.) for biomarker development. Conf. Paper for the Ann. Conf. DGG & BHGL, Stuttgart (online). <https://doi.org/10.5288/dgg-pr-10-04-kw-2021>

Wellpott K, Herde M, Winkelmann T, Bündig C (2024) Liquid in vitro culture system allows gradual intensification of osmotic stress in *Solanum tuberosum* through sorbitol. P Cell Tiss Org Cult 157,12. <https://doi.org/10.1007/s11240-024-02720-w>

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Heterologous Lhcx expression in the diatom *Phaeodactylum tricorutum*M. A. Wunsch<sup>1</sup>, J. M. Buck<sup>2</sup>, P. Kroth<sup>2</sup>, B. Lepetit<sup>1</sup><sup>1</sup>Universität Rostock, Institut für Biowissenschaften, Rostock, Germany<sup>2</sup>Universität Konstanz, Biology, Konstanz, Germany

As phytoplankton, diatoms living in coastal or upwelling regions are exposed to strong fluctuations in light quality and quantity. To minimise cellular damage during exposure to high irradiance levels, these algae possess several photoprotective mechanisms. One of them is the Non-Photochemical Quenching (NPQ), which is the thermal dissipation of excessively absorbed light energy. The fastest sub-type of NPQ is the rapidly inducible and reversible energy dependent quenching (qE). Besides requiring specific xanthophylls, qE in diatoms compulsory needs the presence of thylakoid membrane-associated Lhcx proteins. These proteins are widespread amongst photosynthetic eukaryotes. We previously demonstrated that one motif within the Lhcx proteins of *P. tricorutum* is critical for qE. Sequence comparisons show that this motif is highly conserved among Lhcx proteins of other diatoms and is even found with a high degree of homology in haptophytes or green algae. Diatoms show species specific numbers of Lhcx genes with often unknown functions. By expressing several Lhcx genes of different algae in a *P. tricorutum* Lhcx1 knockout background lacking qE capacity, we aim to investigate a putative universal quenching mechanism

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Identification of genes involved in silica biomineralization in Stramenopile

D. Wulf<sup>1,2</sup>, F. Wang<sup>1</sup>, C. Sakurai<sup>1</sup>, S. Wakao<sup>1,3</sup>, K. K. Niyogi<sup>1,2,3</sup>

<sup>1</sup>UC Berkeley, PMB, Berkeley, CA, United States

<sup>2</sup>Howard Hughes Medical Institute, Berkeley, CA, United States

<sup>3</sup>Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, United States

CO<sub>2</sub> emissions are likely to lead to a rise in global temperatures beyond the 1.5 °C goal of global warming set by the Paris agreement. This goal is not achievable without carbon sequestration. Photosynthesis fixes CO<sub>2</sub> using sunlight energy, half of which occurs in the oceans, especially by diatoms that make up ~40% of oceanic phytoplankton biomass. Most of the fixed CO<sub>2</sub> is released back into the atmosphere, but a portion of it is sequestered in the deep sea, a process called the Biological Carbon Pump. Besides performing photosynthesis, diatoms produce a biomineralized silica cell wall that needs to be regenerated upon cell division. This silica shell formation enables growth, protection from predation, and leads to sedimentation of the cells. The sedimentation sequesters CO<sub>2</sub>. By identification of genes involved in silica shell formation, the efficiency of this sequestration pathway can be increased in the future using genetic tools.

To identify possible genes involved in silica shell formation, the model diatom *Thalassiosira pseudonana* and the synurophyte *Synura petersenii* were selected as models to study silica biomineralization. *T. pseudonana* requires silicon to grow and regenerates the shell during each cell division. *S. petersenii* can grow without silicon and makes silica scales depending on silicon availability. These differences were leveraged to perform a comparative transcriptomics study.

*T. pseudonana* cells were synchronized and the transcriptome studied with a high-resolution time course through two rounds of cell division. *S. petersenii* was grown without silicon, and the transcriptome time course was generated during silica scale biogenesis after addition of silicon. By comparing genes linked to cell division in *T. pseudonana* to silicon-induced genes in *S. petersenii*, we aim to identify conserved genes involved in silica biomineralization between these species. To identify possible silicification regulators, gene regulatory network inference was performed in *T. pseudonana*. These candidate genes will be investigated in high-throughput approaches in the future.

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Supplemental Irrigation: A Critical Strategy for Boosting Crop Productivity and Starch Functionality

W. Liang<sup>1,2,3</sup>, K. Herburger<sup>1</sup>, Y. Zhong<sup>4</sup>, A. Blennow<sup>2</sup>, Y. Liao<sup>3</sup>, X. Wen<sup>3</sup>, Y. Liu<sup>3</sup>

<sup>1</sup>Rostock University, Rostock, Germany

<sup>2</sup>University of Copenhagen, Copenhagen, Denmark

<sup>3</sup>Northwest A&F University, Yangling, China

<sup>4</sup>Max-planck insitution, Potsdam, Germany

Increasing agricultural water use efficiency is a critical strategy for addressing the global water deficit. Supplemental irrigation (SI) has been identified as an effective means of achieving this goal while maintaining crop yields. However, there is a lack of understanding regarding the impact of SI on crop quality, which is an important consideration. This research focuses on the effects of SI on crop growth, grain yield, and starch quality in winter wheat.

1. The Questions:

How much water can be saved by SI?

What impact will SI have on wheat growth?

Will it reduce grain yield while saving water?

How does SI affect the winter wheat quality?

2. The results demonstrate that SI can save 12% of water amount, and maintain the grain yield while saving water. SI has significant effects on crop starch quality, which underscores the importance of understanding the impact of different agricultural environmental conditions on starch quality. SI has little effect on yield and starch molecular structure in wheat, but significantly decreases the relative crystallinity of starch and increases the relative content of B-type granules. Additionally, the study found that tillage practices can influence the effect of SI on starch, with flat tillage enhancing the effect on granule-specific surface area and viscosity. In vitro digestion analysis showed that SI significantly enhanced the resistant starch content of starch, indicating SI-treated wheat provides starch with the trait of slow-digestion characterization.

3. Conclusion

Supplemental irrigation had a positive impact on both the yield and quality of winter wheat grain, as well as on its starch properties. Supplemental irrigation had an impact on the starch properties of winter wheat by reducing granular crystallinity, increasing the presence of B-type granules, and increasing the content of resistant starch.

Agricultural modification is considered the fifth method of starch modification, in addition to chemical, physical, enzymatic, and genetic methods. One of its main advantages is the ability to operate at a large scale and low cost. Various environmental conditions, such as water stress, heat stress, high nitrogen, salinity, shading stress, and CO<sub>2</sub> stress, can significantly impact starch biosynthesis, structure, and functionality.

Agricultural modification has the potential to produce a range of functional modified starches that can meet the growing demand for eco-friendly products.

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Influence of light on the interaction between *Chlamydomonas reinhardtii* and *Pseudomonas protegens*

A. Dierberger<sup>1</sup>, M. Rose<sup>1</sup>, R. Nagel<sup>1</sup>, T. Jakob<sup>1</sup>, S. Sasso<sup>1</sup>

<sup>1</sup>Leipzig University, Plant Physiology, Leipzig, Germany

Microbial communities are shaped by their abiotic environment as well as biotic interactions with other microorganisms. Secondary metabolites are important factors that influence the dynamics of these communities. We established the green alga *Chlamydomonas reinhardtii* and the heterotrophic bacterium *Pseudomonas protegens* Pf-5 as a model system to study antagonistic algal-bacterial interactions [1]. In this model system, *P. protegens* secretes secondary metabolites that negatively interfere with calcium signaling, deflagellate the algae, cause morphological changes, degrade the eyespot and arrest the growth of *C. reinhardtii* [1, 2, 3]. In our current work, we investigated how different light conditions affect this antagonistic model interaction. It was observed that the abiotic factor light affects the abundance of different secondary metabolites of *P. protegens*. We reasoned that bacterial photoreceptors may regulate the accumulation of these metabolites and identified several candidate genes in the genome sequence of *P. protegens*. Multiple putative photoreceptor knock-out mutants were constructed. These mutants are currently being analyzed and the results will be presented.

References

- [1] Aiyar, P. et al, (2017). Antagonistic bacteria disrupt calcium homeostasis and immobilize algal cells. *Nat. Commun.* 8, 1756.  
 [2] Rose, M.M. et al, (2021). The bacterium *Pseudomonas protegens* antagonizes the microalga *Chlamydomonas reinhardtii* using a blend of toxins. *Environ. Microbiol.* 23, 5525–5540.  
 [3] Hotter, V. et al, (2021). A polyene toxin produced by an antagonistic bacterium blinds and lyses a Chlamydomonad alga. *Proc. Natl. Acad. Sci. USA* 118, 1-9. (33)

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Rafting kelps *Macrocystis pyrifera* and *Egregia menziesii* from California: Physiological condition, ultrastructure, microchemistry and sensitivity to high UV treatment

A. Holzinger<sup>1</sup>, T. Roach<sup>1</sup>, C. Permann<sup>1</sup>, P. Charalambou<sup>2</sup>, N. Gierlinger<sup>2</sup>, D. Deheyn<sup>3</sup>

<sup>1</sup>University of Innsbruck, Department of Botany, Innsbruck, Austria

<sup>2</sup>University of Natural Resource and Life Sciences, Department of Bionanosciences, Vienna, Austria

<sup>3</sup>UCSD, Scripps Institution of Oceanography, La Jolla, CA, United States

Brown algae such as *Macrocystis pyrifera* and *Egregia menziesii* are usually sessile organisms that form large kelp forests. However, along the Californian coast many detached thalli are found, which may be part of their natural dispersal strategy or caused by increasing ocean temperatures due to climate change. Therefore, we tested the physiological condition of field collected rafting thalli, collected in July 2023. *M. pyrifera* had an  $Fv/Fm$  value of  $0.63 \pm 0.02$ , an  $ETR_{max}$  value of  $11.23 \pm 2.89$  and contained  $1.1 - 2.4 \mu\text{mol g}^{-1}\text{DW}$  chlorophyll a (chl a) and  $0.5 - 1.2 \mu\text{mol g}^{-1}\text{DW}$  chlorophyll c (chl c). *E. menziesii* had an  $Fv/Fm$  value of  $0.70 \pm 0.14$ , an  $ETR_{max}$  value of  $16.02 \pm 2.00$  and contained  $1.8 - 3.0 \mu\text{mol g}^{-1}\text{DW}$  chl a and  $0.9 - 1.5 \mu\text{mol g}^{-1}\text{DW}$  chl c. When exposed to an artificial UV stress treatment for 75 min (UVA:  $8.46 \text{ W m}^{-2}$ , UVB:  $4.19 \text{ W m}^{-2}$ , UVC:  $9.42 \text{ W m}^{-2}$ , PAR:  $50-54 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), the chl a and chl c contents as well as the chl a: chl c ratio were not significantly changed in both investigated species. However,  $ETR_{max}$  values decreased drastically in *E. menziesii* to  $5.45 \pm 4.80$  and were close to zero in *M. pyrifera*. After UV stress a significantly lower fucoxanthin level was found in *M. pyrifera* ( $p=0.04$ ), and a significant increase in the de-epoxidation ratio of the xanthophyll cycle pigments was found in both species.

The ultrastructure of *M. pyrifera* and *E. menziesii* blades showed typical brown algal features with a central medulla region and peripheral meristoderm containing chloroplasts, mitochondria and many Golgi bodies indicative of physiologically active cells. The ultrastructure of UV stressed cells did not change drastically, but showed damaged chloroplasts with dilated thylakoid membranes and accumulations of plastoglobules. Raman spectroscopy of *M. pyrifera* and *E. menziesii* allowed to detect the chemical signature of the protein and lipid rich cell contents in the meristoderm, which showed changes in the pigment composition after UV stress. The cell walls had a characteristic chemical signature containing cellulose, alginate and fucoidan which was very prominent in the medulla region, but not influenced by UV.

Overall, the present study indicates that rafting thalli of both species were physiologically active, showed an intact ultrastructure and microchemistry which was sensitive to experimental stressful UV treatment.



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Rare red bloom on a permanent snow field in Iceland: Ecophysiology of *Rosetta* sp. (Chlamydomonadales, Chlorophyceae)

D. Remias<sup>1</sup>, L. Procházková<sup>2</sup>, S. Gindorf<sup>1</sup>

<sup>1</sup>University of Salzburg, Dep. of Environment & Biodiversity, Salzburg, Austria

<sup>2</sup>Charles University, Praha, Czech Republic

Blooms of psychrophilic microalgae cause green, golden-brown or reddish snow discolorations, taking place during the melting season in mountainous and polar regions. Most prominent is the red snow phenomenon, where cells of green algae accumulate large amounts of the secondary carotenoid astaxanthin. Traditionally, these spherical unicells were summarized as cf. "*Chlamydomonas nivalis*", presumptively a collective species, and it was reported worldwide. A lack of cultures to study life cycles and the similar morphologies of the dominating immotile stages played a role for these earlier assumptions. With the onset of the molecular age, it was demonstrated that several distinctive taxa can make red snow, and most members belong to the chlamydomonadacean genera *Sanguina*, *Chlainomonas* or *Chloromonas*.

In this study, we likely rediscovered a rare species with the striking feature of a flagellate with sack-like outer envelope, *Smithsonimonas abbotti*, which was originally described by from Alaska (Kol 1942). It was collected in 2017 and 2023 from the same permanent, high-alpine snow field in Iceland. We investigated the alga's ecophysiology in terms of cell ultrastructure (TEM), photosynthesis and pigments. Marker sequencing was performed for the taxonomic classification and metagenomics for assessment of the algal community. The aim was not only to reveal the biodiversity of cryoflora lying behind a traditional collective species, but also to show that snow algae apparently developed several times similar cytological strategies to cope with the habitat of melting snow.

*rbcL* aligns the bloom to the genus *Rosetta*, which was recently erected based on single-cell sequencing of red spherical cells with striking cell-wall ornamentations (Engstrom et al. 2024). The results show an active metabolism based on inorganic carbon uptake. Chromatography revealed that astaxanthin derivatives are highly abundant, and the peak signatures are similar to the carotenoid-esters present in *Chlainomonas*. In summary, this chloromonade is well adapted to harsh conditions close to the freezing point in combination with exposure to high solar irradiation.

References

Kol, E. (1942). The snow and ice algae of Alaska. *Smithsonian Miscellaneous Collections* 101(16): 1-36, 6 pls.

Engstrom, C.B., Raymond, B.B. Albeitshawish, J., Bogdanovic, A., Quarmby, L.M. (2024). *Rosetta* gen. nov. (Chlorophyta): Resolving the identity of red snow algal rosettes. *Journal of Phycology*. 2024;60:275–298.

P 364

New mechanisms regulating the carbon-concentrating mechanism in cyanobacteria

P. Walke<sup>1</sup>, M. Hagemann<sup>1</sup>

<sup>1</sup>University of Rostock, Plant Physiology, Rostock, Germany

Cyanobacteria evolved oxygenic photosynthesis approximately 2.5 billion years ago. Still, they are responsible for up to 20% of global CO<sub>2</sub> fixation. As response to declining CO<sub>2</sub> and raising O<sub>2</sub> partial pressures, cyanobacteria evolved an inorganic carbon-concentrating mechanism (CCM). A set of bicarbonate transporters and CO<sub>2</sub> hydrating complexes enrich bicarbonate (HCO<sub>3</sub><sup>-</sup>) in the cytoplasm. The vast majority of the CO<sub>2</sub>-fixing enzyme RubisCO is located in the so-called carboxysome, where inwardly diffusing HCO<sub>3</sub><sup>-</sup> is quickly converted by carbonic anhydrases to fixable CO<sub>2</sub>.

The cyanobacterial CCM is tightly regulated responding to changing environmental conditions like CO<sub>2</sub> availability or light. In *Synechocystis* sp. PCC 6803, rapid regulation of the bicarbonate transporter SbtA is mediated by the PII-like regulator SbtB. SbtB itself seems to integrate various signals from second messengers, the redox state and most likely phosphorylation.

To examine these regulatory properties, strains lacking all known carbon uptake systems but SbtA are used. Here, we compare the affinity of the photosynthetic carbon response with theoretical estimations resulting in a more profound understanding of the physiological impact of the CCM. To achieve this, we used a variety of methods to quantify aquatic photosynthetic gas exchange of different strains. The mutant Δ5, lacking all carbon uptake systems, is not viable under ambient air. Reintroducing any of the systems is sufficient to rescue the viability. We additionally introduce heterologous transporters.

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### Developmental modifications of plasmodesmata in non-seed plants

K. Ehlers<sup>1</sup>, L. Wegner<sup>1</sup>

<sup>1</sup>Justus-Liebig-University, Institute of Botany, Gießen, Germany

Within the complex body plans of seed plants, highly dynamic networks of plasmodesmata (PD) mediate intercellular communication and control - amongst others - developmental processes. Since information is scarce for non-seed plants, we systematically investigated developmental modifications of PD numbers, structure and function in the five model species *Anthoceros agrestis* (hornwort), *Marchantia polymorpha* (liverwort), *Physcomitrium patens* (moss), *Selaginella moellendorffii* (spikemoss), and *Ceratopteris richardii* (fern).

PD counts performed with transmission electron microscopy revealed that all bryophytes and the fern adjust their PD numbers during cell (wall) expansion by postcytokinetic twinning of existing PD, giving rise to additional secondary PD (Wegner and Ehlers 2024). This mechanism, which has been described before for angiosperms, seems to be an ancient evolutionary trait that most likely emerged already in the most recent common ancestor of all land plants. Only the spikemoss lacked secondary PD formation, which hints at a specific evolutionary trait loss in this taxon, in contrast to its lycophyte sister groups Lycopodiales (clubmosses) and Isoetales (quillworts) (Imaichi and Hiratsuka 2007).

A developmental transition of originally narrow type I PD into wider type II PD with clearly visible internal substructures (Nicolas et al. 2017) seems to be another ancient trait that occurred with all investigated model species, except for the hornwort. In accordance with modelling approaches (Deinum et al. 2019), this PD modification may serve the adjustment of PD permeation efficiencies during wall thickening growth. Instead of forming type II PD, *A. agrestis* develops pit pairs with local, non-thickened wall areas traversed by type I PD.

Fluorescence recovery after photobleaching confirmed that the developmental modifications of the PD structures coincide with a reduction of the PD size exclusion limit in *P. patens*, as observed before for angiosperms.

Our work demonstrates that developing tissues of non-seed plants also possess dynamic PD networks which can be adjusted to varying requirements by means of ancient mechanism giving rise to secondary PD, as well as to structural and functional PD modifications.

#### References

Deinum et al. 2019; DOI 10.7554/eLife.49000

Imaichi and Hiratsuka 2007; DOI 10.3732/ajb.94.12.1911

Nicolas et al. 2017; DOI 10.1038/nplants.2017.82

Wegner and Ehlers 2024, submitted to *Planta*

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### Characterization of a durable biopolymer from *Chlamydomonas reinhardtii* zygospores

P. Ranganathapura Basavaraju<sup>1</sup>, V. Rohr<sup>2</sup>, C. Song<sup>2</sup>, R. Nagel<sup>1</sup>, J. Matysik<sup>2</sup>, S. Sasso<sup>1</sup>

<sup>1</sup>Institute of Biology, Leipzig University, Plant Physiology, Leipzig, Germany

<sup>2</sup>Institute for Analytical Chemistry, Leipzig University, Analytical Chemistry, Leipzig, Germany

*Chlamydomonas reinhardtii* is a single-celled model alga (Chlorophyta) that thrives in temperate soils. While gametogenesis and zygote formation have been studied extensively in *C. reinhardtii*, only a handful of publications have investigated the development of the zygote into a dormant zygospore. We know the indispensability of the *PKS1* gene during the formation of the zygotic cell wall, which facilitates the maturation of newly generated zygotes into zygospores (Heimerl et al. (2018), *The Plant Journal*, 95, 268–281). Zygospores represent a quiescent phase of the alga, marked by their resilience against challenging environmental conditions. The objective of this project is the purification and structure elucidation of the resilient biopolymer present in the cell wall of *C. reinhardtii* zygospores. Following a series of organic solvent washes and refluxing with various acids and bases, 1.2 mg of a highly resistant biopolymer was obtained from 12.9 g of initial fresh weight cell material. Diverse analytical techniques, including solid-state <sup>13</sup>C-nuclear magnetic resonance, and Fourier transform infrared spectroscopy were used to investigate the chemical structure of the purified polymer. Our preliminary analysis elucidated an aliphatic polymer consisting of hydroxylated fatty acids crosslinked via ester and ether bonds to form a resistant polymeric network. Our structure is similar to the polymer model proposed for zygospores of *Chlamydomonas monoica* (Blokker et al. (1999), *Planta*, 207, 539–543). To determine the length distribution and molecular weight of the polymer building blocks, we will degrade and analyze the polymer using pyrolysis-GC/MS. Additionally, Raman spectroscopy will be used for further structure elucidation. The protocol is being optimized in order to scale up production and purify more polymer. Detailed structure elucidation and examination of its mechanical properties will pave the way for beneficial applications in the future.

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Evolutionary “Hotspots” in Flavonoid Biosynthesis

M. F. Marin-Recinos<sup>1</sup>, B. Pucker<sup>1</sup>

<sup>1</sup>Institute of Plant Biology and BRICS, Plant Biotechnology and Bioinformatics, Braunschweig, Germany

Flavonoids are a diverse group of specialized metabolites found in almost all plant taxa, known for their antioxidant properties. Their biological functions span reproduction and protection against biotic and abiotic stressors. Among flavonoids, anthocyanins are notable for their roles in plant coloration. The visible phenotypes of anthocyanin mutants have facilitated extensive studies on the biosynthesis and its regulation, revealing a well-conserved process among plants. The synthesis of anthocyanins and proanthocyanidins, as terminal branches of the flavonoid biosynthesis pathway, share common precursors through a series of enzymatic reactions. A blockage at a specific step in the pathway can redirect the metabolic flow, leading to the production of a different class of flavonoids. Understanding evolutionary changes in biosynthesis pathways are of significant interest beyond flavonoid biosynthesis.

To identify the “genetic hotspots” responsible for substrate relocation leading to color variation, a systematic comparison of 230 studies including anthocyanin-pigmented and non-pigmented varieties was conducted, densely covering the taxonomic diversity of flowering plants. The contribution of different structural and regulatory genes to the intraspecific pigmentation differences was quantified. Notably, the MYB transcription factors play a crucial role in the specificity of the MBW complex, the central activator of anthocyanin biosynthesis, which includes MYB, bHLH, and WD40 proteins. According to our results, MYBs are most often responsible for the differences in anthocyanin content. When structural genes were responsible for the absence of anthocyanins, this was most often due to a lack of DFR activity. Our findings highlight the susceptibility of transcriptional regulation to evolutionary changes and its significance in the development of novel coloration phenotypes.

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DNA methylation, a regulator of keystone enzyme of chlorophyll biosynthesis in *Synechocystis* sp. PCC 6803

N. Schmidt<sup>1</sup>, S. Watanabe<sup>2</sup>, R. Sobotka<sup>3</sup>, W. R. Hess<sup>4</sup>, M. Hagemann<sup>1</sup>

<sup>1</sup>University of Rostock, Institute of Biosciences, Plant Physiology, Rostock, Germany

<sup>2</sup>Tokyo University of Agriculture, Department of Bioscience, Tokyo, Japan

<sup>3</sup>Centre Algatech, Institute of Microbiology, Třeboň, Czech Republic

<sup>4</sup>University of Freiburg, Genetics and Experimental Bioinformatics, Freiburg i. Br., Germany

In bacteria epigenetics regulates DNA repair, cell replication and gene expression by DNA methylation provided by DNA methyltransferases (MTases). *Synechocystis* sp. PCC 6803 harbors at least five functional MTases [1]. The aim of this project is to reveal the purpose and impact of the genomic DNA methylation in cyanobacteria.

Mutants lacking the MTase M.Ssp6803II (*slr1790*) possesses an altered phenotype. The cells are decreased in size, contained less chlorophyll a and are sensitive for UV exposure. However, this phenotype is unstable, after long-term cultivation of the  $\Delta$ *slr1790* strain single clones displaying wild-type-like phenotype (suppressor clones) [2]. Whole genome sequencing of this suppressor clones revealed a single nucleotide exchange in the promoter of *slr1790*. This gene encodes the protoporphyrinogen IX oxidase (HemJ), a keystone enzyme of the chlorophyll biosynthesis [3]. Partial knockout of HemJ displays a reduced chlorophyll content and is complemented by overexpression of homolog enzyme.

Transcriptome data of the original  $\Delta$ *slr1790* clones showed significantly reduced amounts of *slr1790* transcripts. HPLC measurements revealed accumulation of phototoxic chlorophyll precursors in  $\Delta$ *slr1790* cells. To verify the hypothesis that the mutated *slr1790* promoter is responsible for the wild-type like phenotype of the  $\Delta$ *slr1790* suppressor clones, *slr1790* promoter/ $\Delta$ *slr1790* double mutants with and without a specific *slr1790* promoter mutation in the methylation site were analyzed. Physiology and chlorophyll precursor accumulation of these strains support the *slr1790* promoter hypothesis. The native promoter double mutant shows similarly high levels of chlorophyll precursors like the  $\Delta$ *slr1790* single mutant. The Chlorophyll precursors in the mutated promoter double mutant are on a wild-type-like level.

Induced expression systems shall enable complementation of  $\Delta$ *slr1790*. In summary, we propose altered expression of *slr1790* as reason for  $\Delta$ *slr1790* phenotype, suspecting indirect regulation by promoter methylation due to M.Ssp6803II.

[1] Hagemann et al. DNA res., 2018, 25, 343-352, doi.org/10.1093/dnares/dsy006

[2] Gärtner et al. Front. Microbiol., 2019, 10, 1233, doi.org/10.3389/fmicb.2019.01233

[3] Kato et al. PNAS, 2010, 107, 16649-16654 doi.org/10.1073/pnas.1000771107

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## Insights into the DFG priority programme MAdLand

J. Fürst-Jansen<sup>1</sup>, S. 2. SPP 2237 „MAdLand“<sup>2</sup><sup>1</sup>Institute for Microbiology and Genetics, Georg-August University, Applied Bioinformatics, Göttingen, Germany<sup>2</sup>SPP 2237 “MAdLand”, Deutschland, Germany

MAdLand (Molecular Adaptation to Land) is about understanding the origins of key traits of land plants and their evolutionary impact on our modern flora. This DFG-funded priority program started in 2019 and is currently in its second funding period (2024 – 2026). Over 30 multidisciplinary research groups are included in the steadily growing MAdLand community. During the initial funding period, these groups uncovered genetic mechanisms in the adaptive evolution of plant morphology, physiology, biochemistry, and biotic interactions using comparative and functional evolutionary approaches. This resulted in over 70 publications on streptophyte algae and non-seed plants, many of which are a collaboration effort between MAdLand research groups, have appeared in highly reputed journals and are already well-cited.

In the second round of funding, these findings will be further elucidated with the help of new tools. The investigation of the role and evolutionary trajectory of signaling pathways based on phytohormones is a common theme across nearly all proposed projects. To support the MAdLand community, a platform for the analytics of specialized metabolites in non-seed plants be established. Additionally, MAdLand maintains close collaboration with DataPLANT (nfdi4plants) for comprehensive research data management. This collaborative effort also entails making the array of plant research tools and resources generated by the MAdLand project freely accessible to the scientific community.

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## Different Lhc proteins in diatoms and their functional diversity

N. Herrmann<sup>1</sup>, J. Sattler<sup>1</sup>, C. Büchel<sup>1</sup><sup>1</sup>Goethe University Frankfurt, Institute of Molecular Biosciences, Frankfurt a. M., Germany

The light harvesting complex (Lhc) protein family comprises antenna proteins for both photosystems (PS) as well as photo-protective proteins working in non-photochemical quenching. Algae usually contain many more Lhc proteins than vascular plants, and one of the intriguing questions is the reason behind this diversity. Besides the well-studied green algae, there are many algal groups present on Earth that are only distantly related to the green lineage and to each other, but contain Lhc as antenna proteins albeit binding different pigments. Most prominent are the Stramenopiles with their best studied group, the diatoms. Their membrane-intrinsic antenna proteins of the Lhc family bind chlorophyll (Chl) *c* besides Chl *a*, and fucoxanthin (Fx) is the main accessory pigment with up to seven molecules per protein of the eponymous Fucoxanthin-Chlorophyll Proteins (FCP). All diatom species contain a much higher number of expressed *Lhc* genes than found in vascular plants. In the centric diatom *Thalassiosira pseudonana* more than 30 FCP proteins exist, for *Cyclotella meneghiniana* 23 FCPs are known, and *Chaetoceros gracilis* and *Phaeodactylum tricorutum* express 22 and 32 FCP, respectively<sup>1</sup>. All are rather similar in pigmentation and, therefore, absorption, and nothing about their functional heterogeneity can be gained by comparing their protein sequence. Even Lhc<sub>x</sub>, proteins closely related to the LhcSR proteins of green algae and presumably involved in photoprotection by non-photochemical quenching, occur in different forms: in the best studied diatom *P. tricorutum* four different *Lhc<sub>x</sub>* genes were identified, whereby only three proteins were demonstrated to be involved in photoprotection<sup>2</sup>. Even localisation of Lhc<sub>x</sub> proteins is diverse, as e.g. Lhc<sub>x6\_1</sub> in *T. pseudonana* is found in PSII and PSI supercomplexes, whereas Lhc<sub>x1</sub> is located in the pool of FCPs<sup>3</sup>. We here compare Lhc<sub>x1</sub> and Lhc<sub>x6\_1</sub> of *C. meneghiniana* with respect to localisation and function, further demonstrating a functional heterogeneity of rather similar Lhc proteins.

## References

<sup>1</sup>Kumazawa M, Nishide H, Nagao R, Inoue-Kashino N, Shen J-R, Nakano T, Uchiyama I, Kashino Y, Ifuku K: *Physiol. Plantarum* 174 (1), e13598 (2022)

<sup>2</sup>Buck JM, Sherman J, Bártulos CR, Serif M, Halder M, Henkel J, Falciatore A, Lavaud J, Gorbunov MY, Kroth PG, Falkowski PG, Lepetit B: *Nat. Commun.* 10 (1), 4167 (2019)

<sup>3</sup>Calvaruso C, Rokka A, Aro E-M, Büchel C: *Plant Physiol.* 183 (1), 67-79 (2020)

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Terraphos: Exploring the Evolution of Phosphate Scouting During Plant Terrestrialization

C. Apel<sup>1</sup>, C. Naumann<sup>1</sup>, S. Abel<sup>1</sup>

<sup>1</sup>Leibniz Institute of Plant Biochemistry (IPB), MSV, Halle a. d. Saale, Germany

Phosphorus is a vital element for all forms of life. However, its presence in the Earth's crust is notably limited. The assimilated form of phosphorous, inorganic phosphate (Pi) is sequestered into insoluble co-precipitates with metal cations by chemical and microbial rock weathering. Thus, accessing the essential but immobile nutrient posed a significant challenge for first emerging land plants (embryophytes) with their simple rhizoid-based systems. Rhizomatous axes of the earliest embryophytes gave rise to the root systems of tracheophytes, including the capacity of growing root tips to monitor Fe-dependent Pi availability in the growth substrate (local Pi sensing). *Arabidopsis* LOW PHOSPHATE RESPONSE 1 (LPR1) is a key player in local Pi sensing and typifies a novel, bacterial-type cohort of Fe-oxidising multicopper oxidases, which are ubiquitous in the land plants. Interestingly, the ancestors of streptophytes acquired LPR1-type MCOs and at least two other genes involved in Fe-dependent root Pi sensing, by horizontal gene transfer (HGT) from soil dwelling bacteria. Beyond its role in Pi sensing, LPR1 affects root hair development, which is controlled by evolutionary conserved processes shared with rhizoid formation. This makes LPR1-type MCOs excellent candidates to study the effects of HGT on the evolution of land plants and their adaptations to dramatically altered geochemical conditions. This project aims to test the predicted ferroxidase activity of LPR1-like MCOs derived from select organisms covering the evolutionary range relevant to plant terrestrialization. To investigate a possible conserved function in local Pi recognition, we will perform complementation analyses with selected *LPR1*-type MCO genes in *Arabidopsis lpr1* knockout lines. In the bryophyte model system *Marchantia*, we will characterize Pi deficiency responses and analyze the function of LPR1-type MCOs for external Pi sensing, by reverse genetics.

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The plant xanthophyll cycle in the diatom *Phaeodactylum tricornutum*

C. Giossi<sup>1</sup>, M. A. Wünsch<sup>2,1</sup>, P. Kroth<sup>1</sup>, B. Lepetit<sup>2,1</sup>

<sup>1</sup>Universität Konstanz, Konstanz, Germany

<sup>2</sup>Universität Rostock, Rostock, Germany

Diatoms belong to the most successful photoautotrophs on earth. They show a remarkable capacity and flexibility for a fast photoprotection mechanism called energy depending quenching (qE). qE relies on the presence of Lhcx/LhcSR proteins, widespread amongst the eukaryotic photosynthetic kingdom, and a functional xanthophyll cycle. In the latter, the xanthophyll diadinoxanthin is converted into diatoxanthin under excess light conditions, which switches on qE. Due to the advent of genome editing tools, molecular aspects of this process could be (re-)studied in a unprecedented manner. This way, we managed to change the diatom specific xanthophyll cycle into a plant/green alga-like violaxanthin-antheraxanthin-zeaxanthin cycle in the model diatom *Phaeodactylum tricornutum*. We then studied qE in this engineered species and compared the capacity of zeaxanthin to provide qE to the original diatoxanthin-based one. The surprising results will be discussed and integrated into a working model.

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The model organisms *Anthoceros agrestis* and *Physcomitrium patens*: Insights into their cell walls and arabinogalactan-proteins

K. K. Mueller<sup>1</sup>, L. Wegner<sup>2</sup>, K. Ehlers<sup>2</sup>, L. Pfeifer<sup>1</sup>, B. Classen<sup>1</sup>

<sup>1</sup>Pharmaceutical Institut, Kiel University, Pharmaceutical Biology, Kiel, Germany

<sup>2</sup>University of Giessen, Botanical Institute, Gießen, Germany

While much is known about angiosperm cell walls, the knowledge about those of bryophytes is still limited. To extend this knowledge, two model organisms, *Anthoceros agrestis* and *Physcomitrium patens*, were analysed for their cell wall composition in this study. Analytical data indicate presence of pectins, probably mainly homogalacturonan. Immunocytochemical experiments demonstrated absence of a rhamnogalacturonan-I (RG-I) backbone, while the response of several antibodies raised against RG-I side chain epitopes was detectable. Antibodies directed against xylans and the XXXG-motif of xyloglucans indicated low amounts of xylans in the hemicellulose fractions of both bryophytes and high amounts of this xyloglucan motif only in those of *Anthoceros*. Cell wall glycoproteins, the arabinogalactan-protein (AGPs), were isolated, characterized and compared with other land plant AGPs. The galactan core structure was similar to those of other bryophyte and fern AGPs<sup>1</sup>, but different to angiosperm AGPs<sup>1</sup>, as 1,6-linked galactose was more or less absent. In the *Physcomitrium* AGP, the furanosidic arabinose (Araf) linkages were mainly terminal (t) or 1,5-linked, while in the *Anthoceros* AGP, t-Araf dominated and was accompanied by low amounts of 1,3-Araf and pyranosidic t-Ara. The unusual 3-O-methylated pyranosidic rhamnose was also detectable in both bryophyte AGPs, comparable to other spore producing land plant AGPs. The analytical findings were complemented by immunocytochemical detection of AGPs in transmission electron microscopy.

<sup>1</sup>Mueller *et al.*, 2023. Fern cell walls and the evolution of arabinogalactan proteins in streptophytes. *The Plant Journal*, 114, 875–894.

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## The role of trehalose 6-phosphate and TREHALOSE-6-PHOSPHATE SYNTHASE in the evolution of land plants

H. Lepper<sup>1</sup>, C. Champion<sup>2</sup>, Y. Coudert<sup>2</sup>, F. Fichtner<sup>1</sup><sup>1</sup>Heinrich-Heine-Universität, Institute of Plant Biochemistry, Düsseldorf, Germany<sup>2</sup>Université de Lyon, 2Laboratoire Reproduction et Développement des Plantes, Lyon, France

The low abundance signaling sugar trehalose 6-phosphate (Tre6P) has gained more and more attention as an important regulator of sucrose levels, influencing metabolic fluxes and plant development. Both Tre6P and its synthesizing enzyme TREHALOSE-6-PHOSPHATE SYNTHASE (TPS) have been shown to regulate metabolism, shoot branching, flowering and embryogenesis, primarily in angiosperms. However, TPS proteins are present throughout the whole green lineage, not just in flowering plants, and the number of them has increased with the transition to landtime. The most predominant catalytically active TPS protein in *Arabidopsis* (*AtTPS1*) is mainly localized in the vasculature which hints at a potential function of Tre6P during vascularization of early land plant ancestors. As part of the MAdLand Community we aim to elucidate the role of Tre6P in early land plants. Therefore Tre6P-levels will be modulated to observe changes in phenotype and metabolite contents in the extant representative of land plants: *Physcomitrium patens*. Furthermore, comparison of the fluctuations of Tre6P within algae of closest relation to land plants leads to an evolutionary understanding of the importance of Tre6P in terrestrialization and vascularization.

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## Uncovering the Evolution of Anthocyanin-Related Glutathione-S-Transferases

M. Borchert<sup>1</sup>, B. Pucker<sup>1</sup><sup>1</sup>TU Braunschweig, Institute of Plant Biology & BRICS, Plant Biotechnology and Bioinformatics, Braunschweig, Germany

Anthocyanins are one of the main pigments giving plants their unique hues. In nature, their vibrant colors are important for interaction between plants and their specific pollinators, while their antioxidant properties help plants under stress conditions. This combination of characteristics has also made anthocyanins attractive as food dyes or supplements. Other potential uses, such as medicinal application, are currently being investigated.

Anthocyanin biosynthesis is a well-studied model for plant biochemical regulation. Despite extensive research, details of its inner workings and evolutionary origins remain only partially understood. A new major enzymatic function was recently revealed: The group of anthocyanin-related glutathione-S-transferases (arGSTs), before thought to just be anthocyanin binding and transport proteins, were shown to possess catalytic activity. Together with anthocyanin synthase the arGSTs were found to be essential for the conversion of leucoanthocyanidins to anthocyanidins. This discovery also enabled heterologous anthocyanin production to function efficiently for the first time. Potential arGSTs were experimentally described for around 20 species, with most of them being dicots.

One of the first GSTs that was found to be involved in anthocyanin biosynthesis was BZ2 from the monocot *Zea mays*. However, comparing this protein to the now established arGSTs revealed significant differences in the catalytic residue positions. This observation prompted further investigation into monocot arGSTs. A phylogenetic analysis of known members of this group and new candidates identified through a global coexpression approach was conducted. Results indicate that some monocot arGSTs, with distinct catalytic residues, have evolved independently from their dicot counterparts. Other monocots possess arGSTs with high residue conservation when compared to dicot and early angiosperm arGSTs. To validate these findings, we plan to test the functional capacity of identified arGST candidates in complementing a TT19-deficient strain. This research not only uncovers the evolutionary diversity of arGSTs but also enhances our understanding of their role in the anthocyanin biosynthesis, offering new insights into plant biochemistry and potential applications in agriculture and food research.

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Visualization of redox dynamics during *Marchantia polymorpha* meristem development using redox sensors

C. Kock<sup>1</sup>, N. Gutsche<sup>1</sup>, J. Helmig<sup>1</sup>, S. Zachgo<sup>1</sup>

<sup>1</sup>Universität Osnabrück, Botany, Osnabrück, Germany

Reactive oxygen species (ROS), particularly hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), are not only fundamental signaling molecules for stress responses, but also regulate plant development by affecting stem cell formation and cell differentiation processes. Currently, knowledge about the impact of ROS affecting transcription factor activities is rapidly increasing in plants. In vascular plants such as *Arabidopsis thaliana*, meristematic tissues establish redox gradients with a more reduced state in the stem cell area. This has not yet been investigated in depth in bryophytes such as the liverwort *Marchantia polymorpha*, which enables comparative studies to analyze the contribution of ROS to the evolution of land plants.

We investigated the specific functions and mechanisms of glutathione (GSH) and H<sub>2</sub>O<sub>2</sub> in regulating stem cell activity and differentiation processes in *Marchantia*. Knockdown mutants of the  $\gamma$ -glutamylcysteine synthetase gene MpGSH1 were generated to study effects of lowered GSH levels. GSH depletion severely impacts root development in *A. thaliana*, MpGSH1 knockdown plants showed no severe phenotype, whereas full knockouts are likely lethal. The GSH redox potential was visualized applying the roGFP2-hGRX1 sensor in *Marchantia*, revealing differential redox potentials in meristematic versus differentiated thallus tissues. In addition, establishment of the biosensor HyPer7 in *Marchantia* allowed the visualization of H<sub>2</sub>O<sub>2</sub> dynamics in meristematic and mature thallus tissue. Treatment with the CLAVATA3/EMBRYO SURROUNDING REGION-RELATED 2 (MpCLE2) peptide is known to lead to an enlargement of the meristematic zone during vegetative growth. We took advantage of the MpCLE2 peptide effect to investigate the impact on redox potential and ROS distribution during an altered, extended meristem development. Together, our results reveal a crucial role of redox gradients and GSH in maintaining *Marchantia* meristem structure and function, while revealing phylogenetic differences in ROS-dependent stem cell regulatory mechanisms between bryophytes and vascular plants.

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Influence of trait flexibility and genome size on diversification of a tropical plant family

S. Bhadra<sup>1,2</sup>, I. J. Leitch<sup>3</sup>, S. Bellot<sup>3</sup>, W. J. Baker<sup>3</sup>, R. E. Onstein<sup>1,2,4</sup>

<sup>1</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, sDiv, Leipzig, Germany

<sup>2</sup>Leipzig University, Leipzig, Germany

<sup>3</sup>Royal Botanic Gardens, Kew, Richmond, United Kingdom

<sup>4</sup>Naturalis Biodiversity Center, Leiden, Netherlands

Functional traits affect adaptability and diversification rates of plants across lineages. However, it is unclear how genomic factors influence functional “trait flexibility” (i.e., evolvability of traits over macroevolutionary times) impacting diversification. Here we hypothesize that evolution of genome size (the total amount of DNA in the nucleus of a cell), due to its biophysical effects, plays a fundamental role in influencing trait flexibility, and hence diversification rates. To address this hypothesis, we integrated genome size, functional trait, and phylogenetic data of the model plant family of palms (Arecaceae) - a pantropical plant family comprising ca. 2,600 species that express wide functional and genome size diversity. Using macroevolutionary and structural equation models, we show that diversification rates of palms increased c. 20 million years ago, concordant with increased rates of genome size and trait evolution. Furthermore, larger genomes positively influenced the rate of genome size evolution, contrary to the idea that large genomes constrain evolution. Finally, we found that higher rate of genome size evolution was associated with greater trait flexibility. Lineages with larger genomes also showed faster leaf size evolution, which led to increased speciation rates. Rates of stem evolution were negatively influenced by genome size that, interestingly, led to increased speciation. Genome size evolution positively influenced fruit size evolution, likely facilitating animal-mediated dispersal promoting lineage persistence, but not necessarily speciation. Our results suggest that genome size evolution acts as a key driver of diversification in palms both directly, and indirectly via its influence on trait evolution. Comparable mechanisms may underlie some of the most enigmatic evolutionary radiations across angiosperms and the ecological success of certain plant lineages.

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**Warming and zooplankton grazing alter the composition and size-structure of a dinoflagellate-dominated community of the Central Baltic Sea**

C. Paul<sup>1</sup>, J. Dutz<sup>1</sup>, A. Kremp<sup>1</sup>

<sup>1</sup>Leibniz-Institute for Baltic Sea Research Warnemuende, Marine Biology, Rostock, Germany

In the Central Baltic Sea, Sea surface temperature (SST) in spring was already up to 2°C higher (2016) compared to the long-term average mean (1990-2016) and is predicted to increase up to 3.5°C until the end of this century (HELCOM 2017). Warming has been suggested to be the main driver of the observed changes in phytoplankton spring bloom biomass and community composition of the past decades in this area. We conducted a mesocosm experiment with a natural spring plankton community from the Bornholm Basin (Central Baltic Sea) under the combined effects of elevated temperature and excluding/including mesozooplankton grazing. We hypothesized that warming will lead to changes in the phytoplankton biomass and species composition, induced by temperature-driven changes in the micro- and mesozooplankton community.

Results show that at the beginning of the experiment the phytoplankton community was dominated by the dinoflagellate *Peridiniella catenata* and the mixotrophic ciliate *Mesodinium rubrum*, which typically follow the diatom bloom in this area. Elevated temperature lead to a faster drop-down of the phytoplankton bloom, independent from zooplankton treatments. Changes in community composition correlated to temperature and/or changes in grazing relations. Specifically, dinoflagellate biomass increased under elevated temperature alone, concomitant with a change from *P. catenata* dominance to *Dinophysis* sp. and *Proto-peridinium* species. Analyses further show that mesozooplankton grazing on microzooplankton ciliates occur earlier in the spring bloom than previously observed and is enhanced at elevated temperature, changing the phytoplankton community size structure from large dinoflagellates to a dominance of small-sized pico- and nano phytoplankton.

Our results suggest that under future climate change the phytoplankton spring bloom community composition of the Central Baltic Sea will be altered due to temperature-induced changes in zooplankton abundances and trophic interactions.

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**Time-resolved oxidative signal convergence across the algae–embryophyte divide**

T. P. Rieseberg<sup>1</sup>, A. Dadras<sup>1</sup>, T. Darienko<sup>1</sup>, S. Post<sup>2</sup>, C. Herrfurth<sup>2</sup>, J. Fürst-Jansen<sup>1</sup>, N. Hohnhorst<sup>1</sup>, R. Petroll<sup>3</sup>, S. Rensing<sup>4</sup>, T. Pröschold<sup>1</sup>, S. de Vries<sup>1</sup>, I. Irisarri<sup>1</sup>, I. Feussner<sup>2</sup>, J. de Vries<sup>1</sup>

<sup>1</sup>Georg-August-University of Göttingen, Applied Bioinformatics, Göttingen, Germany

<sup>2</sup>Georg-August-University of Göttingen, Plant Biochemistry, Göttingen, Germany

<sup>3</sup>Max Planck Institute for Biology Tübingen, Department of Algal Development and Evolution, Tübingen, Germany

<sup>4</sup>University of Freiburg, Centre for Biological Signalling Studies (BIOSS), Freiburg i. Br., Germany

500 MYA plant terrestrialization fundamentally affected our climate and life on earth. The increased fluctuation and intensity of abiotic factors in terrestrial habitats and the rising oxygen levels created by the first land plants themselves required an elaborate molecular toolkit to mitigate these oxidative stressors. To infer the essential components of this toolkit enabling the conquest of land, we conducted time-resolved stress experiments with the model organism moss *Physcomitrium patens* and the two genome-sequenced Zygnematophyceae algae *Zygnema circumcarinatum* and *Mesotaenium endlicherianum* – sister to all land plants. We combined transcriptomics, metabolite profiling (chlorophylls, carotenoids (RP-C30-HPLC-UV-Vis) and apocarotenoids (HS-SPME-GC-MS)), photophysiology and morphology to obtain a holistic picture. Carotenoids can be considered as one of the major antioxidants of plant biology and are present in any photosynthetic organism. Thus, utilizing their oxidative breakdown products called apocarotenoids as a stress-intensity read-out likely was highly beneficial for plant terrestrialization. Utilizing state-of-the-art inference models (SWING-RF) our data shows a so far unknown connection of LRR kinase gene expression with the small volatile apocarotenoid β-ionone conserved across the algae-embryophyte divide. Generally, combining a bouquet of methods in a huge time-resolved data set broadens our knowledge about several conserved stress-mitigation hubs of which many were long believed to be land plant-specific.

Reference

Rieseberg TP<sup>##</sup>, Dadras A<sup>\*</sup>, Darienko T, Post S, Hohnhorst N, Herrfurth C, Fürst-Jansen JMR, Petroll R, Rensing SA, Pröschold T, de Vries S, Irisarri I, Feussner I, de Vries J# (2024a) Time-resolved oxidative signal convergence across the algae–embryophyte divide. bioRxiv Preprint <https://doi.org/10.1101/2024.03.11.584470>



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Plant defense against pests under Arctic light conditions

A. Mithöfer<sup>1</sup>, C. Wekesa<sup>1</sup>, R. Halitschke<sup>2</sup>, L. Jaakola<sup>3</sup>

<sup>1</sup>Max Planck Institute for Chemical Ecology, Plant Defense Group, Jena, Germany

<sup>2</sup>Max Planck Institute for Chemical Ecology, Mass Spectrometry and Metabolomics, Jena, Germany

<sup>3</sup>The Arctic University of Norway, Tromsø, Norway

The anticipated effects of global warming extend to altering the growth conditions for plants and crops in regions situated at high latitudes, particularly those beyond 60° N, such as the Arctic. Consequently, there will likely be shifts in the natural composition of plant and pest communities, as herbivorous arthropods expand into these territories. Such expansions may instigate novel interactions between species that previously had minimal overlap, introducing fresh challenges in combatting herbivore attacks. Interestingly, plant species thriving in high latitude environments historically face reduced levels of herbivory in comparison to those found at lower latitudes. Our hypothesis is that this phenomenon stems from a gradient of inherent chemical defense mechanisms towards the Northern regions (1). We further hypothesize that this heightened defense is facilitated by elevated levels of the defense-associated phytohormones such as jasmonate. Given its dependence on light for biosynthesis, the extended daylight hours characteristic of Arctic summers may foster the accumulation of jasmonate and consequently trigger downstream physiological responses. To test these hypotheses, we conducted LC-MS/MS based metabolic profiling, gene expression analysis employing RNAseq, and classical feeding assays comparing Bilberry plants (*Vaccinium myrtillus*) that naturally grow in temperate and arctic regions. Preliminary metabolic studies have revealed significant differences in metabolic composition and expression intensity between plants of the two regions. The variability in metabolic expression, including jasmonates, throughout the summer season is notably lower in the arctic region, indicating a steady metabolic rate likely attributed to the consistent light regime during the summer. Among the metabolites overexpressed in the Arctic compared to the temperate region, approximately 70 of them exhibited sustained overexpression throughout the summer months, with the majority involved in the shikimate-phenylpropanoid pathways, recognized for their role in plant defense against insect herbivory.

References

(1) Mithöfer A, Riemann M, Faehn CA, Mrazova A, Jaakola L (2022): Plant defense under Arctic light conditions: Can plants withstand invading pests? *Front Plant Sci* 13, 1051107

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PhyloRSeq++: A Phylogenomic Pipeline for Investigating the Evolutionary Relationships of Streptophyte Algae Using Transcriptomic Data

M. Bierenbroodspot<sup>1</sup>, T. Darienko<sup>1</sup>, S. de Vries<sup>1</sup>, J. Fürst-Jansen<sup>1</sup>, H. Buschmann<sup>2</sup>, T. Pröschold<sup>1,3</sup>, I. Irisarri<sup>4</sup>, J. de Vries<sup>1</sup>

<sup>1</sup>Georg-August-University Göttingen, Institute for Microbiology and Genetics, Department of Applied Bioinformatics, Göttingen, Germany

<sup>2</sup>University of Applied Sciences Mittweida, Faculty of Applied Computer Sciences and Biosciences, Section Biotechnology and Chemistry, Molecular Biotechnology, Mittweida, Germany

<sup>3</sup>University of Innsbruck, Research Department for Limnology, Mondsee, Austria

<sup>4</sup>Section Phylogenomics, Centre for Molecular Biodiversity Research, Leibniz Institute for the Analysis of Biodiversity Change (LIB), Museum of Nature, Hamburg, Germany

Streptophytes, primarily known for including the diverse group of embryophytes (land plants), also encompass various freshwater and terrestrial algae essential for understanding the origins of key traits in land plants. Among these, the Klebsormidiophyceae stand out. Thriving in a wide range of habitats—from tree barks and rocks to extreme environments like the Atacama Desert and Antarctic—Klebsormidiophyceae exhibit filamentous structures and remarkable resilience as pioneers of terrestrial ecosystems.

However, the absence of a robust phylogenetic framework for Klebsormidiophyceae has impeded our understanding of their evolutionary history. In our recent study, we performed a phylogenomic analysis using advanced models to address systematic biases. We developed a comprehensive phylogenomic framework by sequencing 24 new Klebsormidiophyceae transcriptomes and integrating them with 14 previously published genomic and transcriptomic datasets.

Analyzing 845 loci with sophisticated mixture models, we classified Klebsormidiophyceae into six distinct genera within a newly proposed three-order system, indicating a deep divergence dating back over 830 million years. Our ancestral state reconstructions reveal (1) a complex evolutionary history with multiple transitions between terrestrial and aquatic habitats, with the stem Klebsormidiales colonizing land before embryophytes, and (2) the likely multicellular and filamentous nature of the last common ancestor of Klebsormidiophyceae, suggesting that sarcinoid and unicellular forms are derived states. Additionally, our findings indicate that the earliest multicellular streptophytes probably emerged around a billion years ago.

After this success, we established the comprehensive pipeline PhyloRSeq++—a phylogenomic pipeline using transcriptomic data from start to finish. Furthermore, we applied PhyloRSeq++ to analyze the Zygnematophyceae, Coleochaetophyceae, and all currently available streptophyte algae transcriptome data. This comprehensive analysis across these groups enhances our understanding of the evolutionary relationships and the early diversification of streptophytes, providing deeper insights into the origins and transitions of key traits within the entire streptophyte clade.

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Gene expression profile of the herbivore-induced stress responses in *Quercus robur*

H. Schroeder<sup>1</sup>, M. Mader<sup>1</sup>, F. Orgel<sup>1</sup>, T. Nosenko<sup>2</sup>, J. Schnitzler<sup>2</sup>, B. Kersten<sup>1</sup>

<sup>1</sup>Thuenen Institute of Forest Genetics, Großhansdorf, Germany

<sup>2</sup>Helmholtz Zentrum München, Research Unit Environmental Simulation, München, Germany

The pedunculate oak (*Quercus robur*) shows high potential for adaption and is therefore a prime candidate to become a pillar of resilient forests in the future to combat the challenges of climate change. The project “Survivor-Oaks” aims to identify oak trees that have increased tolerance to different biotic and abiotic stresses including herbivory, mildew infestation and drought. Tolerant trees will be selected for the establishing future climate seed orchards. Here, we characterize oak trees based on their expression profiles with respect to herbivory. The goal of this study is to compare the expression-level response of oak trees to infestation by two different insects: a specialist, the green oak leaf roller, *Tortrix viridana*, and a generalist, the gypsy moth, *Lymantria dispar*.

In earlier projects, oaks tolerant or susceptible to *T. viridana* have been identified based on defoliation rates in several high-infestation years. We conducted two separate feeding experiments with the two above mentioned insects using three tolerant and three susceptible oak clones. High-throughput RNA-seq data have been generated and analysed by mapping the data versus a new chromosome-level reference genome of *Q. robur* and differential gene expression analysis with Deseq2. Functional studies were performed using Gene Ontology over-representation and enrichment analyses of genes expressed differentially in response to the two herbivores. The stress responses revealed as well similarities but also differences in the reaction of *Q. robur* to *T. viridana* and *L. dispar*. (e.g., an enrichment of terpene synthase-related terms or regulation of the jasmonate pathway).

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Saltational evolution: a case study in *Capsella bursa-pastoris*

G. Theißen<sup>1</sup>, F. Rümpler<sup>1</sup>, P. Nutt<sup>1</sup>, J. Ziermann<sup>1</sup>, A. Hoffmeier<sup>1</sup>, J. Patzer<sup>1</sup>, L. Gramzow<sup>1</sup>

<sup>1</sup>Matthias Schleiden Institute / Friedrich Schiller University Jena, Genetics, Jena, Germany

Over the last two decades, the *Stamenoid petals* (*Spe*) variants of shepherd’s purse (*Capsella bursa-pastoris*) have been developed into a model system for studying saltational evolution [1, 2]. *Spe* plants represent homeotic mutants in which the petals are transformed into functional stamens, whereas all other organs of the flower are as in the wildtype. The ecophysiological mechanisms that enable *Spe* plants to co-exist in sympatry with wild-type plants in natural populations over decades are relatively well understood [3-7]. The molecular mechanism that generated *Spe* plants in the first place remain elusive, however. We used comparative sequence analysis, classical gene mapping approaches, gene expression studies and genome editing by CRISPR-Cas9 to elucidate the underlying mutation. Here we report that one *Spe* variant was generated by a 6.8 kb insertion of a non-LTR retrotransposon 2 kb upstream of the coding region of one of the two *AGAMOUS*-loci (*CbpAGa*) of *Capsella bursa-pastoris*. This generated a semi-dominant mutant allele (*CpbAGa-Spe*) that is ectopically expressed in the second floral whorl. Since *AGAMOUS* genes provide the floral homeotic C function involved in specifying stamens [8], this finding readily explains the homeotic transformation of second whorl organs into stamens in *Spe* plants, as previously hypothesized [1, 2]. Our results support the view that a radically deviant variant, that has historically even been considered as a new species (*Capsella apetala*), can occur by a simple genetic change within one generation which is the hallmark of saltational evolution.

References

1. Nutt, P., et al. (2006) *Pl. Syst. Evol.* **259**, 217-235
2. Hintz, M., et al. (2006) *J. Exp. Bot.* **57**, 3531-3542
3. Ziermann, J., et al. (2009) *Planta* **230**, 1239-1249
4. Hameister et al. (2009) *Mol. Ecol.* **18**, 2659-2667
5. Hameister, S., et al. (2013) *Flora* **208**, 641-647
6. Hameister and Neuffer (2017) *Plant Ecol. Evol.* **150**, 67-75
7. Neuffer et al. (2020) *PeerJ* **8**:e9226
8. Theißen, G., et al. (2016) *Development* **143**, 3259-3271

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The adaptive potential of the leaf economics spectrum in the Brassicaceae - Role of photosynthetic carbon assimilation biochemistry in adaptation to urban environments

T. Blank<sup>1</sup>, U. Schlüter<sup>1</sup>, A. Weber<sup>1</sup>

<sup>1</sup>Heinrich-Heine-Universität, Düsseldorf, Plant biochemistry, Düsseldorf, Germany

The leaf economics spectrum (LES) is a set of six photosynthesis-correlated traits that covaries across environmental conditions and taxonomic groups. For example, variations in the LES can be used as a measure of how a species adapts to its current environment, i.e. to identify whether it is driven by a resource acquisitive or a resource conservative strategy.

The genus *Diplotaxis* consists of several wild and a small number of recently developed crop species. Especially three species, *D. tenuifolia*, *D. viminea* and their hybrid *D. muralis*, are of special interest for being closely related but still highly diverse. The differences between their modes of photosynthesis (C3 vs C3-C4 intermediates) qualify them as valuable targets for studies on photosynthesis-correlated adaptation to abiotic stress. An early pre-experiment with one or two accessions of each species demonstrated variations of LES traits and dependence on soil type. Further extensive studies on a larger set of accessions will be performed to gain insights into the influence of abiotic stresses on the LES.

To prepare these studies, new accessions were gathered during the natural growth period in 2023. During 5 field trips through (almost) the entire Germany new accessions were collected from a spectrum of previously known and newly identified habitats. The field trips were planned *in silico* by using a combination of public database information and a publicly available imaging tool. As a result, a total of 90 accessions were obtained. As far as possible the collections consisted of:

Seeds for *ex situ* preservation and future studies of the LES traits

Leaf material for early genotyping and elemental analysis

Soil samples together with climatic data to find patterns in the species ecological niche

*In situ* photosynthetic data using a porometer and a multispeQ

Images for determination of *in situ* phenotypes and microenvironments

The collected accessions are currently cultivated in a common garden setup together with reference accessions from the Neuffer Brassicaceae Germ Plasm Collection, Osnabrück for an initial phenotyping. Within each accession up to 6 plants are maintained and allowed to perform open pollination. DNA from all maintained plants will be collected for next generation sequencing and the F1 generation will be used for further studies.

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Polyploidy in Arid plants: Transient or Coexisting? Their Ways to Evolutionary Success

S. Arora<sup>1</sup>, M. Barupal<sup>1</sup>

<sup>1</sup>Jai Narain Vyas University Jodhpur, Botany, Jodhpur, India

Polyploidy, the duplication of an entire genome, is a surprisingly common phenomenon in nature. While it's often associated with harsh environments and invasive species, it also carries significant drawbacks for the organism. Polyploids might occupy a different ecological niche than diploids, reducing competition. Some models propose that polyploidy leads to "reciprocal gene loss," where one copy of a duplicated gene is lost in each lineage following a polyploidization event. This could potentially drive rapid diversification. This review explores the question: can polyploidy be a successful evolutionary path for plants in these harsh conditions? While polyploidy might offer advantages like increased stress tolerance, it can also lead to fertility issues and require adjustments in gene regulation. Understanding the cost-benefit balance of polyploid traits and the trade-offs between different characteristics is crucial. Modern coexistence theory suggests mechanisms like niche differentiation and evolutionary adaptations might allow polyploids to carve out a sustainable space within the ecosystem. Recurrent polyploidization events and the role of chance also warrant investigation. For instance, polyploidy might lead to the evolution of traits that enable plants to exploit specific microclimates within the desert, such as increased water storage capacity or deeper root systems to access underground water reserves. Additionally, polyploidy can trigger the resurrection of dormant genes, potentially leading to the emergence of novel adaptations beneficial for desert survival. Furthermore, recent studies highlight the potential role of recurrent polyploidization events in shaping desert plant diversity. The repeated duplication of genomes over generations might create a pool of genetic variation that can be acted upon by natural selection. This could lead to the rapid evolution of new lineages specifically adapted to the harsh desert environment. The role of chance events, such as hybridization between polyploids and diploids, also warrants investigation, as it could further contribute to the diversification and establishment of polyploid desert flora. In this review, we have explored the multifaceted relationship between polyploidy and the evolutionary success of plants and their intricate mechanisms of the interplay between stress tolerance, niche differentiation, recurrent polyploidization events in arid environments.

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#### Root evolution during maize domestication

I. Lopez Valdivia<sup>1,2</sup>, M. Vallebuena-Estrada<sup>3</sup>, H. Rangarajan<sup>2</sup>, K. Swarts<sup>3</sup>, B. Benz<sup>4</sup>, M. Blake<sup>5</sup>, J. Singh Sidhu<sup>2</sup>

S. Perez-Limon<sup>2</sup>, R. Sawers<sup>2</sup>, H. Schneider<sup>1</sup>, J. Lynch<sup>2</sup>

<sup>1</sup>IPK Gatersleben, OT Gatersleben, Germany

<sup>2</sup>Penn State University, Köln, Germany

<sup>3</sup>Gregor Mendel Institute of Molecular Plant Biology GmbH, Viena, Austria

<sup>4</sup>Texas Wesleyan University, Texas, TX, United States

<sup>5</sup>The University of British Columbia, Vancouver, Canada

Roots are essential for plant adaptation to changing environments, yet the role of roots in crop domestication remains unclear. This study examines the evolution of root phenotypes from teosinte to maize, a transition resulting in reduced nodal root number (NRN), multiseriate cortical sclerenchyma (MCS), and increased seminal root number (SRN). We reconstructed the root phenotypes of maize and teosinte, as well as the soil and atmospheric environments of the Tehuacan Valley - an important site of maize domestication - over the last 18,000 years using a combination of ancient DNA, paleobotany, and functional-structural modeling. Our results reveal that increasing Holocene atmospheric CO<sub>2</sub> concentrations permitted the appearance of reduced NRN and MCS between 12000 to 8000 years before present (yBP), promoting deeper root systems. The advent of irrigation by 6000 yBP switched nitrogen distribution from topsoil to subsoil domains, a change which increased the utility of reduced NRN and MCS. Comparison of allelic frequencies among ancient samples ranging from 5500 to 500 yBP suggest that increased SRN may have appeared around 3500 yBP, coinciding with a period of increased human population, agricultural intensification, and soil degradation. Our results suggest that root phenotypes that enhance plant performance under nitrogen stress were important for maize adaptation to changing agricultural practices in the Tehuacan Valley. Our results support the hypothesis that anthropogenic modifications to the soil environment shaped the root phenotypes of modern maize.

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#### Comprehensive Transcriptome Atlas of *Marchantia polymorpha*: Insights into Gene Regulation and Stress Response

W. Halpape<sup>1,2</sup>, A. Busch<sup>3</sup>, B. Laker<sup>2</sup>, N. Gutsche<sup>3</sup>, F. Althoff<sup>3</sup>, B. Verwaaijen<sup>2,4</sup>, S. Zachgo<sup>3</sup>, A. Bräutigam<sup>2</sup>

<sup>1</sup>PLUS Universität Salzburg, Pflanzenphysiologie, Salzburg, Austria

<sup>2</sup>Universität Bielefeld, Computational Biology, Bielefeld, Germany

<sup>3</sup>Universität Osnabrück, Division of Botany, Osnabrück, Germany

<sup>4</sup>Martin-Luther-University Halle-Wittenberg, Department of Genetics, Halle a. d. Saale, Germany

Liverworts, a prominent group within the bryophytes, are characterized by their unique morphology and the absence of true roots, seeds, or a vascular system. *Marchantia polymorpha* genome lacks evidence of whole-genome duplications over the past 430 million years. Despite having only about a quarter of the transcription factors of *Arabidopsis thaliana*, a model seed plant, *Marchantia* includes nearly all known transcription factor families.

We present a comprehensive transcriptome atlas for *Marchantia*, encompassing over 609 RNA-seq samples collected from various developmental stages and in response to diverse abiotic and biotic stimuli. Using machine learning techniques, we inferred a gene regulatory network to elucidate the functions of *Marchantia* transcription factors. Comparative analysis with *Arabidopsis* revealed both conserved and divergent functions of transcription factors between the two species.

Our results provide a valuable resource for the research community. The abundance of genes of interest can be examined under different stress conditions and developmental stages. Network analysis offers functional predictions for half of the transcription factors. Additionally, differential gene expression analyses across various stress conditions and temporal backgrounds facilitate the identification of genes relevant to specific conditions.

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Distribution and carbon content of root biomass of annual and perennial crops in agroforestry systems

C. Reuse<sup>1</sup>, M. Langhof<sup>1</sup>, B. Stever-Schoo<sup>1</sup>

<sup>1</sup>Julius Kühn Institute, crop production and soil science, Braunschweig, Germany

Agroforestry systems are seen as a measure to adapt agriculture to climate change, but can also have a climate protection function. Perennial plants, such as trees, store carbon in above- and below-ground biomass over a certain period of time and can therefore serve as carbon sinks in these systems. These are becoming increasingly important in times of climate change, as atmospheric carbon (C) can be sequestered here and thus the climate-damaging greenhouse gas carbon dioxide can be reduced. Studies of root biomass in agroforestry systems are still rare. In alley cropping, fast-growing trees are grown alternately with arable land or grassland in strips and harvested every 3 to 7 years for energy or raw material production. To reduce competition for light, nutrients and water between arable crops and trees, it makes sense to grow plants that are adapted to these conditions. The perennial fiber nettle (*Urtica dioica* L. convar. *fibra*) colonizes semi-shady places such as forest edges. Their benefits can be found, for example, in the textile, fiber, cosmetics and food industries as well as in medicine, horticulture and the energy sector - but so far only as a niche product. The root biomass, root carbon content and structural components (lignin, cellulose, hemicellulose) of the two perennial crops poplar and fiber nettle as well as the annual crop maize were comparatively investigated on an alley cropping agroforestry system in northern Germany. The calculation of the area-specific carbon and nutrient quantities was based on root samples up to a soil depth of 160 cm. The root masses of poplar were the highest (53 t DM/ha) and those of maize the lowest (8 t DM/ha). Particularly in deeper soil layers, poplar showed significantly higher root biomasses than maize and nettle. In poplar, around 30 % of the root biomass was assigned to the <2 mm diameter class, and 35 % each to the >5-<10 mm and >10 mm classes. The roots from 5 mm diameter showed a very wide C/N ratio of 136 to 147 compared to 36 for nettle roots and 60 for maize roots due to a high proportion of lignocellulosic components. It can therefore be assumed that not only a higher, but also a longer-term carbon sequestration takes place in the poplar strips than in the comparison crops.

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How much are traits that influence plant fitness fixed due to genetic adaptation or derived during acclimation?

V. Soares<sup>1</sup>, S. Rosbakh<sup>2</sup>, T. Roach<sup>1</sup>

<sup>1</sup>University of Innsbruck, Institute of Botany, Innsbruck, Austria

<sup>2</sup>University of Copenhagen, Department of Plant and Environmental Sciences, Frederiksberg C, Denmark

Sexual plant regeneration is the most temperature-sensitive stage of the plant life cycle, and air temperature is one of the few environmental variables that is consistently elevation-dependent. Plants can respond to environmental factors, such as temperature, within a generation through phenotypic plasticity or across generations via adaptation. It is unclear how much differences in sexual reproductive traits across populations from different altitudes of the same species are adapted (genetically predetermined) or vary due to acclimation. Here, we investigated how key reproductive traits of the same species are influenced by elevation. We hypothesize that (1) species that distribute over large elevation gradients possess a high level of plasticity in sexual reproductive traits, and (2) the biotic and abiotic conditions at high (e.g., short growing season, lack of pollinators) and low (e.g., heat stress, fungal infection) elevation negatively impact specific traits relevant to fitness and species distribution. Reciprocal transplantation of elevation-associated ecotypes of *Arabidopsis arenosa* and genotypes of *Silene vulgaris* were used to test how many traits that influence fitness are fixed due to adaptation (genetic) or derived during acclimation (plasticity). Plants were grown in two common gardens in Tyrol, Austria, at 610 m a.s.l. (valley garden) and at 1960 m a.s.l. (alpine garden). Phenology was majorly impacted by elevation (temperature), with *A. arenosa* plants having finished flowering in the valley garden long before they had started in the alpine garden. Clear phenotypic differences were apparent between high and low-elevation ecotypes in both common gardens, showing a high level of adaptation in rosette diameter, flowering spike length, and seed yield. Currently, we are measuring vegetative traits on plants in both common gardens, and analyses are in progress to investigate the impact of elevation on gametophyte and seed traits.

References

Steinacher, G. and Wagner, J. (2012). Effect of temperature on the progamic phase in high-mountain plants. *Plant Biol (Stuttg)* 14, 295-305  
 Wos, G., Arc, E., Hülber, K., Konečná, V., Knotek, A., Požárová, D., Bertel, C., Kaplenig, D., Mandáková, T., Neuner, G. et al. (2022). Parallel local adaptation to an alpine environment in *Arabidopsis arenosa*. *Journal of Ecology* 110, 2448-2461.

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Habitat potential modelling of *Teucrium chamaedrys* in Iran and the effect of climate change on its distributionM. Keshavarzi<sup>1</sup>, F. Farajollahi<sup>1</sup>, A. Mehrabian<sup>1</sup>, H. Mostafavi<sup>1</sup><sup>1</sup>Alzahra university, Plant Sciences, Tehran, Iran

Climate change has a crucial effect on the habitats of sensitive species. Species distribution models try to formulate and illustrate the different aspects of climate change on the present and future species distribution. *Teucrium* (Lamiaceae) with 15 species in Iran is of medicinal importance in traditional medicine. These species are rich in tannins and polyphenols are commonly used as edible and medicinal plant and flavoring agents. This species has a natural distribution in different habitats of Iran, especially in the provinces around the southern coast of the Caspian Sea. This study aims to model the distribution of *Teucrium chamaedrys* in Iran using the MaxEnt model under two representative concentration pathways (RCP 2.6 and RCP 8.5) for the years 2050 and 2080. The objective is to identify the crucial bioclimatic ( $n=5$ ), topographic ( $n=3$ ) and edaphic variables that influence their distribution and predict how their distribution might change under various climate scenarios. The findings reveal that the most significant variables affecting *T. chamaedrys* are Precipitation of the Warmest Quarter and Mean Temperature of the Coldest Quarter. The MaxEnt modelling demonstrates excellent performance, as indicated by all the area under the curve (AUC) values exceeding 0.9. Based on the projections, it is expected that *Teucrium chamaedrys* will experience negative area changes in the coming years. These results can serve as a valuable tool for developing adaptive management strategies for habitat management and sustainable development due to global warming. According to habitat loss, this species needs special attention and conservation despite its present least concerned situation.

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Effect of nitrogen deposition on reproductive potential of *Juniperus communis* L. and *Taxus baccata* L.E. Pers-Kamczyc<sup>1</sup>, E. Małderek<sup>1</sup>, J. Suszka<sup>1</sup>, J. Kamczyc<sup>2</sup><sup>1</sup>Institute of Dendrology Polish Academy of Sciences, Department of Genetics and Environmental Interactions, Kórnik, Poland<sup>2</sup>Poznan University of Life Sciences, Department of Game Management and Forest Protection, Poznan, Poland

Nitrogen is an element necessary for plant growth and a key component of forest ecosystems. It is one of the basic nutrients that plants need to synthesize proteins, enzymes and other essential molecules. It is also considered a critical limiting factor to plant growth in many forest ecosystems, meaning its availability can significantly impact ecosystem productivity and health. Over the last century, increased agricultural and industrial activity has significantly impacted the global nitrogen cycle, and the level of global nitrogen deposition in soil has increased and is expected to increase significantly in the coming years.

A long-term experiment was conducted at the Institute of Dendrology of the Polish Academy of Sciences, which analyzed the effect of long-term nitrogen availability on the reproductive potential of common yew and common juniper as a model species of woody plants representing different habitat preferences as well as dioecious plants. The experiment used vegetatively propagated individuals of both sexes, which were kept under long-term fertilization conditions. We analyzed quantitative and qualitative parameters of seeds and pollen grains, the germination ability of seeds and pollen grains, the seedling growth potential, as well as content of elements and GC-MS metabolome profile.

It was observed that plants grown in long-term fertilization conditions produced more seeds, but their quality was reduced, which resulted in lower germination capacity. The obtained results indicate a significant negative impact of nitrogen deposition in the soil on the reproductive potential of both species and, consequently, on the sustainability of their populations in the future.

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P 402

Exploring the role of polysaccharide root exudates and biochar in the aggregation of coarse-and fine-textured arable soils

O. A. Leal<sup>1</sup>, M. Sader<sup>2</sup>, G. Salahee<sup>1</sup>, D. Drobietz<sup>2</sup>, N. Siebers<sup>1</sup>, N. Brüggemann<sup>1</sup>, A. Kuhn<sup>2</sup>, H. Klose<sup>2</sup>, M. Wrona<sup>2</sup>, S. D. Schrey<sup>2</sup>

<sup>1</sup>Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences / Agrosphere (IBG-3), Jülich, Germany

<sup>2</sup>Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences / Plant Sciences (IBG-2), Jülich, Germany

Plant roots exude photosynthetically fixed carbon via roots to the soil. Here, the root exudates help to shape the rhizosphere and modify soil structure. Up to 80% of root exudates consist of polysaccharides which are well-known as components of plant cell walls but understudied as part of the root exudate profile. Biochar is a soil amendment that provides multiple services, including improving the structure of soils with low agricultural productivity. In this study, we aimed to analyze the spectrum of polysaccharides released by plant roots and determine their role in soil aggregate formation, particularly when using biochar as a soil amendment.

We tested maize root exudates as well as a range of commercially available polysaccharides on soil adhesion to a positively charged nitrocellulose membrane using four arable soil types with contrasting texture (sand, sandy loam, silt loam), pH (4.6–7.4), and organic carbon content (3.0–21.0 g kg<sup>-1</sup>), with and without biochar amendment (5–15 Mg ha<sup>-1</sup>). We further studied the effects of a 12-week incubation of a soil-biochar blend on soil adhesion, compared to freshly mixed soil-biochar soil samples. Microaggregate stability and CO<sub>2</sub> release from polysaccharide-soil mixtures was measured. Monosaccharide linkage analyses of maize root exudates was carried out to elucidate their composition.

Our findings show distinct pattern in polysaccharide – soil interaction. Polysaccharides with effective binding properties showed this potential across all tested soils. While some polysaccharides displayed no soil specificity (binding all soils uniformly), others exhibited a high degree of specificity as they were less effective in sandy soils compared to those of finer texture. Overall, biochar increased soil adhesion. However, after a 93-day incubation period, biochar led to a pH drop in the sandy soils, likely reducing soil adhesion. Using xyloglucan as a model polysaccharide we further show that stability of microaggregates is increased, and CO<sub>2</sub> release is reduced compared to glucose as an easily available sugar. Thus, more complex exudates may persist longer in soil, thereby favoring soil aggregate formation.

Our results show the potential and relevance of plant root-exuded polysaccharides interacting with soil particles and biochar. Promoting soil aggregation, particularly in marginal soils, by elucidating interactions between root exudates, soil types and biochar may provide a nature-based solution for sustainable agriculture.

PL 002

A chemical effector influencing stomatal development

A. Herrmann<sup>1,2</sup>, K. Sepuru<sup>1,2</sup>, H. Endo<sup>3</sup>, A. Nakagawa<sup>3</sup>, S. Kusano<sup>4</sup>, P. Bai<sup>1,2</sup>, A. Ziadi<sup>3</sup>, H. Kato<sup>3</sup>, A. Sato<sup>3</sup>, J. Liu<sup>5</sup>, L. Shan<sup>5</sup>

S. Kimura<sup>6</sup>, K. Itami<sup>3</sup>, N. Ushida<sup>3</sup>, S. Hagihara<sup>3,4</sup>, K. Torii<sup>1,2,3</sup>

<sup>1</sup>University of Texas at Austin, Howard Hughes Medical Institute, Austin, Germany

<sup>2</sup>University of Texas at Austin, Department of Molecular Biosciences, Austin, United States

<sup>3</sup>Nagoya University, Institute of Transformative Bio-Molecules, Nagoya, Japan

<sup>4</sup>RIKEN Center for Sustainable Resource Science, Wako, Japan

<sup>5</sup>Texas A&M University, Department of Biochemistry and Biophysics, College Station, United States

<sup>6</sup>Kyoto Sangyo University, Faculty of Life Sciences and Center for Plant Sciences, Kamigamo-Motoyama, Japan

Plants are essential for life on Earth, offering habitat, food, oxygen, carbon dioxide absorption, and water cycle regulation. Stomata, the turgor-driven microscopic valves composed of guard cells, control the exchange of gases and water between plants and their environment. Thus, the precise density, distribution, and development of stomata are vital for plant survival. A phenotype-based chemical screen with *Arabidopsis* wildtype seedlings identified a novel bioactive small compound, inducing stomata development. Treatment of kC9 increase the number of stomata in a dose dependent manner. Phenotypic analyses of loss of function mutants of different stomatal pathway components treated with kC9 indicate that kC9 function downstream of ERECTA family members (ERf) but upstream of the basic helix-loop-helix (bHLH) transcription factors SPEECHLESS (SPCH) and SCREAM (SCRM) different from previously reported small molecules. Further analyses revealed that kC9 can both directly inhibit MPK6 by binding competitively to its ATP-binding pocket, hence, perturbing MAPK cascade activation, and disrupt the MPK6 and SCRM interaction. This inhibition of MAPkinase activation extends to pathogen-associated molecular pattern (PAMP) triggered immune signaling pathways, as evidenced by diminished flg22-induced MPK3/6 phosphorylation. Moreover, flg22-mediated immune signaling nullifies kC9's effect on stomatal development in a narrow developmental time frame, highlighting the intricate temporal regulation of MAPK pathway interactions during development. Our findings suggest kC9 as a novel tool to dissect MAPK pathway specificity and reveal an unexpected cross-talk between stomatal development and immune signaling pathways.

PL 003

## The Program Center MetaCom @ IPB

J. Ziegler<sup>1</sup>, G. Balcke<sup>1</sup>, P. Stark<sup>1</sup>, K. Vahabi<sup>1</sup>, S. Neumann<sup>1</sup>, H. Uthe<sup>1</sup><sup>1</sup>Leibniz-Institute of Plant Biochemistry, Program Center MetaCom, Halle, Germany

The Program Center MetaCom is a new interdisciplinary research program at the Leibniz-Institute of Plant Biochemistry and integrates measurements on high-performance analytical equipment with strong expertise in the fields of natural product chemistry, metabolomics, and chemo- and bioinformatics. Exploiting the possibilities of various analytical techniques (LC-time-of flight [TOF] MS, LC-trapped ion mobility spectrometry time of flight MS [TIMS-TOF], LC-triple quadrupole-MS, GC-MS, GC-triple quadrupole-MS, NMR, LC-UV, LC-FLD, LC-pulsed amperometric detection [PAD]), many research questions regarding metabolite analysis are covered, ranging from discovery metabolomics and targeted metabolite profiling to structural elucidation of compounds and bioinformatics in order to comprehensively explore the chemical basis of physiological effects. In this respect, we assist researchers in metabolite-related experiments by guiding through a complete metabolomics / metabolite profiling workflow including experimental design, sample processing, data recording, data interpretation, as well as data processing and visualization. State-of-the-art analytical instruments combined with a variety of detection methods enable highly sensitive quantification and identification of a comprehensive set of metabolites ranging from highly polar to highly apolar ones (e.g. phytohormones, glucosinolates, phytoceramides, terpenes, amino acids, organic acids, phosphorylated compounds etc...) extracted from different sources (e.g. any kind of plant tissue, plant organs, plant cells and etc...).

PL 004

## Evolutionary conservation of the interplay between COP1 and GLKs during terrestrialization

M. Neidert<sup>1</sup>, M. Hansen<sup>1</sup>, M. Kreiss<sup>1</sup>, U. Hoecker<sup>1</sup><sup>1</sup>University of Cologne, Institute of Plant Sciences, Cologne, Germany

In plants the COP1/SPA complex is a key regulator of light signalling by marking photomorphogenesis-promoting transcription factors for degradation. The main function of COP1 as an E3 ubiquitin ligase is highly conserved throughout the phylogenetic tree, but its targets and their involvement in plant physiology and development vary from species to species. Especially during terrestrialization light signalling networks appear to have further evolved to allow adaptation to changing light intensities and spectral compositions.

We consider Golden2-like (GLK) proteins as possible targets of COP1 and contributors to chloroplast development in the bryophyte *Physcomitrium patens*. We previously showed that COP1 and GLK from *P. patens* interact *in planta*, and that COP1-binding reduces the GLK-mediated transactivation in yeast. Here, we show by domain mapping that binding of COP1 and GLKs is evolutionarily conserved for the orthologs from the streptophyte algae *Mesotaenium endlicherianum*, but that different protein domains are involved. Moreover, for *M. endlicherianum* orthologs, GLK-mediated transactivation is not influenced by COP1-binding in yeast. Evolutionary divergence of COP1-interactions could indicate necessary adaptations of light signalling cascades that were needed for plant terrestrialization.



PL 005

### The Role of the Florigen Activation Complex (FAC) in Floral Development in Arabidopsis

M. Romera Branchat<sup>1,2</sup>, C. Pocard<sup>1,3</sup>, C. Vincent<sup>1</sup>, M. Cerise<sup>1</sup>, V. Falavina<sup>1</sup>, A. Pajoro<sup>1,4</sup>, H. Gao<sup>1</sup>, R. Franzen<sup>1</sup>, G. Coupland<sup>1</sup>

<sup>1</sup>Max Planck Institute for Plant Breeding Research, Department of Plant Developmental Biology, Cologne, Germany

<sup>2</sup>Institut für Biologie und Biotechnologie der Pflanzen (IBBP), Münster, Germany

<sup>3</sup>French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Maisons-Alfort, France

<sup>4</sup>Institute of Molecular Biology and Pathology, National Research Council, Dept. Biology and Biotechnology „C. Darwin“ Sapienza University, Rome, Italy

In Arabidopsis, the florigen activation complex (FAC), comprising the bZIP transcription factor FD and the florigen protein FLOWERING LOCUS T (FT), is known to regulate gene expression in the shoot apical meristem (SAM) to trigger flowering (Abe et al., 2005; Andrés and Coupland, 2012; Romera-Branchat et al., 2020). Using confocal analysis and *in situ* hybridization, we show that FD and FDP proteins and mRNAs are expressed in overlapping patterns in the floral meristem from stage 3 onwards, and that FT protein is also present at this stage. Furthermore, we demonstrate that *fd fdp* and *ft tsf* double mutants exhibit similar floral phenotypes, affecting floral organ number, floral meristem size, pedicel growth and organ identity such as leaf-like traits in sepals.

By analysing the expression of several MADS-domain genes related to the ABCE model of floral organ identity, we found that specifically the four class-E MADS-box *SEPALLATA* genes are reduced in expression in the double mutants *fd fdp* and *ft tsf*. In agreement with this result, single and multiple combinations of *sep* mutants displayed similar phenotypes to the ones observed in *fd fdp* and *ft tsf*, such as the presence of bifurcated trichomes on sepals and alterations in organ number in each of the whorls (Biewers, 2014). Additionally, we show that the double mutants *fd fdp* and *ft tsf* also have reduced *AGAMOUS* (*AG*) expression in the floral meristem center, which correlates with a de-repression of *WUSCHEL* (*WUS*) expression in the floral bud. Thus, the larger meristem observed in *fd fdp* and *ft tsf*, with its altered shape, correlates with higher and extended expression of *WUS*, which is known to determine meristem stem cell maintenance (Fletcher, 2018).

FD binding to *SEP* genes suggests that reduced *SEP* expression is a primary defect in *fd fdp* and *ft tsf*, whereas *AG* reduction might be indirect via down-regulation of the *SEP* genes, which are known to activate *AG*. Our findings highlight a critical role for the FAC in floral patterning and floral meristematic growth, beyond its role in flowering induction. Additionally, our results suggest that FT/TSF and FD/FDP are critical to setting proper floral organ number and sepal size robustness, opening new lines of investigation.

#### References

- M., Goto, K., and Araki, T. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* (80-. ). 309:1052–1056.
- Andrés, F., and Coupland, G. (2012). The genetic basis of flowering responses to seasonal cues. *Nat. Rev. Genet.* 2012 139 13:627–639.
- Biewers, S. M. (2014). *Sepallata* genes and their role during floral organ formation Advance Access published 2014.
- Fletcher, J. C. (2018). The CLV-WUS Stem Cell Signaling Pathway: A Roadmap to Crop Yield Optimization. *Plants* 7.
- Romera-Branchat, M., Severing, E., Pocard, C., Ohr, H., Vincent, C., Née, G., Martinez-Gallegos, R., Jang, S., Lalaguna, F. A., Madrigal, P., et al. (2020). Functional Divergence of the Arabidopsis Florigen-Interacting bZIP Transcription Factors FD and FDP. *Cell Rep.* 31.

PL 006

### Elucidation of Exocyst-Driven Tethering in Plants

A. Allahham<sup>1</sup>, M. Werres<sup>1</sup>, M. Trujillo<sup>1</sup>

<sup>1</sup>Aachen RWTH University, Department of Biology, Institute of Molecular Plant Physiology, Aachen, Germany

Exocytosis is a cellular process by which cargo such as soluble peptide hormones and small molecules are delivered to the apoplast, and integral membrane proteins, and lipids are incorporated into the PM. It entails various steps, of which tethering is crucial for directing the secretory vesicle and its cargo, to a specific exit site at the PM. Tethering is orchestrated by the exocyst, an octameric complex, conserved among eukaryotes. Its EXO70 has received substantial attention due to its lipid binding activity function in the exocyst, and its post-translational modifications which regulate the exocyst. In plants, the EXO70 subunit has expanded into large families of paralogous, raising the possibility that they have acquired specific functions, which has been supported by recent research.

Of note, EXO70s paralogues potentially form oligomers *in vivo*, an observation supported by results from the Human EXO70. Moreover, in humans, oligomerization was linked to EXO70 recruitment to the exocyst. However, the nature and the role of EXO70 oligomerization in its recruitment to the complex, and exocyst function, are still largely unknown. Our aim is therefore to understand on a mechanistic level, the basis of EXO70 neo-functionalization. In this context, our work has shown that EXO70 paralogues form homo- and hetero-oligomers, which are dependent on divergent factors. We are also analysing the factors mediating recruitment to the complex including the divergent domains present in EXO70 paralogues. Functional analysis of these domains further supports the specialization of EXO70s paralogues in plants. Therefore, we have identified factors that may contribute to EXO70 specialization within the function of the exocyst complex.

PL 007

**Epigenetics protects male germline development in plants**

 H. Jiang<sup>1</sup>, S. Zhou<sup>1</sup>, J. Cheng<sup>1</sup>
<sup>1</sup>Universität Potsdam, Institut für Biochemie und Biologie, Potsdam-Golm, Germany

Male germline development is a critical process in plants, underpinning the formation of pollen grains essential for sexual reproduction and genetic continuity. This development involves multiple rounds of cell division, characterized by dynamic chromatin states. Advances in genomic technologies have revealed extensive genome-wide epigenetic changes during this process, underscoring the importance of epigenetic modifications. However, the specific mechanisms by which chromatin and epigenetic modifications regulate male germline development remain largely elusive. I will discuss how the removal of histone H3K9me<sub>2</sub>, a methylated form of histone H3 at lysine 9 and a marker of heterochromatin, facilitates male germline development in plants. Our previous research demonstrated that the H3K9 demethylases IBM1 and JMJ27 regulate male meiosis<sup>1</sup>, a key stage in male germline development. Here, we extend these findings to show that IBM1 and JMJ27 are also crucial for pollen wall formation, a structure vital for pollen function and resilience under environmental stress. We discovered that H3K9 demethylation supports gene expression through both genome-wide demethylation, which potentially enhances transcriptional efficiency across tissues, and locus-specific H3K9 demethylation, tailored to the unique chromatin state during pollen wall formation. This demethylation process is initiated by transcription factors that recruit H3K9 demethylases to target genes essential for pollen wall formation. Our results suggest that dynamic H3K9 methylation levels require cell type-specific demethylation, which is essential for male germline development in plants. These findings provide new insights into the epigenetic regulation of male germline development and highlight the pivotal role of H3K9 demethylation in ensuring the proper formation and function of pollen grains.

**Reference**

<sup>1</sup>Cheng JP, Xu LH, Bergér V, Bruckmann A, Yang C, Schubert V, Grasser KD, Schnittger A, Zheng B, Jiang H\* (2022) H3K9 demethylases IBM1 and JMJ27 are required for male meiosis in *Arabidopsis thaliana*. *New Phytol.* DOI: 10.1111/nph.18286.

Our aim is to uncover the role of H3K9 demethylation in regulating wall formation, shedding light on both upstream and downstream components of this regulatory pathway.

PL 008

**Wild cabbage (*Brassica incana*) as potential gene pool for climate change adaptation in Brassica crops**

 B. Salopek Sondi<sup>1</sup>, K. Baotic<sup>1</sup>, I. Orehovec<sup>1</sup>, K. Majsec<sup>1</sup>, N. Bauer<sup>2</sup>, J. Drmic<sup>2</sup>, M. Tkalec<sup>2</sup>, N. Jasprica<sup>3</sup>, N. Major<sup>4</sup>  
 T. K. Kovačević<sup>4</sup>, S. Goreta Ban<sup>4</sup>
<sup>1</sup>Ruder Bošković Institute, Zagreb, Croatia

<sup>2</sup>University of Zagreb, Faculty of Science, Zagreb, Croatia

<sup>3</sup>University of Dubrovnik, Institute for Marine and Coastal Research, Dubrovnik, Croatia

<sup>4</sup>Institute for Agriculture and Tourism, Poreč, Croatia

*Brassica* species are among the most widely consumed vegetables due to their high nutritional value and richness in various valuable health-promoting compounds (polyphenols, glucosinolates, carotenoids, etc.). Climate change has a significant impact on biodiversity and agricultural production worldwide, negatively affecting the growth and development of plants and consequently the yield and quality of the harvest. Drought, heat and increased soil salinity have become important abiotic stress factors for many crops, including brassicas, especially in the Mediterranean region. Therefore, it is crucial to learn more about tolerance mechanisms and to identify reliable stress markers for the selection of tolerant species. Wild representatives growing in nature under extreme environmental conditions can serve as excellent model plants and as a potential gene pool for breeding strategies for Brassica cultivars adapted to often unpleasant environments. To explore the mechanisms of abiotic stress tolerance in brassicas, several kale ecopopulations (*B. oleracea* var. *acephala*) from Croatia as well as populations of wild relatives (*B. incana*) identified on the Adriatic islands were exposed to drought, heat, elevated salinity and combined stress factors. Oxidative stress markers such as proline and lipid peroxidation were determined spectrophotometrically, specialized metabolites were studied by reverse phase separation on a UPLC-ESI-QqQ instrument (Shimadzu Nexera), and selected stress responsive genes (NAC, DREB, HSF) were analyzed by RT-qPCR. The response to particular abiotic stress is species/variety specific. In general, kale was more sensitive to applied abiotic stresses compared to wild relatives. In the sensitive ecopopulations the applied stress factors resulted in a stronger response and a significantly greater change in the measured stress markers such as proline, MDA, stress hormones than in the more tolerant ecopopulations whose response was more resilient. Salinity and osmotic stress caused more drastic changes compared to temperature stress. In combined stress conditions, high temperature may mitigate harmful effect of salinity and drought. More tolerant varieties accumulated more polyphenolic compounds under stress conditions that is in agreement with antioxidant activity.

Keywords: abiotic stress, kale, wild *Brassica* relatives, metabolites, stress responsive genes

PL 010

Investigating BaYMV isolates in Germany: analyzing the diversity of the VPg-regions

C. J. Strauch<sup>1</sup>, P. Bauer<sup>1</sup>, A. Niehl<sup>1</sup>

<sup>1</sup>Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

The yellowing disease in barley is caused by a complex of two viruses, barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV). Infection of barley fields with BaYMV can lead to yield losses of 50% in infested areas. The soil-borne vector *Polymyxa graminis* transmits BaYMV and forms resting spores containing infectious virus particles. To avoid crop losses due to BaYMV, resistant barley cultivars are used by farmers. The resistance genes *rym4* and *rym5* are used intensively in barley resistance breeding. However, BaYMV has efficiently overcome *rym4* resistance in the past and currently, virus isolates, which can efficiently replicate in resistant plants with different backgrounds are being observed. An important factor, which can determine the resistance-breaking feature, is the viral VPg-region. We examine the VPg-region in the BaYMV genome by comparing virus-isolates from different fields and different barley cultivars. We expect to obtain insight into the viral diversity and response to resistance genes of these virus isolates. Additionally, to understand virus-plant interaction on molecular cell-level, we perform protein-protein-interactions studies between plant and virus proteins. We intend to identify changes in the virus isolates characteristic for resistance breaking and we will use this information to further monitor the presence and spread of these resistance breaking virus isolates in fields in Germany.

PL 011

Growth strategies of *Chlorella vulgaris* in seawater for the production of biomass and lipids suitable for biodiesel

R. Rautenberger<sup>1</sup>, A. Détaïn<sup>2,3</sup>, K. Skjånes<sup>4</sup>, P. S. C. Schulze<sup>2,5</sup>, V. Kiron<sup>2</sup>, D. Morales-Sánchez<sup>2,6,7</sup>

<sup>1</sup>Norwegian Institute of Bioeconomy Research (NIBIO), Division of Food Production and Society, Ås, Norway

<sup>2</sup>Nord University, Faculty of Biosciences and Aquaculture, Bodø, Norway

<sup>3</sup>Université de Bourgogne Franche-Comté, UFR Science de la Vie, de la Terre et de l'Environnement, Dijon, France

<sup>4</sup>Norwegian Institute of Bioeconomy Research (NIBIO), Division of Biotechnology and Plant Health, Ås, Norway

<sup>5</sup>University of Algarve, GreenCoLab—Associação Oceano Verde, Campus of Gambelas, Faro, Portugal

<sup>6</sup>UiT – The Arctic University of Norway, The Norwegian College of Fishery Science, Faculty of Biosciences,

Fisheries and Economics, Tromsø, Norway

<sup>7</sup>Universidad Nacional Autónoma de México, Instituto de Biotecnología, Cuernavaca, Morelos, Mexico

*Chlorella vulgaris* is a freshwater microalga that synthesises large amounts of saturated lipids, which makes it suitable for production of bioenergy and biofuels. Since its cultivation usually requires freshwater, it competes with agriculture, economic development and ecological conservation for this limited natural resource. This study investigated the possibility of the partial replacement of freshwater by seawater (50%) in the growth medium for a more sustainable biomass and lipid production. *Chlorella vulgaris* 211-11b was cultivated as shake-flask cultures in Bold's Basal Medium (BBM) formulated with 50% freshwater and 50% seawater under photoautotrophic, mixotrophic and heterotrophic conditions for eight days with glucose as organic carbon source in the latter two cases. The alga's best growth performance and highest lipid contents (49% DW<sup>-1</sup>), dominated by palmitoleic and oleic acid, occurred under mixotrophic rather than photoautotrophic and heterotrophic conditions. This study demonstrates a more economic and ecologically sustainable biomass and lipid production of *C. vulgaris* by saving 50% freshwater, which is available for other purposes.

PL 012

Transgenerational plasticity in clonal plants is fitness relevant for *S. polyrhiza* and its herbivore the waterlily aphid.

A. M. Chávez Argandoña<sup>1,2</sup>, A. Schreyer<sup>1</sup>, P. Prüsener<sup>2</sup>, M. Huber<sup>1,2</sup>

<sup>1</sup>University of Mainz, Institute of Organismic and Molecular Evolution, Mainz, Germany

<sup>2</sup>University of Münster, Institute of Plant Biology and Biotechnology, Münster, Germany

Transgenerational plasticity is an increasingly recognized phenomenon in clonal plants, yet its relevance in fitness is mostly unclear, particularly in natural environments. Using the clonally reproducing duckweed *Spirodela polyrhiza*, we assessed whether copper excess altered plant fitness and phenotypes upon different transgenerationally recurring stresses. When monoclonal plants were pre-treated with copper excess outdoors and subsequently grown for five generations under control condition prior to recurring stress, copper pre-treatment tended to increase plant fitness in recurring copper excess but not in other conditions. When the same treatment was applied to different genotypes indoors, copper pre-treatment altered plant growth rates in a genotype- and stress-dependent manner, with particularly strong effects upon recurring copper excess and aphid herbivory. Copper excess enhanced the levels of antioxidative defensive anthocyanins, levels that were transgenerationally inherited. Copper pre-treatment also primed the accumulation of jasmonates and its precursor OPDA, almost doubling their concentrations upon recurrent copper excess and aphid herbivory. Nevertheless, copper pre-treatment increased the fitness of its herbivore, the waterlily aphid, up to 50%. We show in this study that transgenerational plasticity affects plants in field conditions, and furthermore, that it is relevant for fitness of both the plant and its native herbivore.

PL 013

*Spirogyra pratensis*: functional genomics of a filamentous algal sister to land plantsE. Goldbecker<sup>1</sup>, A. Holzhausen<sup>2</sup>, T. Darienko<sup>1</sup>, D. Varshney<sup>3</sup>, A. Marques<sup>4</sup>, S. G. Consortium<sup>1,5,6,7,8,9</sup>, K. von Schwartzberg<sup>5</sup>  
J. de Vries<sup>1</sup>, S. Rensing<sup>3</sup><sup>1</sup>Georg-August-Universität Göttingen, Applied Bioinformatics, Göttingen, Germany<sup>2</sup>Martin Luther University Halle-Wittenberg, Dept. of Crop Physiology, Halle a. d. Saale, Germany<sup>3</sup>Albert-Ludwigs-Universität Freiburg, Freiburg i. Br., Germany<sup>4</sup>Max Planck Institute for Plant Breeding Research, Dept. of Chromosome Biology, Köln, Germany<sup>5</sup>Universität Hamburg, Aquatic Ecophysiology and Phycology, Hamburg, Germany<sup>6</sup>Universidad de Málaga, Málaga, Spain<sup>7</sup>Christian-Albrechts-Universität zu Kiel, Kiel, Germany<sup>8</sup>Universität Innsbruck, Innsbruck, Austria<sup>9</sup>Université Paris-Saclay, Paris, France

About 500 million years ago, the ancestor of all living land plants began its conquest of the terrestrial habitat, forever changing the surface of the Earth. By using genomics in a comparative and complementary approach, we can infer which traits enabled plants this conquest of land. To do so, we not only need to look at the genomic content of land plants, but also at those of their closest living relatives. Phylogenomic analyses performed over the last decade have recovered the Zygnematophyceae as the algal sister lineage to land plants. One of the most iconic members of the Zygnematophyceae is *Spirogyra pratensis*, a filamentous alga named for its intricate, spirally arranged chloroplasts. Understanding the molecular biology of this alga has been hampered by a lack of genome data.

Here, we present the first assembled genome of *Spirogyra pratensis*. *Spirogyra* has the smallest genome of all streptophyte algae thus far sequenced. Despite its small size, the *Spirogyra* genome contains several characteristic genes that were, until recently, considered to be specific to land plants. To investigate the latter, we exposed *Spirogyra* to a bifactorial gradient of the two important cues, light and temperature, and performed global differential gene expression as well as co-expression network analysis. Our data shed light on pathways for intracellular signaling in response to external cues that existed before land was dominated by plants.

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