

The 8th Scandinavian Plant Physiology Society PhD Students Conference

Conference Book





Uppsala, Sweden 2014

The 8th Scandinavian Plant Physiology Society PhD Students Conference Book by The 8th SPPS PhD Students Conference Organizing Committee and various contributors

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 Phytohormones - 2.Computational Biology and Bioinformatics – 3.Development – 4. Ecology and Environmental Changes - 5.Applied Plant Biology - 6.Biotic Interactions - 7.Response to Abiotic Stress - 8.Genome Defense and Epigenetics



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Välkommen to the 8th SPPS PhD Students Conference!

The Scandinavian Plant Physiology Society (SPPS) was brought into being to promote experimental Plant Physiology, arrange and support scientific meetings and thus, strengthen the interest in and the growth of Plant Science. The SPPS is the major community for plant physiologists in Scandinavia since 1947.

With the SPPS PhD Student Conference in particular, we aim to create an open, informal and enthusiastic atmosphere to facilitate the interaction among the international community of PhD students within the field of Plant Science, as well as to provide the students with the opportunity to meet world-renowned scientists.

We hope that the eight scientific sessions with internationally recognized plenary speakers, as well as the short PhD student talks and poster presentations will not only highlight current major scientific questions, but also the approaches of different subfields of Plant Science to answer these questions.

Many thanks to our sponsors for enabling us to subsidize this meeting's expenses, and especially to the SPPS board for their financial and logistical support. We would also like to thank the invited speakers who agreed to participate, to share their knowledge and to discuss their research with the students.

Varmt välkommen to all the participants and thank you for joining us here in Uppsala to share your insights into Plant Science! We hope you have a nice time during the conference!

On behalf of the organizing committee,

Andrea Claes

Some Swedish Phrases

Curadiah

Swedish Välkommen Hej Hej då! Hur mår du? Bara bra tack. Och du? Ja Nej En till öl, tack! Skå!! Smaklig måltid! Ursäkta! Förlåt! Tack så mycket! Var så god. Snälla	English Welcome Hello Goodbye! How are you? I'm fine, thanks. And you? Yes No Another beer please Cheers/Good health! Bon appetit (Have a good meal) Excuse me! Sorry! Thanks! Thanks a lot! You are welcome. Please
Swedish letter(s) ch ck g g g g g g k 4 q sch ti(on) tj v, w x z	English sound sh k g before a, o, u, å, or unstressed e j before e, i, y, ä, ö and after l or r k before t j soft ch sound, before e, i, y, ä, ö k sh sh(oon) soft ch sound V ks s

Swedish

Tack så mycket för att du deltar i vår konferens. Det var ett stort nöje att tillbringa dessa dagar med er alla.

English

Thank you very much for participating in our conference. It was a great pleasure to spend these days with you all.

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Conference Program

Detailed Program

Monday, June 16th

16:00 - 19:00	Registration	Lobby
19:00 - 21:00	Welcome Dinner	
21:00:	Mingle	

Tuesday, June 17th

09:00 - 10:30	Session 1: Phytohormones (PH)	Conference room
09:00 – 09:45	Jürgen Kleine-Vehn Keynote Lecture	
	Auxin-dependent differential growth regulation	
09:45 – 10:00	Xu Jin	
	Auxin transport during leaf abscission in Populus	
10:00 – 10:15	Thomas Vein	
	Chemical genomics uncovers a partial agonist of auxin controlling apical hook development	
10:15 – 10:30	Hana Rakusova	
	Mechanisms of auxin feed-back on PIN3 polarity for hypocotyl tropism in Arabidopsis	
10:30 – 11:00	Coffee Break	Festvåningen
11:00 – 12:15	Session 2: Computational Biology and Bioinformatics (BI)	Conference room
11:00 – 11:45	Veronica Grieneisen Keynote Lecture	

	How to make Heads or Tails of Cell and Tissue Polarity in the "Omics Era"	
11:45 – 12:00	Klára Hermanová	
	Genome evolution and phylogeny of Australian crucifers	
12:00 – 12:15	Minerva Trejo Arellano	
	Characterisation of the epigenetic landscape of CAF-1 mutants in Arabidopsis thaliana	
12:15 – 13:30	Lunch	
13:30 – 15:15	Session 3: Development (DE)	Conference room
13:30 – 14:15	Jörg Becker Keynote Lecture	
	Reshaping the (epi)genetic landscape of Arabidopsis pollen for genome stability and transgenerational inheritance	
14:15 – 14:30	Dmitry Kremnev	
	PEP activity and expression of photosynthesis genes required for embryo and seed development in Arabidopsis	
14:30 – 14:45	Sacha Escamez	
	'The Black Spot' – death strikes the predicted target cells in xylem thanks to a new intercellular signalling, autophagy and Metacaspase9	
14:45 – 15:00	Patricia Lang	
	Identification and characterization of new co- factors of the plant miRNA biogenesis pathway	
15:00 – 15:15	Henrik Serk	
	Understanding lignin formation in Arabidopsis xylem vessels	
15:15 – 15:45	Coffee Break	Festvåningen
15:45 – 17:00	Panel Discussion 1: Communicating Science Moderated by Peter Sylwan	Conference room
17:00 – 19:30	Uppsala City Tour	

19:30 - 21:00	Dinner	
21:00:	Mingle	

Wednesday, June 18th

09:00 - 10:30	Session 4: Ecology and Environmental Changes (EC)	Conference room
09:00 – 09:45	Ted Turlings Keynote Lecture	
	Trophic interactions in the rhizosphere: applying chemical ecology to develop novel strategies for root pest control	
09:45 – 10:00	Ai-fang Wang	
	Impact of increasing winter and spring waterlogging on the morphology of silver and pubescent birch seedlings in boreal forest	
10:00 – 10:15	Swathi Vurrakula	
	Interactions between carbon and nitrogen metabolism in Arabidopsis WT and gln1;2 mutants under elevated atmospheric carbon dioxide	
10:15 – 10:30	Ashutosh Pandey	
	Impact of ozone on rice (Oryza sativa L.) and mustard (Brassica campestris L.) cultivars grown in the ambient field conditions of India	
10:30 – 11:00	Coffee Break	Festvåningen
11:00 – 12:15	Session 5: Applied Plant Biology (AB)	Conference room
11:00 – 11:45	Eva-Mari Aro Keynote Lecture	

Photosynthesis in direct biofuel production

11:45 – 12:00 Lizhi Long

	The metal ion transporter HvIRT1 is required for manganese uptake and translocation in barley	
12:00 - 12:15	Lauri Nikkanen	
	Overexpression of chloroplast thioredoxin NTRC promotes leaf growth and reveals partial redundancy between plastidial thioredoxin systems	
12:15 – 13:45	Lunch	
13:45 – 15:30	Session 6: Responses to Abiotic Stress (AS)	Conference room
13:45 – 14:30	Stéphane Maury Keynote Lecture	
	Plant response to abiotic stress: how epigenetic can participate?	
14:30 - 14:45	Matleena Punkkinen	
	The role of SNF1-related protein kinase regulatory subunit γ in ABA-dependent signalling pathways in Arabidopsis thaliana	
14:45 – 15:00	Mengshu Hao	
	Cytosolic pH is a major modulator of mitochondrial NADPH oxidation in plants	
15:00 – 15:15	Nageswara Rao Mekala	
	TAP-dependent opposite phosphorylation of PSII core and LHCII proteins in high light prevents the disorganization of the thylakoid membrane	
15:15 – 15:30	Zoltán Takács	
	The role of polyamine catabolism associated hydrogen peroxide and nitric oxide in salt stress-induced cell death in tomato plants	
	Coffee Break	Orangeriet
15:30 – 16:45	Poster Session Sponsored by the Linnean Centre of Plant Biology in Uppsala	Orangeriet
16:45 – 18:00	Panel Discussion 2: Plant Molecular Biology in Everyday Life Moderated by Carl-Gustaf Thornström	Conference room

	Linné Garden and House Tour	
18:00 – 19:30	Communicating Science Workshop	Conference room
19:30 - 21:00	Dinner	
21:00:	Mingle	

Thursday, June 19th

09:00 - 10:30	Session 7: Biotic Interactions (IC)	Conference room
09:00 - 09:45	Steve Whisson Keynote Lecture	
	Effectors, expression regulation, and nutrients required for pathogenicity in Phytophthora infestans, the late blight pathogen of potato	
09:45 - 10:00	Martin Palmqvist	
	Nano-titania aided colonization of Brassica napus by the Plant Growth Promoting Rhizobacteria Bacillus amyloliquefaciens strain UMBC 5113	
10:00 - 10:15	Moona Rahikainen	
	PP2A-CPK interaction regulates innate immunity in Arabidopsis	
10:15 – 10:30	Diep Tran	
	Molecular mechanism of autoimmunity triggered by a pair of NB-LRRs	
10:30 - 11:00	Coffee Break	Festvåningen
11:00 – 12:00	Session 8: Genome Defense and Epigenetics (GD)	Conference room
11:00 – 11:45	Lionel Navarro Keynote Lecture	
	Transcriptional control of immune-response genes by DNA methylation and demethylation and its relevance in plant disease resistance	

11:45 – 12:00	Zuzana Mynarzová		
	An effect of demethylating agents on Petunia x hybrida varieties		
12:00 - 12:15	Prizes and Closing Remarks	Conference room	
12:15 – 13:30	Lunch		

Panel discussions

Panel Discussion 1 – Communicating Science				
Moderator	:	Peter Sylwan (Scientific Journalist)		
Panel	:	Eva-Mari Aro, Stéphane Maury, Ted Turlings, Jürgen Kleine-Vehn, Veronica Grieneisen		
Panel Discussion 2 – Plant Molecular Biology in Everyday Life				
Moderator	:	Carl-Gustaf Thornström (Associate Professor in Genetic and Plant Breeding Policies)		
Panel	:	Lionel Navarro, Stephen Whisson, Jörg Becker, Björn Ingemarsson		







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Abstract Word Cloud





Phytohormones

Keynote Lecture PH - S1

Auxin-dependent differential growth regulation

Elke Barbez, Chloe Beziat, Kai Dünser, Elena Feraru, Mugurel Feraru, Christian Löfke, Michel Ruiz Rosquete, David Scheuring, Lin Sun, and <u>Jürgen Kleine-Vehn</u>

Multicellular plants require particularly defined cellular strategies for tissue patterning and expansion, because the encapsulating cell wall literally binds neighbouring cells to each other. This interdependency limits cellular migration and, therefore, imposes outstanding importance to cell size determination and supracellular growth regulation. The phytohormone auxin is central to these regulations, but we still lack a comprehensive understanding of how auxin instructs cellular decision and how these responses are coordinated to allow tissue and organ growth.

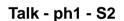
We are combining cell biological, physiological and developmental genetics approaches to decipher auxin-dependent growth regulation on a sub-cellular (Barbez et al., Nature 2012), tissue (Löfke et al., JIPB 2013) and organ level (Ruiz Rosquete et al., Curr Biol 2013).

On a cellular level auxin activity is controlled on multiple levels, including auxin metabolism and compartmentalization (Barbez and Kleine-Vehn, TIPS 2013). In our lab we unravel the role of the endoplasmic reticulum (ER) in auxin signalling. We are currently dissecting the function of PILS putative auxin carriers at the ER and thereby revealing the developmental importance of ER-based auxin biology. Ultimately, auxin balances cellular division and expansion rates, controlling cell size. Our lab is particularly interested in molecular components that precisely instruct auxin-dependent cell size determination, allowing differential cell size regulation in neighbouring tissues. These levels of regulations jointly allow auxin to control differential tissue expansion, enabling complex responses, such as directional organ growth. To exemplify auxin-dependent organ growth, we study the auxin-dependent control of the root architecture. We currently investigate how auxin steers the radial expansion of root systems, contributing to soil exploration.



Here I will present our novel insights into auxin-dependent differential growth regulation.

Notes:



Auxin transport during leaf abscission in Populus

Xu Jin, Urs Fischer

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Leaf abscission is an important trait for biomass production and seasonal acclimation in deciduous trees of temperate regions. Various plant hormones are involved in the timing of abscission. For example, ethylene signaling is required to induce hydrolysis of cell walls, while an auxin gradient was suggested to act upstream of ethylene on the onset of leaf abscission. Besides pharmacological application of auxins on cut surfaces of explants, experimental evidence for such a gradient is however lacking.

We established an experimental system on intact Populus trees, which allows us to induce abscission synchronously under controlled conditions. Leaf blades were bagged in aluminum foil and abscission was recorded daily. Cumulative abscission followed a sigmoidal curve for dark-



induced leaves, whereas control leaves in transparent bags of the same weight as the aluminum foil bags were not separated from the stem. Abscission was preceded by senescence in the petiole but not in the leaf.

Local auxin applications directly onto the abscission zone, as well as onto the distal end of the petiole, delayed dark induced abscission indicating that auxin could range not only as a short but also as a long distance signal. Similarly, an inhibitor of polar auxin transport retarded separation from the plant body. Shortly after dark-induction a new auxin response maximum on the abaxial side of the petiole, highlighting the incipient abscission zone. This auxin response maximum progressively moved from the abaxial to the adaxial side of the petiole, preceding the maturation of the abscission zone, presumably providing positional information for the formation of the abscission zone. Microarray data identified various auxin carriers to be down-regulated after dark induction. Immunolocalizations of those carriers will reveal if their subcellular localization and expression can explain the novel auxin response maximum.

Notes:



Talk - ph2 - S3

Chemical genomics uncovers a partial agonist of auxin controlling apical hook development

<u>Thomas Vain</u>¹, Deepak Kumar Barange^{1,2}, Qian Ma¹, Malgorzata Langowska¹, Alexandre Ismail⁵, Per Anders Enquist², Sibo Tao³, Cristina Castillejo Mangado³, Renhou Wang³, Yi Zhang³, Mark Estelle³, Thomas Moritz¹, Fredrik Almqvist², Laurens Pauwels^{4,5} and Stéphanie Robert¹

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Auxin is perceived in the nucleus by the SCFTIR1 ubiquitin E3 ligase complex by increasing the affinity of the TIR1 receptor to the Aux/IAA proteins. Aux/IAA proteins are then ubiquitinated and degraded by the 26S proteasome releasing auxin responsive genes. However, comprehension of auxin signaling is limited by the redundancy, the pleiotropic effects or the essential function of auxin related genes. Here, we report on a chemical genomics screen targeting an upstream regulator of SCFcomplexes. It reveals four Developmental Regulators (DR) acting as partial agonists of auxin. Inducing the expression of the auxin responsive synthetic promoter pDR5::GUS in an ectopic manner, these DRs create a large range of phenotypes at the seedling level. Our preliminary in vitro results show that the DR4 is able, depending of the TIR1 - Aux/IAA combination, to either promote or inhibit the formation of the co-receptor complex. DR4 abolishes apical hook formation without affecting hypocotyl elongation and is here used to show how auxin perception controls the apical hook development. The apical hook is a developmentally programmed structure formed to protect meristematic cells during seed



germination. DR4 structure activity relationship experiments led us to validate the importance of a highly specific structure of the DR4 to achieve its partial auxin action. We used time lapse imaging to show the needs of functional TIR1/AFBs proteins for the DR4 activity. Aux/IAA gain-of-function mutant phenotype corroborate the in vitro data letting us identify the TIR1/AFBs – Aux/IAA co-receptor combinations involved during apical hook development. Furthermore pharmacological approaches coupling DR4 and auxinole, an antagonist of auxin, validated the importance of a full auxin perception system to control the sequential phase of differential cell growth during apical hook development. Our set of partial agonists of auxin is opening new ways to understand the control of Arabidopsis development regulated by auxin perception.

Notes:



Mechanisms of auxin feed-back on PIN3 polarity for hypocotyl tropism in Arabidopsis

Hana Rakusová, Hélène S. Robert, Jiří Friml

Tropism is a mechanism for plants to adapt to environmental changes such as light (phototropism) and gravity (gravitropism). Gravitropism is a growth reaction orienting plant's development parallel to earth's gravitational field. Roots grow with the gravity vector, whereas



shoots grow against it. Gravity-induced redistribution of the phytohormone auxin mediates tropic responses both in roots as in shoots. In both instances PIN3 auxin transporter re-localizes in response to gravistimulus to redirect the auxin flow and achieve asymmetric auxin distribution. Regulations of PIN3 subcellular localization and subsequently PIN3dependent auxin transport are thus crucial process for tropic bending.

The present study reveals the role of auxin feed-back on PIN3 polarity in terminating the gravity-induced hypocotyl bending. We show that PIN3 localization in the endodermal cells of hypocotyls is re-arranged by increased auxin concentration, and leads to the PIN3 polarization to the inner side of endodermal cells. This presumably reduces auxin accumulation at the bottom hypocotyl side and terminates the cells elongation and hypocotyl bending. This phenomenon involves feed-back between auxin distribution and PIN3 intracellular trafficking as well as PIN3 protein stability. Moreover, we addressed the cellular and molecular mechanisms involved in these auxin-dependent changes in PIN3 localization. We demonstrate that the established regulators of vesicle trafficking, cell polarity and auxin signaling are necessary for PIN3 polarization and fine-tuning of hypocotyl gravitropism.

We provide a previously unexplained concept of how auxin regulates PIN3 relocation resulting in reduction of auxin asymmetry at the bottom part of hypocotyl and in the termination of hypocotyl bending. We also provide insights into molecular components of the signalling cascade that connects gravistimulus and auxin effect to PIN3 repolarisation in the hypocotyl.

Notes:



Poster - S1

A forward genetic screen identified new regulators in auxin-dependent degradation of auxin transport proteins in Arabidopsis thaliana

<u>Radka Zemová</u>¹, Marta Zwiewka¹, Hélène S Robert¹ and Jiří Friml^{1,2}

The plant hormone auxin is a major player for the regulation of plant growth development including embryo and root patterning, lateral organ formation and growth responses to environmental stimuli. Auxin is polarly cell-to-cell transported by the action of specific auxin influx (AUXIN-RESISTANT1 (AUX1) proteins) and efflux (PIN-FORMED (PIN) proteins) carriers, whose subcellular localizations indicate the direction of the auxin flow. Auxin itself regulates its own transport by modulation of the expression and subcellular localization of the auxin transporters. Short auxin treatment activates the transcription of PIN and AUX1 genes and stabilizes PIN proteins at the plasma membrane, whereas prolonged auxin application promotes the turnover of PIN proteins and their vacuolar degradation. In this study we took advantage of forward genetics, which opens up the possibility of identifying molecular components playing a role in these processes. In order to identify new mutants with impaired auxin transport or showing disorders with routing of auxin carriers, we used EMS mutagenized Arabidopsis transgenic line PIN2::PIN2-GFP AUX1::AUX1-YFP eir1 aux1 and we looked for mutants with stronger fluorescent signals after prolonged treatment with the synthetic auxin 2,4dichlorophenoxyacetic acid (2,4-D). The detailed analysis of 3 candidate mutant lines will be presented.



Poster - S2

Specific Aux/IAA-ARF complexes regulate adventitious root initiation in Arabidopsis

Salma Chaabouni¹, <u>Abdellah Lakehal</u>¹, Emilie Cavel¹, Ilaria Pigozzo¹, Daniel Ioan Pacurar¹, Irene Perrone¹, Rozenn Le Hir² and Catherine Bellini^{1,2,3}

¹Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, SE-90781 Umeå Sweden ²Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech 78026 Versailles Cedex, France ³Corresponding author: Catherine.Bellini@umu.se

Adventitious roots (AR), also called shoot-born roots, are crucial for vegetative propagation of elite genotypes in horticulture and forestry. AR formation is a plastic polygenic trait controlled by multiple environmental and endogenous factors. The fine-tuning of AR initiation in the Arabidopsis hypocotyl is regulated by a complex crosstalk between auxin and jasmonic acid (JA) (1). This signaling pathway include three AUXIN RESPONSE FACTOR genes (ARF6, ARF8 and ARF17) (2) acting upstream of JA signaling. In this study, we report a genetic analysis, which shows that several null allele mutants in the Aux/IAA genes developed more AR than the wild type, suggesting that these genes are likely repressing AR initiation through the interaction with ARF6 and ARF8. The analysis by qPCR of the expression profile of these Aux/IAA genes revealed that they are more expressed in the hypocotyl compared to shoot and roots. In addition co-immunoprecipitation technique confirmed that at least three of the identified Aux/IAA proteins physically interact with ARF6 and/or ARF8. Our findings highlight novel molecular players in the control of AR initiation in Arabidopsis hypocotyl.

(1) Gutierrez et al. 2012, Plant cell, 24: 2515-2527. (2) Gutierrez et al. 2009, Plant cell, 21: 3119-3132.



Poster - S3

Effect of exogenous application of selected phytohormonal substances on the production of thiol compounds - GSH, GSSG of Spirea japonica in containers

Martin Jonáš, Petr Salaš, Maksat Matraimov, Hana Sasková

Department of Breeding and Propagation of Horticultural Plants, Faculty of Horticulture Lednice, Mendel University in Brno, Czech Republic jonas.ma@seznam.cz

The experiment was established in order to eliminate the effect of stress factors acting on woody plants cultivated in containers. In the experiment was evaluated effect of exogenouse application of phytohormonal substances. Phytohormones affecting the impact of stress on plants were used for the purpose. Abscisic acid, 24- epibrassinolid, kinetin and spermine were applied by spraying the leaf in three concentrations (0,01 mg.l-1, 0,1 mg.l-1 a 1 mg.l-1) at model woody plant Spirea japonica in containers. Phytohormones play critical roles in regulating plant responses to stress. They are essentials mediators in triggering some plant responses to abiotic stress. Abiotic stress leads to morphological, biochemical, physiological and molecular changes in plants. The experiment was focused on determine of reduced glutatione (GSH) and oxidative glutathione (GSSG) in treated variants in compare with nontreated variant. Thiol compounds are one of the most important side products during the reaction of plants on abiotic stress. The determination of thiol compounds was carried out on HPLC. There were observed diferences between treated and nontreated variants in production of thiol compounds.



Poster - S4

The Arabidopsis Zinc Finger Protein 3 interferes with ABA and light signaling in seed germination and plant development.

<u>Mary Prathiba Joseph</u>¹, Csaba Papdi¹, László Kozma-Bognár¹, István Nagy¹, Marta López-Carbonell², Csaba Koncz³, and László Szabados¹

¹Biological Research Centre, H-6726-Szeged, Hungary, ²University of Barcelona, 08028-Barcelona, Spain, ³Max-Planck-Institut fur Zuchtungsforschung, 50829-Cologne, Germany, maryprat@brc.hu papdi@brc.hu kozmab@brc.hu nagyi@baygen.hu mlopez@b.edu koncz@mpiz-koeln.mpg.de szabados@brc.hu

Seed germination is controlled by environmental signals, including light and endogenous phytohormones. Abscisic acid (ABA) inhibits, whereas gibberellin (GA) promotes germination and early seedling development, respectively. Here we report that ZFP3, a nuclear C2H2 zinc finger protein acts as a negative regulator of ABA- suppressed germination. Accordingly, regulated over-expression of ZFP3 and the closely related ZFP1, ZFP4, ZFP6 and ZFP7 zinc finger factors confers ABA insensitivity to seed germination while the zfp3 zfp4 double mutant displays enhanced ABA susceptibility. Reduced expression of several ABA-induced genes, such as RAB18 and transcription factor ABI4 in ZFP3ox seedlings suggests that ZFP3 negatively regulates ABA signaling. Analysis of ZFP3ox plants revealed multiple phenotypic alterations, such as semidwarf growth habit, defects in fertility and enhanced sensitivity of hypocotyl elongation to red but not to far-red or blue light. Analysis of genetic interactions with phytochrome and abi mutants indicates that ZFP3 enhances red light signaling by photoreceptors other than phyA, and additively increases ABA insensitivity conferred by the abi2, abi4 and abi5 mutations. These data support the conclusion that ZFP3 and the related ZFP subfamily of zinc finger factors regulate light and ABA responses during germination and early seedling development.



Poster - S5

Importance of auxin transport, biosynthesis and conjugation in regulating protonemal growth in Physcomitrella patens

<u>Eric Pederson</u>¹, Mattias Thelander¹, Katarina Landberg¹, Tom Viaene², Jiri Friml^{3,4} and Eva Sundberg¹

¹Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences, PO-Box 7080, SE-750 07 Uppsala, Sweden ²Department of Plant Systems Biology, VIB, Department of Plant Biotechnology and Genetics, Ghent University, 9052 Gent, Belgium ³Institute of Science and Technology Austria (IST Austria), 3400 Klosterneuburg, Austria ⁴Mendel Centre for Genomics and Proteomics of Plants Systems, CEITEC MU - Central European Institute of Technology, Masaryk University, CZ-625 00 Brno, Czech Republic

The moss Physcomitrella patens is an established model organism for the bryophytes and early land plants. A multicellular filamentous system (protonemata) represents the first stage of the gametophytic part of the moss lifecycle and consists of two different types of filaments that grow by apical cell divisions, the photosynthetically active chloronema and colonyspreading caulonema. Protonemal filaments show a cell identity gradient with the proximal cells being more chloronemal-like and a gradual transition towards caulonemal cell identity along the filament. The transition, which occurs only in tip cells, is positively regulated by the plant hormone auxin. Further, the P. patens genome sequence revealed that the auxin machinery genes known from angiosperms are present in this moss species. Thus, homologues of PINs and AUX1/LAX proteins important for auxin transport, SHI/STY, YUCCA and TAA1/TAR proteins participating in the regulation or processing of auxin biosynthesis, GH3 regulating auxin conjunction and homeostasis are all encoded by the Physcomitrella genome. Here we show that all these homologues are expressed in the filamentous tissues with increasing intensity towards the tip in the filamentous tissue. This is true for the main filaments but also the side branches. The presumptive auxin gradient formed appear important for division of apical cells and for timing the transition from chloronema to caulonema identity of the apical cell to a stage when a critical number of



chloronema cells providing photysynthates to caulonema has formed during colony growth.





Computational Biology and Bioinformatics

Keynote Lecture BI - S5

How to make Heads or Tails of Cell and Tissue Polarity in the "Omics Era"

Veronica Grieneisen

Systems Biology, within the era of "omics", is often perceived as the study of large complex networks of interacting genes and proteins. In this talk I will address how, in order to understand spatiotemporal behaviours in morphogenesis, it can be insightful to simplify networks to a few components and rather study the complexity that emerges in allowing different levels of organization to interact within spatial contexts. I will illustrate this spatial multi-level approach through the problem of cell and tissue polarity. Our mathematical studies, combined with experimental work on pavement cells in the leaf epidermis and tissue polarity in roots. reveals that non-linear feedbacks and signalling arise through "invisible" nodes in a biochemic/genetic network due to space and interactions between cells. Finally, I will show how insights derived from modelling molecular interactions has prompted us to collect temporal sequences of imaging data sets to answer key guestions, and how this brings us into a novel realm of "Morphomics", with new and fascinating methodological challenges.

Notes:



Talk - bi1 - S6

Genome evolution and phylogeny of Australian crucifers

<u>Klára Harmanová</u>, Milan Pouch, Terezie Mandáková & Martin A. Lysak

RG Plant Cytogenomics, CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, CZ-625 00, Brno, Czech Republic

The Brassicaceae (Crucifereae) comprises 49 tribes, 321 genera and 3660 species and belongs to the largest plant families. Polyploidization, often accompanied by hybridization, has been of major importance in evolution of flowering plant. Here we investigate the importance of these processes in evolution of tribe Microlepidieae, comprising most crucifer genera endemic to Australia. We used partial sequences of three nuclear single-copy genes to identify putative parental genomes of the allopolyploid ancestor of the tribe. To identify single nucleotide changes in alleles of chalcone synthase (Chs), LUMINIDEPENDENS (LD) and Phytochrome A (PHYA) genes, PCR-SSCP (Polymerase Chain Reaction -Single-Strand Conformation Polymorphisms) was applied to more than 20 Australian crucifer species. Although we did not obtain more than one allel in some species, generally our results based on the Maximum likelihood and Bayesian reconstructions suggest, that the Australian genera descended from an allopolyploid ancestor formed by hybridization between species from tribe Smelowskieae and Crucihimalayeae.

This work was supported by CEITEC (CZ.1.05/1.1.00/02.0068) and by the European Social Fund (CZ.1.07/2.3.00/20.0189)

Notes:



Talk - bi2 - S7

Characterisation of the epigenetic landscape of CAF-1 mutants in Arabidopsis thaliana.

Minerva Trejo Arellano

The basal unit of information storage in the cell is at the level of the DNA sequence. Additional regulatory strata are possible because of the packaging of DNA in a highly compacted chromatin structure. Furthermore, DNA nucleotides can carry chemical modifications that change the interaction with histones, the scaffold proteins of chromatin, and therefore, the level of compaction or DNA accessibility. Multiple feedback loops exist in the dynamics of deposition of DNA modifications and histones and their modifiers. Chromatin Assembly Factor (CAF-1), for example, loads Histone 3.1/Histone 4 (H3.1/H4) dimers and has been proposed as a platform to recruit enzymes to maintain DNA methylation and histone modifications. Plants that lack a functional CAF-1 exhibit a transgenerational aggravation of the phenotype that ultimately leads to sterility of mutant plants by the sixth generation. Transcriptome profiling identified up-regulation of pathogen response genes such as PR1 in CAF-1 mutants. The altered expression of PR1 is accompanied by an increase in chromatin accessibly and a reduced nucleosome occupancy on its 5' end. These approaches provided an insight into the functional role of CAF-1 as preserver of a repressive chromatin state. However, genome-wide chromatin studies are needed in order to elucidate the whole epigenetic landscape of the transgenerational aggravation of the phenotype in CAF-1 mutants. With this aim, bisulphate sequencing was performed in order to characterise changes in the DNA methylation status. Subtle changes in DNA methylation were detected across generations with an overall decrease of CG methylation concomitant with the presence of H3K9me2.



Computational Biology and Bioinformatics (BI)

Surprisingly, some genes exhibited an increase in CpG methylation level accompanied by an increase in expression. Further functional studies are needed in order to correlate the changes in DNA methylation with altered chromatin structure. In this regard, MNAse sequencing is proposed as the tool to depict the alterations in nucleosome arrangements. Characterising the molecular status of the chromatin in CAF-1 mutants will provide a broader scenario of the role played by chromatin maintenance complexes into the correct transmission of information across generations.

Notes:

Poster - S6

Discovering the sex locus in Salix viminalis

Pascal Pucholt, Ann-Christin Rönnberg-Wästljung and Sofia Berlin

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The transition from autosomal chromosomes to sex chromosomes involves several fundamental biological processes and is not well understood yet. Plants are particularly suitable for studying the processes during evolution of sex chromosomes as they possess sex determination systems of various ages, which make it possible to study these evolutionary processes in action. Willows (Salix spp.) are dioecious with male and female flowers on separate plants and do not possess heteromorphic sex chromosomes, which make them excellent model



Computational Biology and Bioinformatics (BI)

species for studies of the genetic control of sex determination. In our guest to learn how diocey evolved and to discover the genetic mechanisms behind sex determination in willows, we aimed at identifying and characterizing loci associated with gender in one Salix viminalis cross. The sex ratio among 527 offspring was close to 1:1. In QTL mapping analyses we found one major locus associated with gender on linkage group 15. An interesting observation was that only markers with maternal origin had a significant association with gender, suggesting that there is a maternal component that is important for sex determination in S. viminalis. We also investigated the haplotype structure in ten markers close or at the sex-QTL and found that all but three female offspring carried one maternal haplotype, while all male offspring except for two, carried the other female haplotype. The two paternal haplotypes were found with equal frequency among the male and female offspring. By analysing individuals with recombinant haplotypes, two markers were found to always be present in females thus suggesting that these two markers are very close to the actual genes controlling sex in this species. We are currently working towards characterizing the sex-QTL region using whole-genome sequence data in order to identify genes with a possible role in sex determination and to learn if this genomic region behaves as a proto sex chromosome with reduced recombination and low genetic diversity.

Key words: salix, evolution, sex determination, QTL analysis





Development

Keynote Lecture DE - S8

Reshaping the (epi)genetic landscape of Arabidopsis pollen for genome stability and transgenerational inheritance

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The life cycle of higher plants is characterized by two morphologically distinct generations: the dominant generation of the sporophyte and the strongly reduced gametophyte generation. It is only during the development of the gametophytes that the plant germline is specified. In Arabidopsis, the male germline is initiated by asymmetric division of haploid uninucleated microspores, giving rise to a vegetative cell enclosing a smaller generative cell that divides before anthesis to originate two sperm cells. The transition between generation-specific cell fates is bound to involve extensive genetic and epigenetic reprogramming.

To reveal the dynamics of (epi)genetic reprogramming during germline specification in Arabidopsis we developed a FACS-based method allowing us to isolate highly pure fractions of microspores as well as vegetative nuclei and sperm cells. Transcriptional profiling of sorted sperm cells showed that transcripts involved in small RNA biogenesis and RNAdirected DNA methylation are enriched in sperm cells, suggesting active epigenetic reprogramming as well as post-transcriptional regulation of gene expression. And indeed, genome-wide methylation profiling of genomic DNA from microspores, vegetative nuclei and sperm cells in conjunction with small RNA analysis indicates that the epigenome in Arabidopsis pollen undergoes targeted reprogramming. This leads to the transcriptional activation of Athila retrotransposons in the vegetative nucleus and accumulation of correspondent mobile 21nt siRNAs in the sperm cells, in which these transposons are hypermethylated. In a similar fashion, targeted demethylation of transposons neighboring maternally imprinted genes and variable epialleles in the vegetative nucleus is contrasted by hypermethylation of these loci in the sperm cells and accumulation of correspondent 24nt siRNAs. Taken together the results



indicate that epigenetic reprogramming during microgametogenesis contributes to transposon silencing, transgenerational recurrence of epialleles and imprinting of maternally expressed genes, guided by siRNA.

Notes:



Talk - de1 - S9

PEP activity and expression of photosynthesis genes required for embryo and seed development in Arabidopsis

Dmitry Kremnev and Åsa Strand

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Chloroplast biogenesis and function is essential for proper plant embryo and seed development but the molecular mechanisms underlying the role of plastids during embryogenesis are poorly understood. Expression of plastid encoded genes is dependent on two different transcription machineries; a plastid-encoded bacterial-type RNA polymerase (PEP) and a nuclear-encoded phage-type RNA polymerase (NEP), which recognize distinct types of promoters. However, the division of labor between PEP and NEP during plastid development and in mature



chloroplasts is unclear. We show here that PRIN2 and CSP41b, two proteins associated with the PEP complex, are essential for proper plant embryo development. Using Co-IP assays and native PAGE we have shown a direct physical interaction between PRIN2 and CSP41b. Moreover, PRIN2 and CSP41b form a distinct protein complex that binds the psaA promoter. The prin2.2 and csp41b-2 single mutants displayed pale phenotypes, abnormal chloroplasts impaired in transcriptional activity and defects in embryo development. The respective csp41b-2prin2.2 homo/heterozygote double mutants produced 25% abnormal white colored ovules and shrunken seeds. Thus, the csp41b-2prin2.2 double mutant is embryo lethal. In silico analysis of available array data showed that a large number of genes traditionally classified as PEP dependent genes are transcribed during early embryo development from the preglobular stage to the mature-green-stage. Taken together, our results indicate that PEP activity and consequently the switch from NEP to PEP activity, is essential during embryo development and that the PRIN2-CSP41b DNA binding protein complex is crucial for PEP activity during this process.



Talk - de2 - S10

'The Black Spot' – death strikes the predicted target cells in xylem thanks to a new intercellular signalling, autophagy and Metacaspase9

Sacha Escamez

The development of multicellular organisms involves programmed cell death to maintain homeostasis of the entire organism. Higher plants' water-conducting xylem tracheary elements (TEs) undergo programmed cell death (PCD) as part of their developmental program. We investigated whether plant metacaspases (MCs) are involved in the control of TE PCD, and found that in Arabidopsis thaliana only MC9 was specifically expressed in developing TEs. Next, expression of MC9 was knocked down by RNA interference in Arabidopsis xylogenic cell cultures where the cells differentiate into TEs and living parenchyma cells. TEs of the MC9 RNAi lines displayed miss-patterned secondary cell walls (SCWs) and impaired clearance after PCD. Surprisingly, we observed that in addition to the TEs also the parenchyma cells died in the RNAi lines. When the RNAi and wild type cells were induced to differentiate as a mix, cell death of parenchyma cells was abolished. We also observed altered cysteine protease activity and increased number of lysotracker-stained bodies in the RNAi versus wild-type TEs, suggesting altered autophagy in the RNAi lines. Consistently, chemically altering autophagy in differentiating wildtype cells resulted in a phenotype similar to MC9 RNAi cells. Together our results suggest that MC9 regulates autophagy in TEs, which is required for proper SCW patterning, restriction of cell death to TEs and post-mortem autolysis in TEs. Moreover, the rescue of the RNAi ectopic cell death phenotype in presence of wild-type cells suggests that MC9 is part of a yet unknown intercellular signaling pathway.



Talk - de3 - S11

Identification and characterization of new co-factors of the plant miRNA biogenesis pathway

Patricia Lang, Pablo A. Manavella, Michael Christie, Jörg Hagmann, Franceli Kulcheski, Detlef Weigel

The biogenesis of miRNAs in plants is a complex process that involves several steps. Between the transcription of a primary miRNA and the final incorporation of the mature miRNA into the RISC, several nuclear processing steps take place. A number of proteins that are involved in miRNA processing and function have been identified so far, some of which are essential and cause lethality in null-mutants, whereas others seem to play partially dispensable roles and only cause a range of morphological defects in mutant plants.

Recently, a new high-throughput screen for the identification of unknown plant miRNA biogenesis cofactors was developed in our lab. The combination of a luciferase-based reporter system and state-of-the-art sequencing technologies enables the rapid identification of mutant genes and reduces the drawbacks of re-discovery of already characterized genes.

Among the identified genes, we isolated a mutant plant presenting defects in the miRNA pathway. The SHORE mapping analysis revealed that the causal mutation lies on a gene encoding an uncharacterized RNA-binding protein (named MSS8, for miRNA-mediated silencing suppressor 8). Confocal microscopy-based co-localization experiments and yeast-2-



hybrid assays showed that MSS8 is able to interact with a known miRNAprocessing factor. Confirming the role of MSS8 in the miRNA pathway, we detected alterations in the steady levels of pre-miRNAs, mature miRNAs and miRNA targets in the mutant background. These results indicate that the protein plays a role in accurate processing of miRNAs.

Notes:

Talk - de4 - S12

Understanding lignin formation in Arabidopsis xylem vessels

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Lignin is the second most abundant biopolymer on earth and is found in the cell wall of specialized cells such as xylem tracheary elements (TEs) which transport water and minerals in vascular land plants. The aim of my study is to better understand TE lignification at the cellular level. To do so, I used Arabidopsis cell suspension cultures which can be induced by adding hormones to differentiate into 40-50% of lignified TEs, while the rest of the cells remain parenchymatic (non-TEs) [1]. Recently, I contributed to show that TE lignification in Zinnia elegans occurred after TE programmed cell death (PCD), in a non-cell autonomous manner,



during which non-TEs provided lignin monomers to dead TEs [2]. Similarly, TE secondary cell wall lignification in Arabidopsis thaliana progressed linearly several weeks after TE-PCD. To assess the cooperative supply of lignin monomers, phenolic compounds were quantified in both intra- and extracellular spaces along TE formation. Extracellular phenolics increased only in cultures induced to become TEs and appeared to be the limiting factor controlling the extent of TE post-mortem lignification. То demonstrate that non-TEs export lignin precursors, monomer synthesis was initially inhibited which resulted in unlignified TEs. When the inhibition was removed after all TEs were dead (only non-TEs remain alive), TEs lignified thereby showing that phenolics exported by non-TEs are lignin precursors. Metabolomic analysis of the extracellular phenolics revealed an increase of lignin intermediates (oligomers) essentially after TE-PCD, confirming the post-mortem progression of TE lignification. To verify TE non-cell autonomous lignification in planta, loss-of-function mutants in CCR (lignin monomer synthesis gene) were complemented by a non-TE specific promoter driving CCR, which rescued the TE lignin reduction of the mutant. Altogether my results confirm that TE lignification in Arabidopsis is post-mortem and is enabled by parenchymatic cells which export lignin monomers to the apoplast for TE lignification.

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[2] Pesquet E, Zhang B, Gorzsás A, Puhakainen T, Serk H, Escamez S, Barbier O, Gerber L, Courtois-Moreau C, Alatalo E, Paulin L, Kangasjärvi J,Sundberg B, Goffner D, Tuominen H. 2013. Non-Cell-Autonomous Postmortem Lignification of Tracheary Elements in Zinnia elegans. Plant Cell, 25, 1314-28



Poster - S7

Identification of proteins involved in the alternative splicing of FLM

Giovanna Capovilla, Markus Schmid

The correct timing of flowering contributes to large extent to the reproductive success of a plant. Previous studies have implicated FLOWERING LOCUS M (FLM), which encodes a MADS-box transcription factor, as one of the genes involved in flowering time control. The FLM transcript has recently been shown to be subject to temperature-dependent alternative splicing. Interestingly, the two main splice-variants, FLM- β and FLM- δ , encode proteins that control flowering in different ways. FLM- β functions as a repressor of flowering, while FLM- δ has the opposite effect.

The aim of this project is to identify proteins that are involved in this temperature-regulated splicing of FLM. To reach this end, different approaches will be followed. The first one would be to design a biotinylated probe on FLM, let it hybridize with the pre-mRNA in a prefixed sample of wild-type plants grown at a chosen temperature and try to pull down the protein-RNA complex using streptavidin-beads. The goal would be to find out sets of proteins that interact with FLM RNA from plants. The second approach is to compare the entire transcriptome of samples grown at different temperature using mRNA-seq technology. The expectation is to find out differential expression of genes involved in alternative splicing, or a different expression on their alternative splice variants, in aim to detect candidates for protein involved in the alternative splicing.



A unifying principle in the metabolism of cyanogenic glycosides in plants

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Cyanogenic glycosides are effective phytoanticipins, involved in plant defence against herbivores due to their ability to release toxic HCN upon tissue disruption. However, there is increasing evidence for the endogenous turnover of cyanogenic glycosides, suggesting their important role in providing a source of reduced nitrogen in plant physiological processes. Metabolite profiling using LC-MS/MS and HR-MS has been carried out in three cyanogenic plant species - cassava, almond, and sorghum – in order to investigate the presence of putative turnover products of cyanogenic glycosides. In total, the formation of 27 structural derivatives of the cyanogenic glucosides linamarin, lotaustralin, prunasin, amygdalin and dhurrin, including di- and triglycosides derived from these compounds, has been demonstrated. The relative abundance of the compounds was assessed in different tissues and developmental stages. Based on results common to the three species, an endogenous turnover pathway for cyanogenic glycosides has been proposed in which reduced nitrogen and carbon are recovered for primary metabolism without the liberation of free HCN. Understanding this "unifying principle" in the metabolism of cyanogenic glycosides, and the significance of the turnover process in plant vital functions, will open up the possibility of regulating the content of cyanogenic glycosides in crops.



The journey of zinc from soil to seed – the role of heavy metal ATPases

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AtHMA2 and AtHMA4 are two heavy metal ATPases localized to the plasma membrane and involved in zinc export. They are expressed in pericycle cells in roots and their involvement in xylem loading for root-toshoot translocation of zinc is well established. However, whether AtHMA2 and AtHMA4 play other roles in zinc transport in the plant is not known. In this study it was found that expression of HMA2 and HMA4 in the vascular tissue of the leaf is confined to the bundle sheath and vascular parenchyma cells. This indicates an involvement in phloem loading by exporting zinc out into the apoplast surrounding the phloem companion cells. Furthermore, HMA2 and HMA4 were found to be highly expressed in developing seeds. The expression of HMA4 is mainly confined to the innermost layer of the seed coat whereas HMA2 is expressed all over the seed. In hma4 and hma2.hma4 knock-out seeds zinc accumulates in the seed coat of developing and mature seeds. Together this indicates that especially HMA4 is important for getting zinc across the apoplastic barrier between the seed coat and endosperm. We propose that during its journey from soil to seed, zinc has to cross at least three apoplastic barriers. Here zinc has to be exported against the electrochemical gradient, meaning that active transport is required. We suggest a model for Arabidopsis in which AtHMA2 and AtHMA4 carry out this function by exporting zinc from: 1) pericycle cells for xylem loading, 2) leaf vascular parenchyma cells for phloem loading, and 3) seed coat cells for postphloem translocation of zinc. This enhanced understanding of how zinc is naturally transported to the developing seed might help in designing improved strategies for zinc biofortification.



Comparative & functional genomics of plant innate immunity using the moss Physcomitrella patens as a model

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Plants and animals have evolved innate immune systems with surface-localized, pattern-recognition receptors (PRRs) that recognize conserved pathogen-associated molecular patterns (PAMPs). MAP kinase cascades transmit and amplify signals from activated PRRs to the nucleus. These cascades consist of a MAP kinase kinase kinase (MEKK), a MAP kinase kinase (MPKK) and a MAP kinase (MPK). In the higher, vascular plant model Arabidopsis thaliana, 4 of its MPKs (AtMPK3/4/6/11) are involved in signalling upon PRR activation. These MPKs are also implicated in abiotic stress responses and in developmental processes. Arabidopsis thaliana has 20 MPKs while the moss Physcomitrella patens has only 8 which may represent a 'basal' set of plant cascades.

P. patens is also a good model for studying plant-pathogen interactions. It is susceptible to pathogenic fungi, oomycetes and bacteria which activate moss defenses including the expression of defense related genes. However, apart from current work from the host group, nothing is known about how P. patens perceives pathogens and activates responses, and moss PRRs or MPKs have not been analyzed. I aim to use the moss P. patens as a comparative genomic model to understand the evolution and regulation of innate immunity in plants. This aim includes the identification of components of the P. patens MAP kinase cascade(s) mediating immunity from PAMP receptors to transcriptional target genes required for defense. I will attempt to understand the role of MPKs in immunity in early land plants and, at the same time, identify new cascade substrates and targets.



Using of Antivirals for Onion Yellow Dwarf Virus Elimination

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Onion yellow dwarf virus is one of the members in garlic virus complex. OYDV is widespread throughout the world and causes serious crop losses and quality deterioration. OYDV was detected in Czech garlic genotype 'Unikát' and chemotherapy was applied for its elimination. Acyclovir, rimantadine or zidovudines at concentrations 25 or 50 mg.l-1 were used as antivirals. Antivirals were applied into media for primary cultures established from garlic cloves through a sterile filter after autoclaving. Cloves were 14 days on this medium. Medium without antivirals was used as a control variant. Meristems were isolated and cultivated on media without antivirals after this time. Young leaves of the new plants were tested by reverse transcription polymerase chain reaction and results were evaluated. The using of antivirals has been successful. We achieved OYDV elimination by the using of every method.

Poster - S12

Unraveling cell wall polarity in pavement cells of Arabidopsis thaliana

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In plants, the epidermis plays an essential role in shaping the entire organism as it is thought to be limiting for growth (Savaldi-Godstein 2008). Another layer of restriction arises from the presence of stiff and cohesive cell walls, which hinder cell-cell movements. Puzzle shape leaf pavement cells displaying interdigitated lobes and indents in the two-dimensional plane of the leaf epidermis, provides a powerful model system to investigate the cellular and subcellular processes underlying cell polarity and shape determination in plant tissues. The formation of these multipolar cells is regulated by the multifunctional phytohormone auxin at the cell plasma membrane via the subcellular compartmentation of Rho GTPases of plants (ROP) signaling pathways (Fu et al. 2002, 2005; Xu at al. 2010), but it remains unclear how such local molecular heterogeneities are translated into the local cell wall properties to generate local shape changes. By probing anticlinal walls on sections using atomic force microscopy (AFM), we determined that cell wall mechanical properties are heterogeneous along the perimeter of pavement cells in Arabidopsis wildtype, but not in the polarity deficient CA-ROP2 line. Strikingly, we observed the presence of a stiffness gradient across the cell wall, suggesting that two contiguous cell walls can retain distinct mechanical properties. Using high-resolution electron microscopy, we showed that although the density of cell wall components was often similar between the lobe and the neck regions of the cells, some of the components were unevenly distributed across the cell wall, thus supporting a scenario in which multipolar pavement cell shape relies on finely tuned modifications of the composition and mechanical properties of cell walls along the perimeter of the cell as well as across the cell wall thickness.

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Poster - S13

WUSCHEL-RELATED HOMEOBOX 8/9 regulates cell division patterns during embryo development in the gymnosperm Norway spruce

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Proper embryo development is crucial as it is when the primary body axes are established. In Arabidopsis, AtWOX8 and AtWOX9, members of the WUSCHEL-RELATED HOMEOBOX (WOX) gene family, are crucial for embryo development. Previous studies have shown that in Norway spruce there is a single PaWOX8/9 gene, which is expressed in embryos. In this work we show that the expression of PaWOX8/9 is high during early and late embryogeny and that the expression decreases when the maturation phase starts. To address the function of PaWOX8/9 during embryo development, we established RNAi lines to knock-down the expression of PaWOX8/9. Using both constitutive and inducible promoters, we could show that down-regulation of PaWOX8/9 during early and late embryogeny disturbs the orientation of cell division plane at the basal part of the embryonal mass resulting in aberrant morphology of the embryos. In addition, the expression of several key cell cycle regulating genes, for example PaE2FAB-LIKE and PaCYCLIN B-LIKE, are affected by the down-regulation of PaWOX8/9. Taken together, we show that PaWOX8/9 performs an evolutionarily conserved function as an essential regulator of the apical-basal embryo pattern establishment.



Keywords: Apical-basal, cell cycle, development, embryogenesis, polarity, spruce, WUSCHEL-RELATED HOMEOBOX

Abbreviations: PEM, Proembryogenic mass EE, Early embryo LE, Late embryo ME, Mature embryo PGR, Plant growth regulator

Poster - S14

The control of plant growth by a novel ubiquitin-mediated mechanism

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There is a substantial body of evidence about gene regulatory networks that control the initiation and differentiation of different cell types in plants, but relatively little is known about regulatory networks that control cell proliferation during organogenesis and contribute to establishing the final size of organs and seeds.

I am studying a novel ubiquitin-mediated peptidase pathway that controls the duration of cell proliferation during organ growth. Mutations in DA1 which encodes a Ubiquitin-Activated Peptidase (UAP) synergistically interact with mutations in genes encoding the RING E3 ligases BB/EOD1 and DA2 to significantly increase organ and seed size in Arabidopsis. This is caused by increased duration of cell proliferation and hence cell numbers in organs.

The RING E3 ligases BB/EOD1 and DA2 multiply-monoubiquitinate DA1, activating its latent peptidase activity. DA1 in turn cleaves the E3 ligases. Based on genetic interactions, it is proposed that DA1 normally functions to increase the activities of the E3 ligases, but it is currently not understood how cleavage increases activity. Part of my project is to elucidate further the interactions of DA1 with a number of proteins involved



in the control of organogenesis. These interactions were previously identified by yeast-2-hybrid screen and pull-down assays.

The first putative DA1 peptidase substrate is TCP15, from the DNAbinding TCP family of transcription factors thought to promote cell proliferation, with TCP15 having a specific role in the control of endoreduplication. The second, known as TMK4 or BARK1, is from a family of receptor-like kinases that sense auxin in conjunction with ABP1 to activate ROP GTPase signalling. BARK1 is also part of the brassinosteroid response pathway.

A second part of my project is to use live-cell imaging in growing leaves of DA1 and EOD1 single and double mutants in order to measure the cellular basis and function of DA1 by tracking cell division patterns in early growth.

Poster - S15

Endocytic vesicle in plant meiotic spindle positioning

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Accurate positioning of spindles is a critical aspect during meiosis, as it ensures each daughter cell has the appropriate size and contains a single nucleus. JASON is involved the spindle positioning in male meiosis II, however, the underlying mechanism remained unknown. Here we show that JASON is mainly localized in the organelle band in male meiosis II, indicating a direct role of JASON in organelle band formation. In root cells, JASON is localized in the endomembrane system, as well as the plasma membrane. However, only the plasma membrane marker PIP4- RFP is localized in organelle band, suggesting that JASON is transported from the plasma membrane to the organelle band by endocytosis in meiosis. These data highlighting a role of endocytosis in plant meiosis will be



explored further.





Ecology and Environmental Changes

Ecology and Environmental Changes (EC)

Keynote Lecture EC - S13

Trophic interactions in the rhizosphere: applying chemical ecology to develop novel strategies for root pest control

Ted Turlings

University of Neuchâtel

Larvae of the beetle Diabrotoca virgifera virgifera (Western corn rootworm) cause tremendous damages to maize roots in the USA. Since its incidental introduction into the Balkan region in the 1990s it has rapidly spread and become a serious problem in Europe as well. We think we have revealed the primary reason why the insect is so successful on maize. The most valuable roots are well-defended with toxic benzoxazinoids The rootworm larvae, however, are completely unaffected by the toxins and use them to identify the most nutritious roots. How then can we fight this pest? One solution may be entomopathogenic nematodes, tiny parasitic worms that kill the larvae within days. These nematodes are attracted to the sesquiterpene E- (β) -caryophyllene, which is specifically emitted from maize roots after rootworm attack. Usina genetic transformation we restored caryophyllene emission in an American maize line that had lost the signal. This resulted in enhanced attraction of nematodes and better protection of maize plants against rootworm damage. I will give examples of how we use our knowledge of the system to develop various novel strategies to apply entomopathogenic nematodes more effectively against rootworms.



Talk - ec1 - S14

Impact of increasing winter and spring waterlogging on the morphology of silver and pubescent birch seedlings in boreal forest

<u>Ai-fang Wang</u>^{1,2}, Marja Roitto¹, Sirkka Sutinen¹, Tarja Lehto², and Tapani Repo¹

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Global climate change is predicted to increase precipitation and snow melt frequency, therefore increasing the risk of waterlogging in winter in Northern Europe. Waterlogging causes oxygen deprivation in soil, it may retard the growth of root systems and decline the whole-tree growth. We studied the responses of silver birch (Betula pendula) and pubescent birch (Betula pubescens) seedlings to excess water in winter and in early spring. A growth chamber experiment was conducted with one-year-old birch seedlings. The experiment consisted of a four-week dormancy period and an eight-week growth period. The treatments were: (1) no waterlogging, (2) four-week waterlogging in the dormancy phase, (3) four-week waterlogging in the growth phase, (4) four-week waterlogging in dormancy phase followed by four-week waterlogging in the growth phase. Each treatment consisted four-week post-treatment phase without waterlogging. Leaf area, stomata, glandular and non-glandular trichome density of leaves were investigated. Basal stem lenticel density was measured after four weeks of growth phase. The black root proportion was analysed at the end of the dormancy waterlogging phase, at the end of the growth waterlogging phase, after two weeks and four weeks growth without waterlogging in growth phase. As a result of waterlogging in the dormancy phase, root surface became black, but it did not have



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consequences in the leaf size, leaf anatomy and stem lenticels density in the following growth phase. Waterlogging in the growth phase increased black root proportion and reduced leaf size in both species, and induced different response of leaf anatomy of both species. Pubescent birch had more non-glandular trichomes in leaves, and more stem lenticels in the bottom of stem than silver birch. This may have promoted the better tolerance of waterlogging in pubescent birch.

Keywords: climate change, waterlogging, birch, root, leaf anatomy, stem lenticel

Notes:

Talk - ec2 - S15

Interactions between carbon and nitrogen metabolism in Arabidopsis WT and gln1;2 mutants under elevated atmospheric carbon dioxide

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Atmospheric carbon dioxide levels are predicted to double by the end of this century which increases net photosynthetic production in C3



Ecology and Environmental Changes (EC)

plants at the expense of photo-respiratory nitrogen recycling, resulting in alterations in carbon and nitrogen metabolism. Increased carbohydrate content along with reduced nitrogen levels and subsequently protein content have implications on plant growth and adaptation to stress. In plants, conversion of ammonium into glutamine by glutamine synthetase (cytosolic GS1 isoforms numbered five (Gln1;1 to Gln1;5) and chloroplastic GS2 in Arabidopsis) is a central step in nitrogen assimilation.

Our objective is to study the role of GS1 isoforms (GIn1;2 isoform in particular) under elevated carbon dioxide. Arabidopsis WT and gIn1;2 mutants are grown hydroponically and in soil with varying nitrogen nutrition, under both ambient (aCO2; 400ppm) and elevated CO2 (eCO2; 800ppm).

Under optimum nitrogen nutrition, a higher ammonium accumulation in roots and shoots of gln1;2 compared to WT was seen under both aCO2 and eCO2 indicating that the Gln1;2 isoform plays an important role. Elevated CO2 doubled the plant biomass in WT plants but not in the gln1;2 mutant, showing that N deprivation under eCO2 results in impaired growth. A similar phenotype was seen in both hydroponic and soil experiments. Under toxic ammonium nutrition, WT plants at eCO2 showed impaired ammonium assimilation and an imbalance in nitrogen-carbon interactions resulting in significantly reduced growth, while gln1;2 showed severely impaired growth relative to WT.

In conclusion, a single gene mutation in GLN1;2 results in significantly reduced growth. This reflects the pivotal role of cytosolic GS in general and that of GLN1;2 in particular in catering to the plants N needs, especially under eCO2 conditions and points towards the need for a deeper understanding of carbon and nitrogen interactions in plants in future environmental scenarios.



Talk - ec3 - S16

Impact of ozone on rice (Oryza sativa L.) and mustard (Brassica campestris L.) cultivars grown in the ambient field conditions of India

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Tropospheric ozone (O3) is a global air pollutant causing adverse effects on plants and crop production. . It is formed by photochemical reactions between nitrogen oxides, methane and volatile organic mostlv generated bv anthropogenic compounds emissions. O3concentration is currently increasing at an annual rate of 0.3 ppb per year globally. India is one of the most vulnerable regions of world to increasing O3 concentrations due to high population, rapid economic growth and favorable meteorological conditions. O3 enters the plant leaves through stomata and generates reactive oxygen species which in turn can decrease photosynthesis, growth, biomass accumulation and crop yield. Earlier studies on Indian crops have highlighted their vulnerability to O3, but genetic variations within the species are scarcely reported.

Two separate experiments, one with 19 cultivars of rice (Oryza sativa), and the other with two cultivars of mustard Brassica campestris (L.), were conducted to study the effect of ambient O3 concentrations in India. EDU (ethylene diurea; [N-(2-2-oxo-1-imidazolidinyl) ethyl]- N'-phenyl urea) was used as a tool in both experiments to assess the effect of O3 on growth, yield and antioxidative defense response. Most of the widely



Ecology and Environmental Changes (EC)

grown cultivars of rice and mustard showed sensitivity to ambient O3 concentrations as reduced biomass and increased oxidative stress. However, the defense strategies against the oxidative stress generated by O3 were found to be cultivar specific. The O3 sensitive cultivars did not necessarily show a decrease in yield, suggesting different carbon allocation patterns. Cultivar with higher biomass and better antioxidative defense did not translate to better yield, while cultivars with fewer benefits on biomass and narrow time span of enzymatic defense showed better yield response. Thus, these studies provide a better understanding on the complex strategies characterizing sensitivity/tolerance to O3 in cultivar of same species, and use this information in breeding for O3 tolerant plants.



Ecology and Environmental Changes (EC)

Poster - S16

Metabolic responses of silver birch (Betula pendula Roth) and hybrid aspen (Populus tremula L. x P. tremuloides Michx) leaves to increased air humidity in Free Air Humidity Manipulation (FAHM) experiment

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Atmospheric water vapor affects the water and energy balance of plants by disturbing gas exchange, inhibiting transpiration and reducing mineral nutrient uptake. We studied metabolic responses of silver birch (Betula pendula Roth) and hybrid aspen (Populus tremula x tremuloides Michx.) to long-term increase in air humidity at Free Air Humidity Manipulation (FAHM) experiment. Leaves were collected during fourth humidification season and foliar metabolites were analyzed by GC-MS. Silver birch and hybrid aspen had similar responses to increased air humidity. However, response was stronger in silver birch than in hybrid aspen. Humidification treatment affected carbohydrate metabolism indicating adjustment to altered carbon and nutrient balance. Antioxidant system was induced by high air humidity indicating oxidative stress.





Applied Plant Biology

Keynote Lecture AB - S17

Photosynthesis in direct biofuel production

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Plants, algae and cyanobacteria are the key autotrophic organisms that produce the food and feed for all human beings and animals. In the future, the ability of solar energy conversion by photosynthetic organisms is expected to be utilized in a much larger scale in production of bioenergy as well as high-value chemicals, in promotion the transition of our societies to efficient and carbon neutral bioeconomy - with ultimate goal to get completely rid of the fossil fuels.

The beauty of oxygenic photosynthetic organisms relies in their capacity to oxidize water molecules by using solar energy and in storing the energy of released electrons in high energy bonds of organic molecules. Photosynthetic light-harvesting and electron transfer reactions are the key factors that specify the photon conversion efficiency (PCE) of oxygenic photosynthetic organisms. Light, under aerobic environments, is an elusive substrate of photosynthesis and thus not only an efficient electron transfer but also efficient photoprotection mechanisms are crucial for photosynthetic organisms. Electron transfer reactions via the two photosystems have remained very similar during evolution from cyanobacteria to higher plants but the light harvesting systems have undergone considerable evolution. Likewise, the regulation of light harvesting but also that of the electron flow from water to terminal acceptors have experienced remarkable changes during evolution of photosynthetic organisms. In order to understand how the PCE can be enhanced in plants, algae and cyanobacteria for production of sustainable target solar fuels, the basic electron transfer pathways and their photoprotection mechanisms have to be mapped upon varying environmental cues and scenarios have to be developed for maximization of PCE in future bioreactor conditions.



Talk - ab1 - S18

The metal ion transporter HvIRT1 is required for manganese uptake and translocation in barley

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Manganese (Mn) deficiency is a serious nutritional disorder in many crop plants throughout the world. We have previously identified HvIRT1 (Iron-Regulated Transporter) as a barley gene encoding a plasma membrane-localized protein capable of transporting Mn, Fe, Zn and Cd when expressed in yeast. However, the in planta role of the HvIRT1 transporter has not been clarified. In the present study, barley hvirt1-RNAi lines were generated. The level of HvIRT1 transcripts in homozygous barley hvirt1-RNAi lines was only about 5% of that in the wild type, thus indicating a very substantial down-regulation. This down-regulation resulted in much lower 54Mn2+ influx in roots of the hvirt1-RNAi lines were grown in soil without Mn-deficiency, the hvirt1-RNAi lines contained approximately 40 % and 30% less Mn in straw and grain tissue, respectively, than did the wild type and corresponding null lines. In an



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alkaline, well-aerated soil inducing Mn deficiency, the hvirt1-RNAi lines showed a strong phenotype with decreased photosynthetic performance and decreased foliar Mn concentrations. In situ hybridizations revealed that in Mn sufficient plants HvIRT1 transcripts were mainly localized at the rhizodermis and pericycle. However, during Mn deficiency, HvIRT1 expression was induced dramatically in the cortex and endodermis. We conclude that HvIRT1 is a metal ion transporter which is required for uptake, translocation and grain loading of Mn in barley.

Notes:

Talk - ab2 - S19

Overexpression of chloroplast thioredoxin NTRC promotes leaf growth and reveals partial redundancy between plastidial thioredoxin systems

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Redox regulation and redox signalling through thioredoxins (TRXs) are pivotal regulatory factors in a plant's development and adaptation to its ever-changing environment. TRXs are ubiquitous in all life forms and regulate several processes in the cell or organelle by reducing intra- or



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inter-subunit disulphide bridges in their target proteins. A TRX gets oxidized as it reacts with a target and needs to be re-reduced to restore its functionality. This is achieved through TRX reductases (TRs), a class of enzymes that in turn receive their reducing power either from NADPH or, in chloroplasts and in cvanobacteria, from reduced ferredoxin produced in the light reactions of photosynthesis. TRXs are especially important in plant chloroplasts, where they are involved in regulation of almost all essential processes of the organelle. The chloroplast NADPH thioredoxin reductase (NTRC) is a unique enzyme that contains both an N-terminal TR domain as well as a C-terminal TRX domain and can, functioning as a dimer, control its own redox state. NTRC regulates chloroplast development and ROS metabolism as well as biosynthesis of aromatic amino acids, chlorophyll and starch. Recently it was proposed that NTRC also has a chaperone function. We have shown (Toivola et al. 2013, Front. Plant Sci. 4: 389) that overexpression of NTRC in Arabidopsis thaliana, in addition to restoring a wild type phenotype in ntrc knockout plants. substantially increases leaf size, biomass yield, accumulation of starch and the number of chloroplasts per cell. Interestingly, a WT phenotype can also be partly recovered by overexpressing a mutated NTRC where either the NTR or TRX domain has been inactivated. This is indicative of previously unknown cross-talk between the NTRC and FTR thioredoxin systems. Our results suggest that overexpression of chloroplast TRXs is potentially applicable to bioengineering plant and algal species for sustainable biofuel production and for increasing the yield of crop plants.



High-throughput screening approach to identify inhibitors against nucleotide-sugar producing enzymes.

Daniel Decker

Many essential plant processes, such as cell wall and sucrose synthesis, require sugars "activated" with a nucleotide-diphosphate, such as UDP. Enzymes involved in this activation. UDP-glucose (UGPase) and UDP-sugar pvrophosphorvlase pyrophosphorylase (USPase), can catalyze the reversible conversion of UTP and a sugar-1phosphate to the corresponding UDP-sugar and pyrophosphate (PPi). UGPase can produce UDP-glucose, while USPase also produces several other UDP-sugars (mainly UDP-galactose, UDP-glucoronic-acid and UDParabinose UDP-xylose). In order to identify inhibitors for these enzymes, we used a high-through-put approach, involving screening of a chemical library containing 17500 compounds. We used an in vitro semi-robotized assay in which PPi was used to couple the activity of UGPase/USPase with luminescence.

In this initial screen, 201 putative inhibitors were identified. After removal of false-positives and validation in two additional assay-systems (forward and reverse reaction of the enzymes), 5 compounds remained. These showed a dose-dependent response (IC50 between 0.5 and 50 μ M) and were acceptable according to the Lipinski rules. My poster will include details of the inhibitor screening approach, as well as planned further kinetic characterizations of the compounds, analyses of analogous compounds (hit expansion), and possible in vitro and in vivo applications will be discussed.



Moving zinc from soil to seed in rice: Increasing zinc content of the rice endosperm by native overexpression of a zinc pump

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Zinc is necessary for the correct function of over 300 enzymes in the human body. Deficiency of zinc is detrimental to human health and remains the fifth most important health risk factor in developing countries according to the WHO. Biofortified rice with increased zinc levels in the endosperm could potentially help to alleviate zinc deficiency in developing countries. Such plants should be designed to move increased amounts of zinc from soil towards the seed endosperm. In the route from soil to seed, zinc must be transported from symplast to apoplast on several occasions: During xylem loading in the root, during phloem loading in source leaves and during translocation from maternal tissue to the progeny tissue in seeds. Export of zinc from the symplast is against the electrochemical gradient and thus requires active transport. These transport steps are bottlenecks in the zinc transport and need therefore be targeted in biofortification strategies. This project focuses on increasing the native expression of the rice heavy metal ATPase Orvza sativa HMA2, a P-type ATPase that exports zinc from the symplast into the apoplast. OsHMA2 has been shown to be involved in xylem loading of zinc, to be present in nodes and to be involved in partitioning of zinc towards the developing seed. Further, experiments performed by our group on the two Arabidopsis thaliana homologues to OsHMA2, AtHMA2 and AtHMA4 indicate involvement in both leaf phloem loading and translocation of zinc from the seed coat into endosperm. Using a multiple gene copy strategy, overexpression of OsHMA2 in its native location holds the potential to increase zinc concentration in endosperm of rice.

Keywords: Plant Heavy Metal ATPase, Zinc transport, Cis-genesis,



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Oryza sativa

Poster - S19

Brachypodium mutants defective in silicon transport as a tool for investigation of silicon effects on cell wall composition

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Silicon (Si) plays a role as beneficial element in plants by improving their growth and resistance towards stress. A major part of the Si is embedded in the cell wall. However, there is limited knowledge about the specific forms of Si and the functional properties of Si in relation to cell wall structure and composition. On this background, the objective of our research is to understand Si deposition mechanisms and interactions with cell wall components in grass species.

Brachypodium mutants defective in silicon influx (bdLsi1-1) were isolated and characterized. The Si concentration in mature mutants was on average 0.37% of the dry matter, which was 80% lower compared to the wild type. At the same time, calcium and zinc levels were significantly higher than in the wild type plants. Cell wall composition was mapped with monoclonal antibodies designed to recognize specific glycan-epitopes. Mutant plants showed higher signal for an antibody which recognizes $(1\rightarrow 6)-\alpha$ -L-arabinan side chains of rhamnogalacturonan I which is one of the main components of pectin, a primary constituent of higher plant cell walls. Enzymatic saccharification efficiency was similar for the wild type and bdlsi1-1 mutant. The release of glucose comprised about 16-20% and that of xylose 11-14% of the dry matter.

We conclude that the Brachypodium bdlsi1-1 mutant is a suitable tool for further studies of Si deposition and interactions with cell wall components, as well as the consequences for enzymatic degradability of



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cell walls. We are currently investigating these relationships in further details, focusing on higher, more realistic Si concentrations and a larger span in cell wall Si concentrations between wild type and mutant plants.





Response to Abiotic Stress

Keynote Lecture AS - S20

Plant response to abiotic stress: how epigenetic can participate?

Stéphane Maury

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Plant response to abiotic stress is a main challenge in agriculture. This is particularly relevant in a context of global climate change. The understanding of physiological as well as genetic/molecular processes controlling plant's response to abjotic stress will help to improve plant breeding. Recently, epigenetic mechanisms such as DNA methylation have been shown to participate to the control of plant development and their adaptation to environment through modifications of chromatin compaction and gene expression profiles. Phenotypic plasticity defines as the different phenotypes for a given genotype in distinct environments is a key process for plant to adapt to their changing environment. This is particularly relevant for perennial plants such as trees that are exposed to repeated fluctuations of their living conditions. In this context, drought is a significant threat to forest health and agro-ecosystem productivity. With the availability of its genome and its important natural genetic and phenotypic variations. Populus became a model tree. Poplars (Populus spp.) are among the fastest growing trees in temperate latitudes. Their high productivity is associated with large water requirements. The concept of water deficit tolerance, when applied to cultivated tree species such as poplars, has been defined as the ability to limit the decrease of biomass production in response to a moderate water deficit. Variations of DNA methylation have been reported between genotypes, tissues but also in response to drought and geographic location origin. Nevertheless, the relationships between gene body DNA methylation, gene expression and the phenotypic plasticity still need clarification. This is the objective of my work in the LBLGC laboratory at the University of Orléans that may ultimately help to improve the actual predictive phenotypic models based on genetic variations for selection.



Notes:

Talk - as1 - S21

The role of SNF1-related protein kinase regulatory subunit γ in ABA-dependent signalling pathways in Arabidopsis thaliana

Matleena Punkkinen, Jian-Kang Zhu, Hiroaki Fujii

Plants regulate their responses to osmotic stress through several signaling pathways, some of which are also activated by abscisic acid (ABA). SNF1-related protein kinase 2s (SnRK2s) are important components in osmotic stress signaling, and SnRK2.2, 2.3 and 2.6 are essential kinases in the ABA-dependent signaling pathway: they are strongly activated by ABA, and the snrk2.2/2.3/2.6 mutant is almost completely insensitive to ABA. The exact regulation mechanism of SnRK2s is, however, still unclear.

In this study we looked into a potential regulator of SnRK2s, KING1, a homolog of the γ -subunit of the yeast SNF1-complex. SNF1 in turn is a homolog of SnRKs, and requires the γ -subunit to be fully active. In a previous large-scale screening study the induction of KING1 expression lead to ABA-insensitivity during germination, which suggests that KING1 is involved in the ABA-dependent pathway. Thus, we analyzed the interaction between KING1 and the ABA-activated SnRK2.6. KING1 was found in pull-down fractions with FLAG-tagged SnRK2.6 in transgenic



Arabidopsis thaliana. Further analysis with recombinant proteins in an in vitro kinase assay showed suppression of the activity of SnRK2.6 by KING1. In vitro experiments, for which where we identified the A. thaliana mutants of KING1 and its closest homolog and produced the double mutant, showed that during germination king mutants had increased sensitivity to ABA.

Notes:

Talk - as2 - S22

Cytosolic pH is a major modulator of mitochondrial NADPH oxidation in plants

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NADPH is a key substance in plant metabolism, by carrying reducing power that maintain whole plant internal redox homeostasis, and support most synthetic pathways. NADPH/NADP+ ratio reflects the redox state of the cell, as dependent on metabolic activities. In the electron transport chain of plant mitochondria, alternative pathways for NAD(P)H oxidation are catalysed by type II NAD(P)H dehydrogenase. NDB1 is a type II NADPH dehydrogenase, which has a high capacity to oxidise cytosolic NADPH and can regulate whole cell NADPH status. Previously, NDB1 was



thought to be Ca2+ and pH dependent, but all studies were done in the presence of a high, unphysiological concentration of Ca2+. No direct determination of K0.5(Ca2+) has been carried out. In this study, K0.5(Ca2+) of the NDB1 from potato and Arabidopsis thaliana was determined under various pHs. The result shows that K0.5(Ca2+) is significant lower with higher pH, which indicates that Ca2+-regulation is subordinate to pH regulation for NDB1. This knowledge is important for further understandings of NADP(H) homeostasis and redox dependence, especially regarding to predict how transgenic plants modified for the NADPH dehydrogenase will vary concerning plant growth and stress responses.

Notes:



Talk - as3 - S23

TAP-dependent opposite phosphorylation of PSII core and LHCII proteins in high light prevents the disorganization of the thylakoid membrane

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In plant thylakoid membrane the **PSII-LHCII** the higher phosphoproteins are phosphorylated under all growth light conditions. Nevertheless, depending on the naturally relevant light intensity, the relative phosphorvlation level between different PSII-LHCII phosphoproteins shows considerable difference. In steady state growth light conditions, both the PSII core and LHCII proteins are moderately phosphorylated. Upon shift to high light conditions, the PSII core proteins are strongly phosphorylated and the phosphorylation level of LHCII proteins is suppressed. On the contrary, low light conditions suppress the phosphorylation of PSII core, but LHCII protein phosphorylation is strongly induced. The reversible and differential phosphorylation of the PSII-LHCII proteins is dependent on the redox regulated interplay between the STN7 and STN8 kinases, and TAP38 and PCPB phosphatases. Physiological reason for such dynamic and opposing regulation of PSII-LHCII proteins has remained elusive. Here we have studied stn7, stn8 kinase and tap38 and pbcp phosphatase mutants and the pgr5 with uncontrolled redox balance under different light intensities. We show that the lack of TAP38 phosphatase as well as the redox unbalance in pgr5 lead to strong concomitant phosphorylation of both PSII core and LHCII proteins (analogous to traditional "state 2") under high light, inducing complete unpacking of PSII-LHCII supercomplexes and strongly increased relative excitation of PSI. Indeed, under high light, where strong PSII core protein phosphorylation is required for fluent unpacking of photodamaged PSII complexes, the LHCII dephosphorylation is required to preserve the



excitation balance between PSII and PSI. We conclude that strong concomitant phosphorylation of PSII and LHCII proteins ("state 2") leads to excess repulsion forces between the hyperphosphorylated PSII core and LHCII proteins, resulting in disorganization of PSII-LHCII supercomplexes, loss of lateral heterogeneity and excitation energy spillover to PSI.

Keywords: High light, Protein Phosphorylation, PSII-LHCII supercomplex.

Notes:

Talk - as4 - S24

The role of polyamine catabolism associated hydrogen peroxide and nitric oxide in salt stress-induced cell death in tomato plants

Zoltán Takács, Ágnes Gallé, Ágnes Szepesi, Péter Poór, Irma Tari

Polyamines are biogenic polycationics, which have positive effect in salt stress tolerance but they can act as prooxidants as well. In the catabolism of polyamines hydrogen peroxide (H2O2) produced which can induce programmed cell death (PCD).

In our experiments hydroponically grown tomato plants were treated with sublethal- (100 mM) or lethal (250 mM) concentration of NaCl in



modified Hoagland solution.

The level of H2O2, diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, EC 1.5.3.14) enzyme activity, which catalysed polyamines oxidation, showed two peaks after 30 min and 2 h after treatment with lethal salt stress. 250 mM NaCl concentration induced an increase in the levels of reactive oxygen species (ROS) and nitric oxide (NO) production in the leaf discs. Lethal NaCl caused significant decrease in the gene expression of PAO at 30 min in shoot and increase in the gene expression of PAO at 30 min in shoot and increase in the gene expression of DAO at 3 h. Spermidine (Spd) and spermine (Spm) content increased after 100 mM NaCl treatment but the accumulation of these polyamines decreased in leaves exposed to 250 mM NaCl.

We also analysed the time dependent effect of specific PAO inhibitor, MDL72527 in leaves of 100 mM and 250 mM NaCI-treated tomato plants. The activity of DAO and PAO enzymes and H2O2 accumulation exhibited only one lower peak at 2 h in the leaves exposed to salt stress after treatment MDL72527. The MDL72527 significantly decreased the ROS and NO concentrations and kept them on a constant low level, in every saline-treatment.

All of these findings suggest that the oxidative stress caused by catabolism of polyamines promote PCD in salt stressed tomato plants, due to the changes in ROS and NO levels.

Notes:



Development of in-vitro methods for the assessment of antioxidant acitvity of low-molecular antioxidants and plant extracts

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Abiotic factors can cause excessive production of reactive oxygen and nitrogen species (RONS) in plants. Plants prevent from RONS functioning due to the antioxidant protective system. Moreover, if this protective antioxidant system is failing, the oxidative stress takes place. This work deals with the determination of antioxidant activity, the reducing power and total polyphernolic compounds in extracts from primary leaves of barley (Hordeum vulgare L. cv. Bonus) in-vitro. Barley was grown up at low (LI – 150 µmol.m-2.s-1) and high (HI – 1000 µmol.m-2.s-1) irradiation. It was exposed to an increased dose of UV-B radiation (0,5 W.m-2) and compared with control plants. For this purpose have been established and calibrated methods of fluorimetric and spectrophotometric determination of antioxidant activity, reducing power and total polyphenolics compounds (methods ORAC, ABTS, DPPH, FRAP and TPC). The calibration of ABTS (2,2'-azinobis(3-ethylbenzothiazolin-6-sulfonate), DPPH (2,2-diphenyl-1picrylhydrazyl), ORAC (Oxygen radical antioxidant apacity) and FRAP (Ferric rediction antioxidant power) was performed using the standard Trolox. Method TPC (total polyphenolics compounds) was performed using as standard gallic acid. We expect that the antioxidant activity will be higher in extracts taken from plants which was exposed to UV-B radiation.



Evaluating of the effectiveness of Hydrogel influence on the growth and development of Tilia platyphyllos L. in the nursery

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Effects of water stress on a plants physiology, including, leaf and gene expression, have been studied extensively. Polyacrylamide (PAAm) Hydrogels are commonly employed to ensure soil hydration in horticulture. The experiment was established at a multipurpose scientific experimental workplace on plots of Faculty of Horticulture in Lednice. Mendel University in Brno. The aim of this work was to evaluate the effect of Hydrogels on morphological and physiological parameters of the Tilia platyphyllos L. In the experiment, each variant used 50 pieces of planting material, repeated experiments were doubled. Used planting technology was traditional. Control plants were grown in naked soil and planted in the conventional nursery manner on an open site. Polyacrylamide Hydrogel was applied in three concentrations: 2 kg m-3, 3.5 kg.m-3, 5 kg.m-3 and potting substrate. Chemical and physical characteristics of the substrate: pH 5.5-7.0; N 250–350 mg.1L.; P2O5 200–250 mg.1L.; K2O 300–400 mg. 1L. The irrigation system was automatic; containers had sensors for measuring humidity and temperature of the substrate. Temperature for automatic irrigation was installed and set to 250C degrees. Morphological and physiological data of this study have shown good results the plants that were planted in the 3.5 kg.m-3 concentration. Stability of polyacrylamide is an expected result and has been observed by other investigators as well.



Green light induces significant accumulation of chlorophyll a isomer in barley plants

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Light quality affects synthesis of plant pigments not only quantitatively but also qualitatively. In the experiments aimed at the effect of the light quality (blue, green, red, white light) on barley plants (Hordeum vulgare L) we observed in plants cultivated at the green light (500-590nm) a significant peak in chromatograms during the reversed phase-HPLC analysis of photosynthetic pigments. This peak was absent or negligible in other barley plant variants, cultivated at blue (420-480nm), red (600-660nm) and white light at the same intensity of irradiance (240µmol.m-2.s-1). The concentration of this pigment of barley cultivated at green light (240µmol.m-2.s-1) was about 0.1mg/g of fresh weight (f.w.) that is approximately ten times less than chlorophyll a concentration. For the followina identification we separated this peak occurring on chromatograms between peaks of chlorophyll b and a using the fraction collector of our HPLC system. We found that the absorption and fluorescence spectra of chlorophyll a and unknown pigment exhibit the same absorption and emission maxima in both the red and the blue spectral region. Mass spectrometry confirmed the same molecular weight of both pigments and supported the hypothesis that unknown pigment should be some type of chlorophyll a isomer. The circular dichroism spectra of chlorophyll a and chlorophyll a isomer will be also presented.



Penetration of radiation of different spectral quality to barley leaf

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The primary photosynthetic pigment of higher plants is chlorophyll a which absorbs light in the blue and red spectral regions. Other auxiliary pigments are carotenes and xanthophylls which absorbs in the blue to blue-green region. Therefore, the blue component of the radiation is absorbed extensively already in the upper leaf layers. The red component penetrates deeper and the green one is absorbed the least.

The depth of penetration of the blue (460 nm), red (620 nm) and green (520 nm) radiation into barley leaf was investigated by means of fluorescence microscope. Measurements were performed on the cross sections when excitation light illumi-nated the surface of the leaf and fluorescence was detected at an angle of 90 °. Four variants of cultivation light were used in the experiment – blue (420 - 480 nm), red (600 - 660 nm) and green (500 - 590 nm). The last variant was the control with ratio of blue:red:green 1:1.5:2, which simulates proportions of these colors in sunlight. The cross sections were prepared from the medium segments of 8 day-old primary leaves.

The results were in agreement with the theory – blue light was strongly absorb-ed already in the upper leaf layers, red and green light penetrated deeper. Different depth of penetration for plants cultivated under blue, red and green light is discussed in relation to their different morphology.



Post-translational modifications of ferredoxin-NADP+ oxidoreductase in Arabidopsis thaliana chloroplast

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Rapid changes in protein function can be achieved through posttranslational modifications, which are known to affect the activity, interaction as well as localization of proteins. Recent studies have shown that, besides protein phosphorylation, many other types of modifications, including acetylation, methylation and glycosylation, are important in the regulation of chloroplast proteins. As chloroplast is the central hub of plant metabolism, identifying these modifications is important in understanding how plants respond to changes in the environment. Both of the two Arabidopsis thaliana leaf-type ferredoxin-NADP+ oxidoreductase (FNR) isoforms, the key enzymes linking the light reactions of photosynthesis to carbon assimilation, exist as two distinct forms with different isoelectric points. Our results show that both FNR isoforms contain multiple alternative N-termini which are partially Na-acetylated leading to the change in the pl. Moreover, both isoforms were found to contain acetylation of a conserved lysine residue near to the active site, while no evidence for in vivo phosphorylation was gained. Experimental evidence and structural modeling show that the identified modifications do not affect the membrane attachment of FNR or its direct interaction with ferredoxin. However, we found that the amounts of different FNR forms change upon transfer from darkness to light, which implies that the modifications are important in regulating FNR function.



Identification and characterization of differential expressed genes in common bean under salt stress.

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Salinity is one of the important abiotic stress factors at all developmental stages in arid and semi-arid soils. Common bean (Phaseolus vulgaris L.) is a major protein source in developing countries and decrease in vield reaches up to 50% due to the adverse effects of salinity. Among the different varieties of common bean, there exist natural salt tolerance, however deep understanding of molecular mechanism of salinity tolerance is still scarse. Thus we first aimed to identify the salt responsive genes in common bean by transcriptome sequencing and functional characterization of certain candidate aenes through overexpression in A. thaliana lines. To observe the effects of salinity, the mutant lines along with wild type plants were grown in hydroponics system for five weeks and NaCI were added to the hydroponics medium by 50 mM increments in three days. The salt application were continued for four days after the salt concentration reaches to 150 mM. The leaf area, leaf water potential, canopy and root fresh weights were used for phenotyping the salinity tolerance. We have overexpressed seven upregulated genes and preliminary results revealed that two of the genes showed improvement on salt tolerance in arabidopsis. Further analysis will be performed on the T3 homozygous lines for these mutant lines.





Biotic Interactions

Keynote Lecture IN - S25

Effectors, expression regulation, and nutrients required for pathogenicity in Phytophthora infestans, the late blight pathogen of potato.

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Diseases of crop plants represent a serious constraint to food production worldwide. This is especially so when a single disease can lead to over €5bn in lost production and control costs, as for late blight disease of potato, caused by the oomycete, Phytophthora infestans. The early stages of P. infestans infection are biotrophic, when living host plant tissue is essential to the pathogen. Accumulating evidence indicates that P. infestans produces a multitude of secreted effector proteins to suppress host defence responses, keeping infected tissue alive, and promoting infection development. We have used microarrays to identify pathogen transcripts that accumulate significantly during different stages of infection. The set of transcripts co-expressed with markers of biotrophic infection is highly enriched for sequences encoding effector proteins, especially the much-studied RXLR class of effectors. In addition, transcripts coding for metabolic proteins and potential regulators of expression during infection were also identified. RNA silencing of over 50 individual infection-induced genes has highlighted a diverse range of genes that are essential for full pathogenicity in P. infestans. While effectors are essential for suppressing plant defences during P. infestans infection, little is known about nutrients it acquires from plant hosts, to grow and complete its lifecycle. Since the majority of P. infestans biomass formed during infection is located in the apoplast, we grew P. infestans in apoplastic fluid extract and determined the changes in gene expression and metabolites present in the extract over time. Confirmation of individual molecules preferentially taken up by P. infestans during growth in planta is underway and progress will be presented.



Notes:

Talk - in1 - S26

Nano-titania aided colonization of Brassica napus by the Plant Growth Promoting Rhizobacteria Bacillus amyloliquefaciens strain UMBC 5113

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The field of nanobiotechnology is growing immensely and is creating opportunities at increasing speeds, not least within agriculture. Important biological processes occur at the nano interface, for example DNA and certain microorganism share this universe with mineral nanoparticles produced by both microorganism and plants. The surface activity of particles and molecules of this size is greatly enhanced compared to materials of larger size, due to increased surface to volume ratio where atoms at the surface have less neighbor interference and with lower coordination have an increased affinity to foreign atoms and to quantum size effects occurring in conducting materials where electrons move freely leading to changing in absorption and fluorescence wavelengths, ionization potentials and electron affinities after the size of the particle



(Roduner E. 2006).

We used titania nanoparticles with positive surface charge to adhere bacteria of spatially variable charge to Rape seed (Brassicae napus) roots which have negative charge. These particles were described in Seisenbaeva G.A. et al (2103) and was earlier proven to be biocompatible in Kessler V.G. et al (2012). With Scanning Electron Microscopy (SEM) we found a quantitatively increased fixation of clusters on the roots were our coupled Energy-Dispersive X-ray Spectroscopy (EDS) analysis confirmed the presence of titanium. By GFP-tagging our bacterium and studying the colonization with Confocal Laser Scanning Microscopy (CLSM) we illustrated the effect of increased adherence by our titania nanoparticles. We compared quantitatively the amount of bacteria by washing the roots and counting colony forming units (CFU).

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Notes:



Talk - in2 - S27

PP2A-CPK interaction regulates innate immunity in Arabidopsis

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Exposure to pathogens triggers the activation of complicated signalling networks in plant cells. These signals originate in various cellular compartments and converge in cytoplasm, where they are regulated by counteracting pairs of kinases and phosphatases and the message is relayed by reversible phosphorylation of proteins. Until now, only few of the kinase-phosphatase pairs regulating the defence responses have been identified. Previously in the project, a pair of a calcium dependent protein kinase (CPK) and a type 2A protein phosphatase (PP2A) has been identified to regulate the defence responses to plant pathogens. PP2A is predominantly trimeric, consisting of catalytic subunit C, scaffold subunit A, and regulatory subunit B. Protein phosphatase 2A regulatory subunit B'y (PP2A-B'y) acts as a negative regulator of plant immunity, Arabidopsis pp2a-b'y knock-down mutant having constitutive activation of defence responses, and PP2A-B'y is believed to control organellar defence signalling in connection to light. In accordance, the CPK is a positive regulator of innate immunity. CPK is transiently activated by phosphorylation upon pathogen recognition, but the phosphatases regulating the signal upstream of the CPK have not been identified. BiFC studies done with full-length and truncated forms of the CPK together with structural modelling suggest that PP2A binds the activated form of the CPK at its calcium binding domain. Moreover, an ingel kinase assays demonstrated that PP2A-B'y negatively regulates CPK activity in Arabidopsis leaves. Based on these results, we suggest a novel mechanism where PP2A limits plant defense through negative regulation of CPK activity.

Notes:



Talk - in3 - S28

Molecular Mechanism of Autoimmunity Triggered by a Pair of NB-LRRs

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Autoimmunity that appears in incompatible hybrids in plants can occur due to epistatic interactions between disease resistance (R) genes from separate lineages. R genes, in many cases, encode nucleotidebinding and leucine-rich repeat (NB-LRR) domain containing proteins, which play a key role in perception and activation of immune responses conferring disease resistance against pathogens. In an incompatible hybrid generated by a cross between the two natural accessions of Arabidopsis thaliana, Uk-3 and Uk-1, both two causal genes were identified to encode NB-LRR proteins - DANGEROUS MIX 1 (DM1) and DANGEROUS MIX 2d (DM2d). In this hybrid, epistatic interaction between DM1 and DM2d results in F1 hybrids suffering from dwarfism, reduced growth rate, and spontaneous cell death in leaves. However, how the NB-LRRs as a pair can trigger the autoimmune responses remains to be investigated.

To understand the molecular mechanism of DM1/DM2d dependent cell death signaling, we used the yeast two-hybrid (Y2H) system for



interaction assays, and the transient and stable expression of the NB-LRRs in Nicotiana benthamiana and A. thaliana, respectively, for functional assays. By using the Y2H system, we defined minimal interaction domains both for the homotypic interaction of DM1 and heterotypic interaction between DM1 and DM2d, and identified mutations that affect the interactions. Our preliminary data suggests that both physical interactions of NB-LRRs and several critical residues that would affect conformation of the protein contribute to induce the signaling. Further experiments are being carrying out to test a hypothesis of asymmetric contribution of the two NB-LRRS to the signaling by (1) fine-mapping residues of the two proteins that can modulate heterotypic- and/or homotypic- interactions, (2) testing functional contributions of these residues to the signaling, and (3) overlaying the experimental information to natural variants of NB-LRRs in A.thaliana to predict the interaction properties of a certain pair.

Notes:



Poster - S26

Analysis of barley gene sequences BCI-4 and BCI-7 in Arabidopsis thaliana with regard to aphid preference and population growth.

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Arabidopsis thaliana was chosen as a model plant to study the transformation effects with two barley genes BCI-4 and BCI-7 with putative defense functions against aphids. BCI-4 gene encodes for a putative calcium binding protein, BCI-7 encodes for a proteinase inhibitor. The two gene sequences were previously described as potential resistance factors against bird cherry-oat aphid (Rhopalosiphum padi) in barley. BCI-4 and BCI-7 expression was shown to be up-regulated in barley plants after aphid feeding, however only in genotypes with moderate resistance to the aphid: BCI-4 and BCI-7 gene transcription products were absent in aphid susceptible genotypes. Aim of this work was to investigate whether a constitutive expression of BCI-4 and BCI-7 would affect plant aphidinteraction. A series of tests was carried out to assess the effects of transformation on the behavior and reproduction of the green peach aphid. Myzus persicae. For experiments with aphids we selected transformant lines with confirmed transgene expression and azygous control lines. Although transgenes were constitutively expressed in all tissues and not specifically in the phloem where the aphids are feeding, we observed various effects of the transformation. In choice tests, transformant BCI-7 plants were avoided and the aphids preferred settling on control plants. Delayed settling when aphids are choosing an appropriate host may result in lower population growth. We could observe lower population densities on BCI-7 transformant plants when compared to control plants, however the effect was present only under certain experimental conditions. No effect was observed on aphid life span and fecundity on BCI-7 transformant plants. Transformation with BCI-4 caused no changes in



aphid behavior or performance on A. thaliana. Our study demonstrated that transformation of A. thaliana with barley genes putatively involved in plant defense against aphids provided a good model to study plant-insect interactions.

Poster - S27

Isolation and characterization of Bacillus amyloliquefaciens UCMB5113 bio-pesticides and their effect against oilseed rape phytopathogens

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Strains of certain Bacillus species can improve plant protection against pathogens. The protective effect may be direct, due to production of antibiotic compounds, or indirect, by priming of plant defense as induced systemic resistance. In this study the effect of compounds secreted from Bacillus amyloliquefaciens subsp. plantarum UCMB5113 were tested for their effects on pathogens and Arabidopsis thaliana plants. In plate tests whole UCMB5113 bacteria and exudates showed antagonistic effect against several fungal pathogens that are commonly infecting plants of the genus Brassica. A crude lipopeptide fraction from Bacillus UCMB5113 applied on Arabidopsis plants resulted in growth promotion and protection against the fungal pathogen Alternaria brassicicola. Use of Arabidopsis signaling mutants and PDF1.2 and VSP2 promoter driven GUS lines showed that the lipopeptide fraction activated some jasmonic acid (JA) dependent responses but growth promotion seems uncoupled from disease suppression. Separation of lipopeptide compounds using reversed-phase HPLC showed several fractions with antifungal activity. Analysis by mass spectrometry identified the most



potent compounds as novel linear forms of fengycins and synthetic peptide mimics confirmed the biological activity. The ability of Bacillus UCMB5113 to stimulate growth and counteract pathogens using direct and indirect modes of action provides an interesting tool for crop production.





Genome Defense and Epigenetics

Genome Defense and Epigenetics(GD)

Keynote Lecture GD - S29

Transcriptional control of immune-response genes by DNA methylation and demethylation and its relevance in plant disease resistance

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In higher eukaryotes, the vast majority of the genome appears to be transcribed, leading to an extraordinary diversity of non-coding RNAs. Whereas the functional significance of these non-coding RNAs is mostly unknown, increasing evidence suggests a role for these molecules in guiding chromatin modifications. In plants, a large portion of non-coding RNAs is processed by the RNA silencing machinery to produce 24 nt siRNAs that guide cytosine DNA methylation of transposable elements (TEs)/repeats leading to their transcriptional silencing. This phenomenon is referred to as RNA-directed DNA methylation (RdDM) and contributes to the transcriptional repression of some developmentally as well as abiotic stress-regulated genes that carry repeats in their vicinity. Importantly, RdDM was also shown to negatively regulate resistance against biotrophic pathogens and, accordingly, this silencing pathway directly controls the transcriptional status of some immune-response genes by directing DNA methylation of promoter-derived repeats. However, the dynamics and biological relevance of such epigenetic-based transcriptional control remain ill-defined. Here, I will report the dual role of DNA methylation/demethylation in maintaining a low basal expression of plant defense genes in the absence of pathogen while also providing a suitable chromatin environment for pervasive transcription upon pathogen detection. I will also present the relevance of such regulatory mechanism in fine-tuning the plant innate immune response. Finally, I will discuss the impact of this regulatory process in possible orchestrating transgenerational inheritance of epigenetic/genetic states that may contribute to genome evolution and rapid adaptation of plants to their environment.



Genome Defense and Epigenetics(GD)

Notes:

Talk - gd1 - S30

An Effect of Demethylating Agents on Petunia × hybrida Varieties

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DNA methylation is one way how the sessile organisms like plants can deal with changing environment around them. By modifying their expression patterns they can affect multiple aspects of plant growth and development. One type of epigenetic change is DNA methylation – by methylation of cytosine it turns into 5- methylcytosine. DNA methylation plays the role of a switch that turns the methylated genes off. If the plant genome is demethylated during the development of the plant, possible distinction in the epigenome between treated and untreated plant can arise, especially during flowering. In this experiment, two varieties of Petunias were treated during germination by several demethylating agents respectively, with varying concentration of the treatment. During the first phase of the experiment some differences in earliness of flowering were detected, both among the variant grown in natural light conditions and in the set subjected to higher light intensity. Variations in both shape and color of the flowers were observed, as well as some changed leaf and



Genome Defense and Epigenetics(GD)

stem morphology. Currently the experiment is heading towards its next phase, pollination of chosen samples and examination of the second generation of hybrids.

Notes:



Poster - S28

Genomic Imprinting in Capsella rubella Endosperm Tissue

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Genomic imprinting is an epigenetic phenomenon causing unequal expression of maternal and paternal alleles dependent on their parent-oforigin. We addressed the question whether imprinted genes are conserved between the closely related Brassica species Arabidopsis thaliana and Capsella rubella that diverged only around 10-14 million years ago. By using Single Nucleotide Polymorphisms to trace parental specific alleles and deep RNA sequencing, we identified hundreds of imprinted genes in the Capsella rubella seed endosperm. Most of the catalogued C. rubella imprinted genes have orthologs in A. thaliana; however, the majority of them are not imprinted in A. thaliana. Conversely, imprinted genes that were previously identified in A. thaliana are not imprinted in C. rubella, revealing that the imprinting status of genes is subject to rapid changes.



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The conference is organized by a joint committee of The Scandinavian Plant Physiology Society and The Linnean Center. The organizing committee is a group of PhD students from Swedish University of Agricultural Sciences (Sveriges lantbruksuniversitet) with different backgrounds.





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