8th Conference of the Polish Society of Experimental Plant Biology

Communication in plants: from cell to environment

12-15 September 2017, Bialystok, Poland

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of the Polish Society of Experimental Plant Biology

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Editors:
Iwona Ciereszko
Andrzej Bajguz

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8th Conference of the Polish Society of Experimental Plant Biology
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8th Conference of the Polish Society of Experimental Plant Biology
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The conference is held under the auspices of

Rector of the University of Bialystok
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Conference Programme

8th Conference of the Polish Society of Experimental Plant Biology

Communication in plants: from cell to environment

12-15 September 2017, Bialystok, Poland

Tuesday, 12 September 2017

12:00 – 18:00	**Registration and poster set up**
13:00 – 15:00	**PSEPB committee meeting**
16:00 – 18:00	**Welcome Reception**
18:30 – 20:30	**Bialystok city tour**

Wednesday, 13 September 2017

09:00 – 18:00	**Registration**
09:30 – 10:00	**Opening Ceremony, Welcome speeches**
10:00 – 11:45	**Keynote Lectures:**
10:00 – 10:45	**František Baluška** (Germany): Neurobiology view of higher plants
10:45 – 11:30	**Thorsten Nürnberger** (Germany): NLP cytolysins – microbial virulence factors and host defense activators
11:30 – 12:00	Coffee break

Session 1: Signalling in plant development and metabolism

Chair: **Iwona Ciereszko** and **Halina Gabryś**

12:00 – 13:30	**Plenary Lectures:**
12:00 – 12:30	**Sjef Smeekens** (Netherlands): The ribosome as a metabolite sensor: Sucrose regulated protein translation and the control of metabolism and growth in plants
12:30 – 13:00	**John Lunn** (Germany): A tale of two sugars – the role of trehalose 6-phosphate in sucrose signalling and homeostasis
13:00 – 13:30	**Jose J. Sanchez Serrano** (Spain): Stem cell renewal or differentiation: a plant's perspective for a crucial decision
13:30 – 13:45	Coffee break

Session 1: Signalling in plant development and metabolism

Chair: **Iwona Ciereszko** and **Halina Gabryś**

13:45 – 14:15 **Plenary Lectures:**

13:45 – 14:15 Marta Derba-Maceluch, Joanna Leśniewska, Xiao-Kun Liu, Magdalena Krzeslowska, Christine Ratke, PrashantPawar, **Ewa Mellerowicz** (Sweden): Plant responses to altered cell wall structure

14:15 – 15:15 **Oral Presentations:**

14:15 – 14:30 **Dorota Kwiatkowska** (Poland), Marcin Lipowczan, Dorota Borowska-Wykręt, Sandra Natonik-Białoń: Layers of growing plant cell walls differ in stiffness and/or in-plane tensile stress

14:30 – 14:45 **Milena Kulasek** (Poland), Agnieszka Rezulak, Wojciech Glinkowski, Jacek Kęsy, Paulina Glazińska: Expression of Auxin Response Factors (ARF) ARF2, ARF3, ARF4 changes over space and time in yellow lupine

14:45 – 15:00 **Julia Zdzieszyńska** (Poland), Marlena Stawska, Piotr Topolewski, Krystyna Oracz: The role of CUL4-related proteasomal proteolysis in germinating *Arabidopsis thaliana* seeds

15:00 – 15:15 Elżbieta Sarnowska, Paweł Ćwiek, Marcin Leszczyński, Nataliia Rusetska, Aleksandra Gos, Michal Szymanski, Marcin Ligaj, Alicja Chrzan, Dominika M. Gratkowska-Zmuda, Anna T. Rolicka, Anna Balcerak, Iga Jancewicz, Ewelina Macech-Klicka, Wagner L. Araújo, Takayuki Tohge, Sebastian P. Sacharowski, Szymon Kubala, Jarosław Steciuk, Bruno Huettel, Anna Maassen, Joanna Szarkowska, Paulina Kondrak, Alisdair R. Fernie, Csaba Koncz, Janusz A. Siedlecki, **Tomasz J. Sarnowski** (Poland): The SWI/SNF ATP-dependent chromatin remodelling complexes are involved in the control of metabolic processes in plants and human

15:15 – 16:15 Lunch provided

Session 2: Symplasmic communication and plant development

Chair: **Ewa U. Kurczyńska** and **Paweł Sowiński**

16:15 – 17:30 **Plenary Lectures:**

16:15 – 16:45 **Yoselin Benitez-Alfonso** (United Kingdom): New insights on the regulation of plasmodesmata cell walls and their consequences for plant development

16:50 – 17:20 **Manfred Heinlein** (France): Plant-virus interactions involved in viral cell-to-cell spread

17:30 – 17:50 Coffee break

Session 2: Symplasmic communication and plant development

Chair: **Ewa U. Kurczyńska** and **Paweł Sowiński**

17:50 – 19:00 **Oral Presentations:**

17:50 – 18:05 Małgorzata Grzyb, **Justyna Wróbel-Marek** (Poland), Ewa Kurczyńska, Jan Rybczyński, Anna Mikuła: Symplasmic communication during development of *Cyathea delgadii* somatic embryos

18:05 – 18:20 **Katarzyna Sokołowska** (Poland), Magdalena Turzańska, Marie-Charlotte Nilsson: The conducting parenchyma cells – an important element of symplasmic transport inside feather moss stems

18:20 – 18:35 **Łucja Kowalewska** (Poland), Radosław Mazur, Szymon Suski, Maciej Garstka, Agnieszka Mostowska: 3D visualization of thylakoid membrane development

18:40 – 19:00 **Beata Zagórska-Marek** (Poland), Magdalena Turzańska, Katarzyna Sokołowska: Chiral events in the development of *Physcomitrella* gametophores

19:00 – 20:00 **Poster sessions 1-2**

20:15 – 22:00 **General Assembly for PSEPB Members**

Thursday, 14 September 2017

Session 3: Plants and abiotic stresses

Chair: **Agnieszka Sirko** and **Danuta M. Antosiewicz**

09:00 – 10:30 **Plenary Lectures:**

09:00 – 09:30 **Céline Masclaux-Daubresse** (France), Qinwu Chen, Anne Marmagne, Liliana Avila-Ospina, Anne Guiboileau: Autophagy machinery, leaf senescence and nitrogen remobilization in *Arabidopsis thaliana*

09:30 – 10:00 **John Hammond** (United Kingdom), Abdul Baten, Justin Bloomfield, Graham King: The role of DNA methylation in regulating plant adaptations to low phosphate availability

10:00 – 10:30 **Ann Cuypers** (Belgium): Metal-induced oxidative challenge: from perception and signal transduction to plant acclimation and growth

10:30 – 12:00 **Oral Presentations:**

10:30 – 10:45 **Arita Kuś** (Poland), Jolanta Kwaśniewska, Robert Hasterok: Multicolour FISH-based analysis of the origin of mutagen-induced micronuclei in *Brachypodium distachyon*

10:45 – 11:00 **Rafał Krela** (Poland), Krzysztof Leśniewicz: Amino acids motifs responsible for activity properties of S1/P1-like nucleases involved in plant PCD

11:00 – 11:15 **Katarzyna Sala** (Poland), Ewa Kurczyńska: Apoplast markers of cell differentiation – spatio-temporal analysis of the autografted *Arabidopsis* seedlings

11:15 – 11:30 **Magdalena Krzesłowska** (Poland), Irena Rabęda, Teresa Lehmann, Ewa J. Mellerowicz, Mirosław Mleczek, Anna Napieralska, Sławomir Samardakiewicz, Dariusz J. Smoliński, Szymon Suski, Adam Woźny: Plant cell wall in Pb accumulation and tolerance

11:30 – 11:45 Kinga Wcisła, Aleksandra Wojcińska, Joanna Siwińska, Ewa Łojkowska**, Anna Ihnatowicz** (Poland): Genome-wide association studies of *Arabidopsis thaliana* responses to disturbed iron and manganese homeostasis

11:45 – 12:00 **Aneta Piechalak** (Poland), Anetta Hanc, Tomasz Skrzypczak, Liliana Ciszewska, Arleta Malecka, Danuta Baralkiewicz, Agnieszka Kutrowska: Chelant-assisted phytoextraction and accumulation of Zn by *Zea mays*

12:00 – 12:15 Coffee break

Session 3: Plants and abiotic stresses

Chair: **Agnieszka Sirko** and **Danuta M. Antosiewicz**

12:15 – 13:30 **Oral Presentations:**

12:15 – 12:30 **Jarosław Tyburski** (Poland), Monika Skorupa, Marcin Gołębiewski, Krzysztof Klamkowski, Katarzyna Wójcik, Waldemar Treder, Andrzej Tretyn: Transcriptomic response to a salt stress and salt shock in leaves of sugar beet (*Beta vulgaris*, ssp *vulgaris*) and its halophytic ancestor, *Beta vulgaris* ssp. *maritima*

12:30 – 12:45 **Wojciech Rymaszewski** (Poland), Denis Vile, Christine Granier, Jacek Hennig: Coupling high-throughput phenotyping and gene expression analyses to study the natural variation of morpho-physiological and molecular responses to long-term water deficit in *Arabidopsis thaliana*

12:45 – 13:00 **Damian Gruszka** (Poland), Anna Janeczko, Michal Dziurka, Ewa Pociecha, Jana Oklestkova, Iwona Szarejko: Barley brassinosteroid mutants provide an insight into phytohormonal homeostasis under control condition and during plant reaction to drought stress

13:00 – 13:15 **Michał Rurek** (Poland), Magdalena Czołpińska, Witold Nowak, Tomasz Pawłowski, Aleksandra M. Staszak, Tomasz Spiżewski, Włodzimierz Krzesiński: Mitochondrial response to the various water deficiency conditions in three cultivars of cauliflower

13:15 – 13:30 **Marzena Kurowska** (Poland), Dorota Świergolik, Iwona Szarejko: The tonoplast intrinsic protein (TIP) gene subfamily of aquaporins in barley: structural features and expression profiles during drought stress

13:30 – 15:00 Lunch provided

Session 4: Signalling in biotic interactions

Chair: **Jacek Hennig** and **Iwona Morkunas**

15:00 – 17:00 **Plenary Lectures:**

15:00 – 15:30 **Sophien Kamoun** (United Kingdom): The long reach of the effectors of plant associated organisms

15:30 – 16:00 **Saskia Hogenhout** (United Kingdom): How bacteria hack plant development to attract insect vectors

16:00 – 16:30 Bettina Johst, Hannah Jaspar, Michael Bitterlich, Philipp Franken, **Christina Kühn** (Germany): Plant factors affecting the symbiosis between arbuscular mycorrhizal fungi and the host plant root system

16:30 – 17:00 Karolina Jarzyniak, Joanna Banasiak, Martin Di Donato, Markus Geisler, **Michał Jasiński** (Poland): Cytokinin transporter from the ABC family in legume rhizobia symbiosis

17:00 – 17:15 Coffee break

17:15 – 18:30 **Oral Presentations:**

17:15 – 17:30 **Ton Timmers** (France), Judith Fliegmann, Nikita Malkov, JulieCullimore, Jean-Jaques Bono: Nod factor perception and signal transduction during endosymbiotic interactions of *Medicago truncatula*

17:30 – 17:45 Mariola Piślewska-Bednarek, Ryohei Thomas Nakano, Kei Hiruma, Marta Pastorczyk, Suthitar Singkaravanit-Ogawa, Paul Schulze-Lefert, **Paweł Bednarek** (Poland): Analysis of gstu13 plants uncouples two putative signaling functions of indole glucosinolate metabolism products in flg22-triggered immune responses in *Arabidopsis*

17:45 – 18:00 **Agnieszka Woźniak** (Poland), Van Chung Mai, Kinga Drzewiecka, Jacek Kęsy, Łukasz Marczak, Dorota Narożna, Renata Rucińska-Sobkowiak, Waldemar Bednarski, Iwona Morkunas: The sequence of enhanced generation of *Pisum sativum* defense signalling molecules in response to pea aphid infestation

18:00 – 18:15 **Justyna Nawrocka** (Poland), Monika Skwarek, Katarzyna Jas, Magdalena Szczech, Urszula Małolepsza: The role of metabolic components, volatile compounds, PR proteins and mechanical strengthening in multilayer protection of cucumber plants against *Rhizoctonia solani* induced by *Trichoderma* atroviride TRS25

18:15 – 18:30 **Zhimin Yin** (Poland): miRNAs and mRNA targets in potato-PVY pathosystems

18:30 – 19:30 **Poster sessions 3-4**

20:30 – 00:00 **Conference Party**

Friday, 15 September 2017

Session 5: Epigenetics and chromatin regulation in plant development

Chair: **Piotr Ziółkowski** and **Marta Koblowska**

09:00 – 10:30 **Plenary Lectures:**

09:00 – 09:30 **Ian Henderson** (United Kingdom): Chromatin and recombination landscapes in plant genomes

09:30 – 10:00 **Dao-Xiu Zhou** (France): Interplay between chromatin modification and metabolism to regulate plant growth and tolerance to stress

10:00 – 10:30 Jakub Dolata, Yanwu Guo, Agnieszka Kołowerzo, Dariusz Smoliński, Grzegorz Brzyżek, Szymon Świeżewski, **Artur Jarmołowski** (Poland): Coupling of RNA Polymerase II transcription elongation with pre-mRNA splicing

10:30 – 10:45 Coffee break

10:45 – 12:00 **Oral Presentations:**

10:45 – 11:00 Daniel Buszewicz, Rafał Archacki, Antoni Palusiński, Maciej Kotliński, Anna Fogtman, Roksana Iwanicka-Nowicka, Katarzyna Sosnowska, Jan Kuciński, Piotr Pupel, Jacek Olędzki, Michał Dadlez, Aleksandra Misicka, Andrzej Jerzmanowski, **Marta K. Koblowska** (Poland): HD2C histone deacetylase binds to SWI/SNF chromatin remodeling complex and act together to regulate *Arabidopsis* heat stress response

11:00 – 11:15 **Tomasz Bieluszewski** (Poland), Weronika Sura, Mateusz Abram, Anna Bieluszewska, Łukasz Gałgański, Jan Sadowski, Piotr Ziółkowski: The impact of mutations affecting NuA4 histone acetyltransferase on the life cycle of *Arabidopsis thaliana*

11:15 – 11:30 **Maciej Lirski** (Poland), Magdalena Kroteń, Maciej Kotliński, Marta Koblowska, Andrzej Jerzmanowski: Linker histones act as powerful lock of heterochromatin activity in *Arabidopsis*

11:30 – 11:45 **Weronika Sura** (Poland), Michał Kabza, Wojciech Karłowski, Tomasz Bieluszewski, Marta Kuś-Słowińska, Łukasz Pawełoszek, Jan Sadowski, Piotr A. Ziółkowski: H2A.Z impact on gene expression in plant response to stress

11:45 – 12:00 **Karolina Chwiałkowska** (Poland), Urszula Nowakowska, Agnieszka Bielska, Anna Szałkowska, Mirosław Kwaśniewski: Progressive methylome modulation during water-deficiency conditions in barley is coupled with transcriptomic responses

12:00 – 12:10 Coffee break

Session 6: NO life without RNS/ROS

Chair: **Agnieszka Gniazdowska** and **Jolanta Floryszak-Wieczorek**

12:10 – 14:00 **Plenary Lectures:**

12:10 – 12:40 **Francisco J. Corpas** (Spain): Nitric oxide in plant peroxisomes under stress conditions

12:40 – 13:05 Tereza Tichá, Lucie Činčalová, Michaela Sedlářová, Lenka Luhová, **Marek Petřivalský** (Czech Republic): S-nitrosylation as a key component of nitric oxide redox signalling in plant development

13:05 – 13:35 **Kim H. Hebelstrup** (Denmark): An overview of the control of NO by phytoglobins in plant development and stress responses

13:35 – 14:00 Jolanta Floryszak-Wieczorek, Karolina Izbiańska, Joanna Gajewska, Jarosław Gzyl, **Magdalena Arasimowicz-Jelonek** (Poland): New insight into the fate of peroxynitrite in plants

14:00 – 15:00 Lunch provided

Session 6: NO life without RNS/ROS

Chair: **Agnieszka Gniazdowska** and **Jolanta Floryszak-Wieczorek**

15:00 – 16:00 **Oral Presentations:**

15:00 – 15:15 **Jarosław Gzyl** (Poland), Karolina Izbiańska, Jolanta Floryszak-Wieczorek, Magda Arasimowicz-Jelonek: Nitric oxide status in roots of soybean seedlings exposed to cadmium

15:15 – 15:30 **Urszula Krasuska** (Poland), Katarzyna Ciąćka, Renata Bogatek, Agnieszka Gniazdowska: Hydrogen cyanide mimics nitric oxide cellular mode of action

15:30 – 15:45 **Marta Gietler** (Poland), Małgorzata Nykiel, Sławomir Orzechowski, Joerg Fettke, Barbara Zagdańska: Proteomic analysis of S-nitrosylated proteins in wheat seedlings with different dehydration tolerances

15:45 – 16:00 **Marlena Stawska** (Poland), Ewa Walczuk, Krystyna Oracz: Interactions between light induced signal and reactive oxygen species during light dependent germination of *Arabidopsis thaliana* seeds

16:00 – 16:20 Coffee break

16:20 – 17:30 **Poster sessions 5-6**

17:30 – 18:00 **Closing Ceremony**

Saturday, 16 September 2017

One day trip, to choose from:

Białowieża Białowieża National Park, lunch

Sokółka Orthodox church – **Kruszyniany** Mosque, lunch in Tatar Jurt – Tatar cuisine
Supraśl Museum of Icons – *additional payment*, Orthodox Monastery

Conference venue:

University of Bialystok,
Faculty of Biology and Chemistry
Institute of Biology
Ciolkowskiego 1J, 15-245 Bialystok
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Keynote Lecture

8th Conference of the Polish Society of Experimental Plant Biology
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L1

Neurobiology view of higher plants

František Baluška

Institute of Cellular and Molecular Botany, University of Bonn, Bonn, Germany
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Plants live in complex environments and their survival is dependent on the reliable sampling and processing of critical biotic and abiotic information. For example, plant root navigation is based on compex sensory signal integration in the root apex zones, especially in the transition zone. This allows growing roots to search for water and nutrients, and avoid or escape dangerous situations. The exploratory nature of root apices requires large energy supplies for signaling, as well as for cell division and elongation. In the case of negative tropisms, for example the light escape and salt avoidance tropisms, roots must respond quickly and these tropisms are energetically costly. Roots use their plant-specific cognition and problem-solving apparatus which allows them to exploit heterogeneous soil for water and mineral nutrition effectively. Plant-specific memory and processing of sensory information are based on classical neuronal molecules and neurotransmitters such as glutamate, GABA, serotonin, melatonin and others.

NLP cytolysins – microbial virulence factors and host defense activators

Thorsten Nürnberger

Center of Plant Molecular Biology, University of Tübingen, Tübingen, Germany
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Members of the superfamily of necrosis and ethylene-inducing peptide 1 (Nep1) like proteins (NLPs) are found in bacteria, fungi and oomycetes. A subset of these proteins causes leaf necrosis on dicot, but not on monocot plants. NLP cytotoxicity was shown to be crucial for microbial virulence and a necrotrophic lifestyle of the producing microbe. X-ray crystallography-based analyses of two microbial NLPs revealed substantial fold conservation of these proteins with cytolytic toxins produced by marine organisms (actinoporins). Actinoporins bind to animal host sphingomyelin prior to membrane pore formation and cytolysis. While plants do not produce sphingomyelins, we show that the target site for NLP toxins is a dicot-specific glycosylated sphingolipid. Binding induces a conformational switch within NLP cytolysins thereby mediating membrane attachment and pore formation. These findings also explain the unusual host selectivity of NLP toxins.

Many NLP proteins harbor a twenty amino acid stretch (nlp20) that mediates host immune activation in *Brassicaceae* including *Arabidopsis*. This peptide is recognized by a ternary immune receptor complex comprising ligand-binding leucine-rich receptor protein RLP23 and two co-receptors, SOBIR1 and BAK1. Stimulus-dependent complex formation will be discussed along with potential use of this and related receptors in engineering crops with heightened immunity to microbial infection.

Plenary Lectures

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PL1.1

The ribosome as a metabolite sensor: Sucrose regulated protein translation and the control of metabolism and growth in plants

Sjef Smeekens

Institute of Environmental Biology, Utrecht University, Utrecht, The Netherlands
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A new paradigm in the control of plant metabolism and growth has emerged involving sequence conserved (SC) peptides encoded by upstream open reading frames (uORF) of mRNAs. Over 40 SC – peptides have already been identified and recent results suggest that each peptide responds to a specific metabolite, which mainly are end products of metabolic pathways. The result of such Metabolite-Regulated Translation (MRT) by SCuORFs is that metabolite concentration determines the level of translation of the main ORF (mORF) and thus the amount of protein produced. MRT involves stalling of ribosomes on the mRNA upstream region, which inhibits mORF translation. mORFs usually encode enzymes, transcription factors or signalling intermediates. The result is a direct and dynamic control over metabolism. We study MRT using sucrose control of translation of selected bZIP transcription factors.

These bZIPs are major regulators of metabolism and operate in the central TOR – SnRK1 – T6P axis.

A tale of two sugars – the role of trehalose 6-phosphate in sucrose signalling and homeostasis

John Lunn

Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany
@ lunn@mpimp-golm.mpg.de

Trehalose 6-phosphate (Tre6P) is an essential signal metabolite in plants that influences leaf growth and senescence, stomatal function, flowering, inflorescence architecture and embryogenesis. Tre6P closely tracks diurnal and externally imposed fluctuations in the levels of sucrose. We propose that Tre6P functions as both a signal and negative feedback regulator of sucrose levels, helping to maintain intracellular sucrose concentrations within an optimal range. This function can be compared with the insulin-glucagon system for regulating blood glucose levels in animals. In leaves, Tre6P regulates photoassimilate partitioning to sucrose during the day and the remobilization of transitory starch reserves to sucrose at night, linking both of these to demand for sucrose from sink organs. In meristems and other growing tissues, Tre6P signals the availability of sucrose for growth, influencing developmental decisions and the fate of imported sucrose. The intertwined relationship between sucrose and Tre6P is captured in the sucrose-Tre6P nexus concept. This model helps us to understand how Tre6P exerts such a profound influence on plant growth and development, and provides a framework for engineering Tre6P metabolism for crop improvement.

PL1.3

Stem cell renewal or differentiation: a plant's perspective for a crucial decision

Jose J. Sanchez Serrano

Centro Nacional de Biotecnologia CSIC, Madrid, Spain
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In all multicellular organisms, stem cells are continuously confronted with the critical decision of dividing for self-renewal or differentiating along any of several cell fate pathways. Differentiation entails the activation of developmental transcription programmes that are repressed in undifferentiated stem cells. In mammals, this involves a shift from stalled to productive transcriptional elongation of developmental genes. In the model plant *Arabidopsis thaliana*, differentiation requires the activity of the transcriptional regulator MINIYO (IYO). IYO interacts with RNA polymerase II and sustains transcriptional elongation in developing organs. Moreover, IYO is rate-limiting for differentiation and it moves from the cytosol into the nucleus in cells at the meristem periphery, possibly triggering their differentiation. However, the genetic mechanisms controlling IYO nuclear accumulation are poorly characterized, and evidence that increased nuclear IYO levels trigger differentiation is largely correlative. The IYO-interacting protein RIMA is homologous to proteins linked to nuclear import of selective cargo in yeast and mammals. The developmental phenotypes and transcriptomic changes caused by IYO or RIMA knockdown suggest a close functional relationship between the proteins. Indeed, RIMA knockdown reduces the nuclear levels of IYO and prevents its pro-differentiation activity, supporting that RIMA-dependent nuclear IYO accumulation elicits cell differentiation in *Arabidopsis*. How the IYO/RIMA complex drives RNA polymerase II into productive transcription to activate cell differentiation is unclear at this moment.

Differential phosphorylation signatures of the RNA polymerase II C-terminal tail may underlie this crucial regulatory step.

Plant responses to altered cell wall structure

Marta Derba-Maceluch[1] • Joanna Leśniewska[2] • Xiao-Kun Liu[1] • Magdalena Krzesłowska[3] • Christine Ratke[1] • Prashant Pawar[1] • Ewa Mellerowicz[1]

1 Department of Forest Genetics and Plant Physiology, Swedish Uniwersity of Agricultural Sciences, Uppsala, Sweden

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Plant cell wall is a dynamic structure, adjusting to endogenous as well as exogenous factors. It is formed by several polymers, each made separate: cellulose microfibrils – synthesized by rosette complexes in plasmalemma and secreted directly to cell wall, matrix polysaccharides– synthesized in the Golgi and exocytosed, and monomers of lignin, cutin, or suberin– delivered via different ways through plasmalemma and polymerized in cell walls. These different processes must be coordinated to ensure the biosynthesis of functional cell wall. Further, during cell expansion, the properties of cell walls must be monitored to allow 100-fold increase in size without rupture. Moreover, the wall of non-growing cells requires adjustments according to mechanical stimuli such as wind, or the body weight. All these processes require that the cell wall status is monitored.

The sensing system called the cell wall integrity (CWI) sensing was described for yeast and a similar system exists in plants as indicated by activation of basal resistance in cell wall defective mutants and in plants treated with cellulose biosynthesis inhibitors (Ellis C 2001 Plant Cell 13:1025; Cano-Delgado A 2003 Plant J34:351). Plant CWI pathway in growing tissues has two types of sensors in the plasmalemma: receptor-like kinases (RLKs) with ectoplasmic domains binding different ligands and stretch-activated Ca2+channels. Among RLKs, wall-associated kinases (WAKs) interact with pectins, and a *Catharanthus roseus* receptor-like kinase 1-like (CrRLK1-L) FERONIA with a hormone peptide – RALF (Haruta M 2014 Science 343:408). CrRLK1-Ls and WAKs act via ROP signaling, MAPK cascades, plasmalemma Ca^{2+} channels, RBOH NADPH oxidases, ROS, and ATPases, leading to wall alkalization, and ultimately to altered gene expression and SA, JA and ET signaling.

Non-growing tissues such as xylem, also exhibit CWI sensing. Mutations in secondary wall CesAs induce resistance to pathogens via different pathways than JA, ET or SA (Hernandez-Blanco C 2007 Plant Cell 19:890). Deacetylation of xylan also triggers biotic resistance using a different pathway than in primary walled cells (Pawar PM-A 2016Plant Biotechnol J14:387). One inducer could be mannooligosaccharides, which suppress secondary wall biosynthesis and stimulate growth (Benova-Kakosova A 2006 Plant Physiol142:696; Zhao Y 2013Plant J 74:473). CWI in xylem frequently triggers tyloses via ET and JA (Leśniewska J 2017 Plant Physiol 173:1409). Intriguingly, suppression of xylan biosynthesis triggers growth and reduces secondary wall formation (Biswal AK 2015 Biotechnol Biofuels8:41; Derba-Maceluch M 2015 New Phytol 205:666; Ratke et al., unpublished). Elucidation of these pathways is an urgent task for xylem biology.

Thus, CWI pathways might differ in different plant tissues. Understanding them is crucial for biotechnological improvement of woody plants, in particular when cell wall is manipulated, for example, for biorefinery applications.

PL2.1

New insights on the regulation of plasmodesmata cell walls and their consequences for plant development

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Cell wall biopolymers play essential roles in the development of plants, fungus and bacteria. A (1,3)-β-glucan polymer, named callose, accumulates around plasmodesmata to restrict intercellular communication but the mechanisms underlying this regulatory function are poorly investigated. To study the physico-mechanical properties of callose at plasmodesmata cell walls, we initiated an analysis of cellulose – (1,3)-β-glucan composite blends using 1H NMR Spectroscopy, rheology, and AFM-nanoindentation experiments. In parallel, changes in the molecular composition of cell walls, as a result of modifications in the expression of plasmodesmata proteins involved in callose synthesis/degradation, were investigated. The effects of these modifications in root developmental traits were also studied. The results indicate unexpected physical and biochemical interactions between cell walls polymers likely linked to their mechanism in the regulation of plasmodesmata permeability and transport. The research highlights the importance of the cell wall environment when considering the effect of changes in plasmodesmata on intercellular communication and organ development.

Plant:virus interactions involved in viral cell-to-cell spread

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Plant viruses and their movement proteins (MP) rely on cellular macromolecular assembly and transport pathways to target plasmodesmata (PD) for cell-to-cell movement. Our team investigates these pathways in the context of virus:host interactions at the level of gene expression and defense responses, and aims to unravel important mechanisms leading to disease. We have found that Tobacco mosaic virus (TMV) interacts with junctions between the cortical ER-actin network and microtubules (MT) for replication and uses the ER-actin network and specific myosin motor proteins for transport to PD. Replication involves the occurrence of double-stranded RNA (dsRNA), which triggers RNA silencing as well as Pattern–Triggered Immunity (PTI), and we are interested to understand how the virus takes control over these defenses to facilitate its spread. Using next generation sequencing approaches, we address the function of virus- and host-derived small RNAs (sRNAs) and their RNA targets, as well as of the viral silencing suppressor in controlling sRNA activity, during virus replication and spread. We also try to understand why certain plants react to a specific virus with disease while other plants are rather tolerant. One way to approach this question is by analysis of recovery, which refers to the occurrence of healthy leaves on symptomatic plants. We identified a virus:host interaction in *Arabidopsis* that initially produces strong developmental symptoms and achieves a tolerant state at later stages. Genetic analysis led to the identification of genes and pathways required for recovery and which may play an important role in tolerance, thus allowing plants to remain healthy in the presence of viruses in the environment.

[1] Niehl A, Wyrsch I, Boller T, Heinlein M (2016) Double-stranded RNAs induce a pattern-triggered immune signaling pathway in plants. New Phytol 211, 1008-1019

[2] Heinlein M (2015) Plant virus replication and movement. Virology 479-480, 657-671.

[3] Heinlein M (2015) Plasmodesmata: channels for viruses on the move. Methods Mol Biol 1217, 25-52 Amari K, Di Donato M, Dolja VV, Heinlein M (2014) Myosins VIII and XI play distinct roles in reproduction and transport of Tobacco mosaic virus. PLoS Pathog 10, e1004448

[4] Peña E, Ferriol I, Sambade A, Buschmann H, Niehl A, Elena SF, Rubio L, Heinlein M (2014) Experimental plant virus evolution reveals a role of microtubule dynamics and TORTIFOLIA1/SPIRAL2 in RNA trafficking. Plos One 9, e105364

[5] Peña E, Heinlein M (2013) Cortical microtubule-associated ER sites: organization centers of cell polarity and communication. Curr Opin Plant Biol 16, 763-773

[6] Kørner CJ, Klauser D, Niehl A, et al (2013): The immunity regulator BAK1 contributes to resistance against diverse RNA viruses. Mol Plant Microbe Interact 26, 152-161

PL3.1

Autophagy machinery, leaf senescence and nitrogen remobilization in *Arabidopsis thaliana*

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Processes allowing the recycling of organic nitrogen and export to young leaves and seeds are important determinants of plant yield, especially when plants are nitrate limited. Because autophagy is induced during leaf ageing and in response to nitrogen starvation, its role in nitrogen remobilization has been suspected. To investigate the role of autophagy in nitrogen remobilization, several autophagy-defective (atg) *Arabidopsis* mutants were grown under low and high nitrate supplies and labeled with 15NO – at vegetative stage in order to determine in a pulse/chase experiment the 15N partitioning in seeds at harvest (1). Results showed that nitrogen remobilization efficiency was significantly lower in all the atg mutants irrespective to biomass defects, harvest index reduction, leaf senescence phenotypes and whatever nitrogen conditions (2). It was also observed that atg mutants accumulate larger amount of ammonium, amino acid and proteins in their leaves than wild type and are depleted in sugars. Over accumulation of proteins in atg mutants occurred despite higher endopeptidase and carboxypeptidase activities can be measured in mutants. The specific over accumulation of the RPS6, RPL13 ribosomal proteins, catalase and glutamate dehydrogenase proteins, and the accumulation of peptides putatively identified as degradation products of Rubisco large subunit and chloroplast glutamine synthetase GS2 led us to conclude that incomplete chloroplast protein degradation results from autophagy defects and that protein degradation through autophagy might be selective (3). Proteomic analyses were carried on in order to identify protease activities and cargos accumulated in atg mutants.

[1] Masclaux-Daubresse C, Chardon F (2011) Exploring nitrogen remobilization for seed filling using natural variation in *Arabidopsis thaliana*. Journal of Experimental Botany 62: 2131-2142.

[2] Guiboileau A, Yoshimoto K, Soulay F, Bataillé M, Avice J, et al. (2012) Autophagy machinery controls nitrogen remobilization at the whole-plant level under both limiting and ample nitrate conditions in *Arabidopsis*. New Phytologist 194: 732-740.

[3] Guiboileau A, Avila-Ospina L, Yoshimoto K, Soulay F, Azzopardi M, et al. (2013) Physiological and metabolic consequences of autophagy defisciency for the management of nitrogen and protein resources in *Arabidopsis* leaves depending on nitrate availability. New Phytologist 199: 683-694.

The role of DNA methylation in regulating plant adaptations to low phosphate availability

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Epigenetic modifications can modulate gene expression and may be inherited through mitosis and meiosis without changes in the underlying DNA sequence, and with capacity for reversibility. These mechanisms can contribute to phenotypic plasticity in a changing environment, without having to rely on genetic variation. The role of DNA methylation in regulating or priming plant regulatory networks for low phosphate (P) availability is a potentially significant and unexplored epigenetic process that may facilitate plant adaptations to low P availability. To test this, we grew Brassica rapa R-o-18 plants hydroponically under glasshouse conditions. Plants were supplied with a full nutrient solution for 7 days. After 7 days, half the plants were transferred to a nutrient solution containing 0 μM P and the remaining plants continued to receive a full nutrient solution (225 μM P). Leaf and root tissues were then destructively harvested 24, 72 and 192 hours after the withdrawal of P from half the plants. RNA and DNA were extracted from all samples, with RNA-seq analysis carried out to identify differentially expressed transcripts. DNA from physiologically P deficient root samples collected 72 hours after P withdrawal were subject to whole genome bisulphite sequencing (WGBS) and compared with controls. The results from the RNA-seq and WGBS will be presented.

PL3.3

Metal-induced oxidative challenge: from perception and signal transduction to plant acclimation and growth

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Over the past two centuries, anthropogenic and industrial activities have led to high emissions of toxic metals into the environment at concentrations significantly exceeding those from origin. Cadmium (Cd) is a non-essential trace element, imposing a serious threat to the environment and human health. Copper (Cu) is an essential redox-active micronutrient that is involved in many physiological processes and hence is required for normal growth and development. The cellular redox state is an important determinant of metal phytotoxicity, therefore the impact of environmentally realistic Cd and Cu concentrations was investigated in *Arabidopsis thaliana* seedlings in relation to oxidative damage and signalling in roots and leaves.

Roots are in direct contact with metals in their surrounding resulting in a high root metal concentration that immediately affects metal homeostasis and sequestration and induces cellular responses. As a redox-active element, Cu can directly cause ROS (reactive oxygen species) formation, whereas this implies indirect pathways (e.g. via NADPHoxidases) under Cd stress. To avoid cellular oxidative damage, plant cells posses a well-equipped antioxidative defence system to maintain the cellular redox state. Both the involvement of enzymatic (e.g. superoxide dismutases) and antioxidant metabolites (e.g. glutathione and ascorbate) are investigated in wildtypes and mutants under metal stress with a focus on metal-specific alterations.

Multiple studies emphasize the existence of extensive crosstalk between different signal transduction pathways in plant cells. Because hydrogen peroxide (H_2O_2) production is an immediate response to increased Cd- or Cu-exposure, it might be a key molecule that can trigger signal transduction events after plant metal exposure. *Arabidopsis* wild-type and signal transduction (e.g. MAPKinase, ethylene..) mutant seedlings are kinetically exposed to metal stress and investigated for metal sensitivity and the interconnection with the cellular redox state.

In conclusion, the metal-induced oxidative challenge is strongly dependent on the chemical properties of the metal and the plant organ considered. The stress intensity determines its involvement in downstream responses in relation to oxidative damage or signalling that ultimately defines plant acclimation and growth.

The long reach of the effectors of plant associated organisms

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Plants are closely associated with a variety of living organisms. Therefore, understanding how plants interact with mutualistic and parasitic organisms is essential for a comprehensive understanding of the biology of plants. The field of plant–biotic interactions has coalesced around an integrated model. Major classes of molecular players both from plants and their associated organisms have been revealed. These include plant immune receptors as well as apoplastic and host-cell-translocated (cytoplasmic) effectors of the invading organism. This talk focuses on effectors, molecules secreted by plant-associated organisms that alter plant processes. Effectors have emerged as a central class of molecules in our integrated view of plant-microbe interactions. Their study has significantly contributed to advancing our knowledge of plant hormones, plant development, plant receptors, and epigenetics. Many pathogen effectors are extraordinary examples of biological innovation; they include some of the most remarkable proteins known to function inside plant cells. I will review some of the key concepts that have emerged from the study of the effectors of plant-associated organisms.

PL4.2

How bacteria hack plant development to attract insect vectors

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Phytoplasmas are obligate bacterial parasites that inhabit the vascular tissues of plants and are transmitted by sap-feeding insect vectors. These parasites induce dramatic changes in plant development, including proliferation of stems (witch's brooms) and the reversion of flowers into leaf-like structures (phyllody). My group has shown that phytoplasmas generate these disease symptoms via the production of an arsenal of virulence proteins, named SAPs, which interact with and promote the degradation of a diverse range of plant transcription factors, including homeodomain proteins.

Interestingly, these SAPs also convert plants into more attractive hosts for feeding and egg laying by phytoplasma insect vectors. Thus, phytoplasma virulence proteins have evolved to interfere with key plant developmental processes and simultaneously promote the fitness of sap-feeding insect vectors on which phytoplasmas depend for spread in nature.

Plant factors affecting the symbiosis between arbuscular mycorrhizal fungi and the host plant root system

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Mycorrhizal plants benefit from the fungal partners by getting better access to soil nutrients like phosphate. In exchange, the host plant supplies carbohydrates to the fungus. The additional carbohydrate demand in mycorrhizal plants was shown to be partially balanced by higher CO_2 assimilation and increased C metabolism in shoots and roots. In order to test the role of sucrose transport for fungal development in arbuscular mycorrhizal (AM) tomato, transgenic plants with down-regulated expression of sucrose transporter genes were analyzed. Plants carrying an antisense construct of the sucrose transporter SlSUT2 repeatedly exhibited increased mycorrhizal colonization and the positive effect of plants to mycorrhiza was abolished. Grafting experiments between transgenic and wild type rootstocks and scions indicated that mainly the root-specific function of SlSUT2 has an impact on colonization of tomato roots with the AM fungus. Localization of SlSUT2 to the periarbuscular membrane indicates a role in retrieval of sucrose from the periarbuscular matrix into the plant cell thereby affecting hyphal development. Screening of an expression library for SlSUT2-interacting proteins revealed interactions with candidates involved in brassinosteroid (BR) signaling or biosynthesis. Interaction of these candidates with SlSUT2 was confirmed by bimolecular fluorescence complementation (BiFC).

Tomato mutants defective in BR biosynthesis were analyzed with respect to mycorrhizal symbiosis and showed indeed decreased mycorrhization. In addition, tobacco and tomato wild type plants were treated with epi-brassinolide or alternatively, with an inhibitor of brassinosteroid biosynthesis. Both treatments significantly affected mycorrhization parameters of wild type roots after inoculation with the AM fungus *Rhizophagus irregularis*. This suggests that BRs affect mycorrhizal infection and colonization.

SlSUT2 forms a protein-interaction network including the membrane steroid binding protein MSBP1, the BRI1-associated receptor kinase BAK1, and the BR biosynthetic sterol reductase DIMINUTO1/DWARF1 (DIM1). We therefore investigate whether SlSUT2 interacting proteins are directly or indirectly involved in the establishment and maintenance of functional symbiotic interaction. Mycorrhization experiments were performed with wild type plants and with transgenic tobacco plants with up- or down-regulation of genes encoding SlSUT2 interaction partners. We suggest that SlSUT2 being inducible by several biotic interactions, constitutes a part of the plant defense strategy via its sucrose retrieval function.

PL4.4

Cytokinin transporter from the ABC family in legume rhizobia symbiosis

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Legume plants have a unique ability to form intimate, intracellular associations with nitrogen-fixing rhizobial species. An exchange of specific signalling molecules between host and microsymbiont constitutes the first step required both, for bacterial infection and the formation of new, root-derived organs, called nodules. Since these two events occur in different root tissues, namely in the rhizodermis and in the underlying cortical cells, their strict regulation and coordination has to exist. An essential role of plant hormones, especially auxins and cytokinins, in modulation of nitrogen-fixing symbiosis has been widely postulated. Activation of cytokinin signalling pathway in the root cortex, is thought to be a key event of the infection process. As a consequence, bioactive cytokinins are biosynthesized in root susceptible zone, and they are a part of positive feedback loop triggering cortical cell division and sustaining nodule organogenesis. Intriguingly, rhizodermal cytokinins are being suspected to act as a mobile signal joining outer and inner root tissue responses. However, dedicated transporters, mediating cytokinin translocation between rhizodermis and root cortex, as well as within cortical cell layers await discovery.

We addressed a question about a presumed role of selected, root expressed full-size ABC (ATP-binding cassette) transporter from the G subfamily in modulation of nitrogen-fixing symbiosis, in model legume plant *Medicago truncatula*. Expression of this transporter is significantly up-regulated upon symbiotic bacteria and isolated NF, as well as cytokinin treatment. Upon *Sinorhizobium meliloti* infection, a promoter activity of the gene of interest is mainly in the root cortex. Additionally, according to recently published tissue-specific transcriptome data, transcript of investigated ABCG transporter accumulates significantly in rhizodermis upon NF treatment. Importantly, analyzed protein localizes to the plasma membrane and exports bioactive cytokinins in an ATP-dependent manner. Disruption of this transporter in Tnt1 mutant line as well as in silenced material impaired nodulation efficiency in comparison with wild-type control plants. In light of the presented data, a putative role of ABC transporters in symbiotic interactions through phytohormone translocation can be proposed.

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Chromatin and recombination landscapes in plant genomes

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Plant genomes can be broadly divided in euchromatin and heterochromatin, which are cytologically defined, and generally correspond to gene versus transposon rich regions. We now appreciate that epigenetic modifications of the genome, for example DNA methylation, underlie differentiation of the genome into these different chromatin states. In addition to associating with different patterns of transcription, it is well appreciated that plant heterochromatin is typically also silenced for recombination. In many of the large grass genomes, including wheat, the majority of the chromosomes consist of non-recombining expanses of heterochromatin, which can cause significant limitations for breeding. Our research investigates the genetic and epigenetic factors that shape recombination in plant genomes. I will present new data where we have profiled *Arabidopsis* recombination factors genome-wide, which has revealed hotspots associated with genes, and also surprisingly, DNA transposons. I will discuss the implications of these finds for plant genome evolution and the relationship between genes and transposons. I will also present new work profiling chromatin states in the hexaploid wheat genome and show how this correlates with recombination. In summary this talk will expire the relationships between chromatin, transcription and recombination, with implications for the stability of plant chromosomes and how we improve crops via breeding.

PL5.2

Interplay between chromatin modification and metabolism to regulate plant growth and tolerance to stress

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Recent data suggest that metabolic flux may impact gene expression through involvement of key metabolites in epigenetic modification of chromatin. For instance, the activity of many chromatin – modifying enzymes is directly dependent on key metabolites such as acetyl-coenzyme A for histone acetylation, S-adenosylmethionine for DNA and histone methylations, and NAD+ for histone deacetylation, among others. Conversely, chromatin regulators (e.g. histone deacetylases) are also involved in posttranslational modification and regulation of metabolic enzymes. This collaborative program between epigenetic dynamics and metabolism has to be further interconnected with environmental cues, which may optimize plant adaptive responses and growth. We will present our recent data in the field and discuss how epigenetic regulators are involved in the process where plant metabolic flux impacts gene expression to optimize plant growth and response to stress.

Coupling of RNA Polymerase II transcription elongation with pre-mRNA splicing

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The interconnection between transcription and splicing is a subject of intense study. We reported that *Arabidopsis* homologue of spliceosome disassembly factor NTR1 is required for correct expression and splicing of DOG1, a regulator of seed dormancy. Global splicing analysis in atntr1 mutants revealed a bias for downstream 5' and 3' splice site selection and an enhanced rate of exon skipping. A local reduction in RNA polymerase II (Pol II) occupancy at misspliced exons and introns in atntr1 mutants suggests that directionality in splice site selection is a manifestation of fast Pol II elongation kinetics. In agreement with this model, we found AtNTR1 to bind target genes and co-localise with Pol II. A minigene analysis further confirmed that strong alternative splice sites constitute an AtNTR1-dependent transcriptional roadblock. Plants deficient in Pol II endonucleolytic cleavage showed opposite effects for splice site choice and Pol II occupancy compared to atntr1 mutants, and inhibition of Pol II elongation or endonucleolytic cleavage in atntr1 mutant resulted in partial reversal of splicing defects. We propose that AtNTR1 is part of a transcription elongation checkpoint at alternative exons in *Arabidopsis*.

PL6.1

Nitric oxide in plant peroxisomes under stress conditions

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Peroxisomes are single membrane-bound subcellular compartments present in almost all types of eukaryotic cells. The basic enzymatic constituents are catalase and H_2O_2-producing flavin oxidase which illustrates its prominent oxidative metabolism [1]. Peroxisomes are also characterized by metabolic plasticity, as their enzymatic content can vary according to the organism, cell/tissue type, development stage and environmental conditions. In plant cells, these organelles house a large number of antioxidative enzymes, such as catalase, superoxide dismutase (SOD), components of the ascorbate-glutathione cycle and several NADP-dehydrogenases, involved in different functions [2]. Nevertheless, accumulating data have shown that plant peroxisomes have the capacity to generate nitric oxide (NO) through an L-arginine-dependent nitric oxide synthase (NOS) which strictly depends on NADPH and requires calmodulin (CaM) and Ca^{2+}. Furthermore, peroxisomal NO, together with other related RNS such as peroxynitrite, has been shown to participate in the response to abiotic stresses such as salinity, arsenic, cadmium or drought indicating that peroxisomes have an active nitro-oxidative metabolism [3,4]. As part of this metabolism, the numbers of peroxisomal proteins which undergo NO-mediated post-translational modifications such as nitration and S-nitrosylation have been also demonstrated under physiological and stress conditions [5]. All these data demonstrate the existence of a very dynamic nitro-oxidative metabolism into plant peroxisomes indicating the relevance of these organelles.

[1] Corpas FJ (2015) Plant Biol (Stuttg) 17:1099-1103.

[2] Leterrier et al (2016) Protoplasma 253: 403-415.

[3] Corpas et al (2009) Plant Physiol 151: 2083-2094.

[4] Corpas & Barroso (2014) Ann Bot 113: 87-96

[5] Palma et al (2015) Ann Bot 1163: 627-636.

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S-nitrosylation as a key component of nitric oxide redox signalling in plant development

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S-nitrosylation, as the attachment of nitroso group to protein cysteine thiols, has emerged as a new type of ubiquitous protein post-translational modification within the complex network of nitric oxide bioactivity. S-nitrosylation as a convergence of signalling pathways of reactive nitrogen and oxygen species impact protein functionality, stability and cellular localization in cells. Moreover, low-molecular weight S-nitrosoglutathione represents a relatively stable and mobile reservoir of NO bioactivity. Structure and activity of multiple plant proteins involved in the transduction of plant hormone signals, control of carbohydrate metabolism, induction of apoptosis and plant responses to stress stimuli is known to be regulated by reversible S-nitrosylation in vivo. S-nitrosoglutathione reductase is considered a key enzyme of the regulation of intracellular levels of S-nitrosoglutathione and indirectly also of protein S-nitrosothiols. It has a crucial role in the maintenance of balanced levels of reactive nitrogen species and participates in the control of cellular redox state. Recent research in model plant systems has been focused to the identification of endogenously S-nitrosylated proteins in unstressed plants and S-nitrosylation patterns in plants exposed to different abiotic and biotic stress factors. Comparative analysis of the plant S-nitrosoproteome under normal and stress conditions represents a valuable tool to obtain more insights to the role of NO in the signalling pathways of plant development.

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PL6.3

An overview of the control of NO by phytoglobins in plant development and stress responses

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Phytoglobins (a.k.a. plant hemoglobins) are prominent scavengers of NO in plant cells and therefore important players in the equilibrium between NO formation and NO removal. In particularly, cell- and tissue-specific expression of phytoglobins serves to control local levels of NO in plant cells. Three classes (1 to 3) of plant phytoglobins have been identified. Functions of the class 3 type are mostly unknown, whereas the class 1 and 2 plant phytoglobins have been associated with modulation of NO. For this, transgenic over-expression or silencing of plant phytoglobins have proven to be excellent tools to modulate levels of NO with the intention of studying biological functions of NO and its crosstalks with other RNS/ROS and with hormones in development and in stresses. Under normoxic conditions the gene expression pattern of plant phytoglobins is related to cell- and tissue-specific regulation of NO levels in responses to different environments and developmental stages. However, during hypoxic stress conditions class 1 plant phytoglobin gene expression is highly upregulated in all plant tissues, where plant phytoglobin plays a role in reducing nitrogen loss through NO emission and to maintain energy status. NO is a central molecule in the distinct signaling pathways of the responses towards necrotrophic or biotrophic pathogens, and plant phytoglobin gene overexpression or gene silencing interferes with progression of infections in very different types of plant-pathogen interactions in a pattern related to modulation of NO levels.

New insight into the fate of peroxynitrite in plants

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Peroxynitrite ($ONOO^-$) as the product of the diffusion-controlled reaction of nitric oxide (NO) and superoxide is a short-lived oxidant and nitrating species. It is widely accepted that an enhanced $ONOO^-$ formation contributes to oxidative and nitrosative stress in various biological systems including plants. However, the mode of $ONOO^-$ action in plant cells seems to be rather different than the well-recognized equivalent in animals. Firstly, in contrast to animal cells, $ONOO^-$ itself seems to be less destructive for plants. Secondly, the accumulating data indicate that $ONOO^-$ might be continuously formed in healthy tissues as an inevitable event of plant cell metabolism. Thirdly, plant cells are adapted to detoxify $ONOO^-$ excess by a broad range of its decomposition mechanisms. Based on these assumptions, $ONOO^-$ formation could provide an important regulatory loop for NO bioactivity under both plant physiological and pathophysiological states provoked by environmental stresses.

The most important mechanism of how $ONOO^-$ can regulate plant responses to developmental and stress stimuli is the modification of tyrosine residues of proteins. In this way it can behave as a positive modulator of the redox regulation within the cell. Importantly, besides proteins it can modify lipids and oligonucleotides as well. Recent results from our laboratory have showed that plant immunity is accompanied by a specific $ONOO^-$-dependent RNA modifications, indicating a novel function of $ONOO^-$ in plants. Performed analysis revealed that nitration of particular mRNAs is not a random process and could contribute to the post-transcriptional regulation of defense gene expression tuned with programmed cell death.

Taken together, the old and new ideas on the chemical, physiological and pathophysiological features of $ONOO^-$ will be discussed to better understand its fate in plant responses to adverse environmental conditions.

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Oral Presentation

8th Conference of the Polish Society of Experimental Plant Biology
Communication in plants: from cell to environment

OP1.1

Layers of growing plant cell walls differ in stiffness and/or in-plane tensile stress

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In living cells of a number of plant tissues, cell wall layers facing the protoplast show a pattern of more or less regular folds when the tissue is isolated and plasmolysed, i.e. when the tensile stress that exists in the wall plane *in situ* is removed. The folds formation is a reversible process and was postulated to take place due to buckling of the inner cell wall layers. We analyze the phenomenon quantitatively for the epidermis or collenchyma of cylindrically-shaped elongating organs: etiolated sunflower (*Helianthus annuus*) hypocotyl; barley (*Hordeum vulgare*) coleoptile; and dandelion (*Taraxacum officinale*) peduncle. The empirical results are used as input data for the computation model where the cell wall is represented by plates embedded in an elastic medium and buckling is due to differences of mechanical properties between the plates. The model shows that both differences in the in-plane initial stress and in Young's modulus of the plates generate fold formation. Comparison of the fold shapes obtained from the model with that of the examined cell walls supports the postulate that differences in in-plane tensile stress of the wall layers *in situ* lead to buckling of the growing cell wall layers after the stress removal.

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Expression of Auxin Response Factors (ARF) ARF2, ARF3, ARF4 changes over space and time in yellow lupine

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Auxin is one of the most powerful phytohormones – it orchestrates plant development and morphogenesis from fertilization and embryogenesis to fruit set and ripening, it is involved in response to biotic and abiotic stresses, as well as in tropisms. Such pleiotropic effect is possible due to changes in: (i) patterns of auxin concentration and (ii) patterns of expression of auxin signal transduction elements over space and time (i.e. these parameters are characteristic for a given tissue at a given time). One of the elements of auxin signal transduction pathway, Auxin Response Factors (ARFs), are transcription factors that function only in the presence of auxin. ARF2, ARF3 and ARF4 are transcription repressors, and take part in: hypocotyl elongation, leaf, flower and inflorescence morphogenesis, time of flowering, seed formation, organ abscission, senescence (ARF2), pistil morphogenesis, lateral root formation (ARF3), establishment of organ polarity (ARF3 and ARF4) and fruit ripening (ARF4) (reviewed in [1]). Results of our previous research ([2]; unpublished data) showed, that these ARFs may be engaged in vegetative and generative development in yellow lupine.

The aim of or study was to describe patterns of ARF2, ARF3 and ARF4 expression in various tissues and developmental stages of yellow lupine. For this purpose, we cultivated plants in phytotron and 27, 36 (vegetative stage) and 48 (generative stage) days after germination. We collected the following tissues: roots, root nodules, cotyledons, leaves, leaf pedicels, hypocotyls, apical buds, and only from 48 day-olds: inflorescence stem, axillary flowers, flowers (separately from all four whorls). Then we homogenized the samples, isolated total RNA, synthesised second strand in reverse transcription and finally performed qPCR analysis. The expression of mentioned ARF genes varied among the tissues and time points, strongly suggesting their involvement in developmental processes in yellow lupine.

We envision, that further research will give us a tool for precise fine-tuning expression of auxin signal transduction elements and/or auxin concentration that will lead to increase in grain yield of yellow lupine and will encourage the farmers to sow this underrated plant.

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[1] Li, S.B., Xie, Z.Z., Hu, C.G., Zhang, J.Z. (2016) A review of Auxin Response Factors (ARFs) in plants. Front. Plant Sci. 7: 47.

[2] Glazińska, P., Wojciechowski, W., Kulasek, M., Glinkowski, W., Marciniak, K., Klajn, N., Kęsy, J., Kopcewicz, J. (2017) De novo transcriptome profiling of flowers, flower pedicels and pods of *Lupinus luteus* (yellow lupine) reveals complex expression changes during organ abscission. Front. Plant Sci. 8:641.

OP1.3

The role of CUL4-related proteasomal proteolysis in germinating *Arabidopsis thaliana* seeds

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Seed dormancy and germination involve coordinated series of processes resulting in the biosynthesis and degradation of defined cellular components. Moreover, a wide range of endogenous factors, such as: phytohormones (i.e. gibberellin – GA, abscisic acid – ABA) and reactive oxygen species (ROS) regulate processes which occur in the seed. Also environmental factors (i.e. light and temperature) has an influence on seeds germination. It is known, that the signal induced by light stimuli interacts with metabolic and/or signaling pathways of phytohormones requiring several changes at the biochemical and molecular levels within the plant cell. It is postulated that proteolysis of specific proteins by 26S proteasome is one of the key mechanisms regulating seed related events. It is known that the decisive step to identify the protein by 26S proteasome is the ubiquitination of the target-substrate subjected further for degradation. This process is carried out in cells by various types of ubiquitin ligases, among which CULLIN4 (CUL4) is one of the key enzyme. The CUL4 belongs to the E3 type ubiquitin ligase, which together with other regulatory proteins form a complex responsible for identification of the substrate protein. It is known that in plants, CUL4 and proteasome are involved in many different biological processes regulating: the level of phytohormones, DNA repair or cell division during a light stress in photosynthetic tissues. However, there is still little known about its function in germinating seeds (not photosynthetic tissue). Therefore, the aim of the present study was to characterize the biological function of CUL4-related complex and proteasomal proteolysis in light-dependent germination of *Arabidopsis thaliana* (Col-0 ecotype) seeds. The *A. thaliana* dormant (unable to germinate in darkness, at 25 oC) and non-dormant (after-ripened) seeds (or isolated embryos) were used in biological assays in light or darkness, in the presence of water or MG132 (an inhibitor of the proteasome activity). The obtained results showed that the response of *A. thaliana* seeds to the presence of MG132 dependent on the dormancy level. To verify, if CUL4-related proteasomal proteolysis is involved in modulation of light-dependent germination, the qRT-PCR analysis of genes coding particular elements of the proteasome and CUL4-based E3 ubiquitin ligase complex were performed in wild type *A. thaliana* seeds imbibed in various light conditions. Additionally, the analysis of the amount of 26 proteasome and ubiquitinated proteins (by Western blot method), as well as the activity of the proteasome complex (using the polyacrylamide in gel fluorescence measurement method) were also performed. The obtained novel results indicated that CUL4-based complex may play an important role in proteasomal proteolysis in regulation of light-dependent germination of A. thaliana seeds and highlight the importance of ubiquitination of specific proteins in this process.

The SWI/SNF ATP-dependent chromatin remodeling complexes are involved in the control of metabolic processes in plants and human

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The SWI/SNF – type ATP-dependent chromatin remodeling complexes (CRCs) are conserved from fungi to mammals and plants. SWI/SNF CRCs regulate the DNA accessibility by control of chromatin structure, activity and organization. The SWI/SNF impairment causes embryo lethality or severe defects in development, including carcinogenesis in animals. Recent study indicated that SWI/SNF complexes play important role in control of various regulatory processes like i.e. hormone signaling pathways and their crosstalk in both human and *Arabidopsis*. Furthermore, it has been reported that different classes of SWI/SNF complexes are involved in regulation of specific processes. Here we show that SWI/SNF CRCs inactivation leads to the altered expression of genes related to various metabolic processes and consistently strongly affects sugar metabolism and aminoacid biosynthesis in *Arabidopsis*. We also demonstrate the existence of direct interaction between SWI/SNF CRCs and key proteins involved in the control of metabolism which is evolutionary conserved in plants and human. This collectively suggests an important role of SWI/SNF CRCs in proper regulation of metabolic processes in both human and plants. Additionally, our findings indicate that metabolic alterations observed in some cancers may be caused by the impairment/loss of SWI/SNF CRCs activity. Last but not least, our study justify using *Arabidopsis thaliana* as attractive and valuable model for searching for common molecular mechanisms controlling highly conserved regulatory processes in Eukaryotes.

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OP2.1

Symplasmic communication during development of *Cyathea delgadii* somatic embryos

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Somatic embryogenesis is used as a model system for studying mechanisms controlling embryo development at the morphological, physiological and molecular levels because of many similarities with zygotic embryogenesis. However, all the research conducted so far has focused on plant species belonging to spermatophytes. Recently, somatic embryogenesis in the tree fern *Cyathea delgadii* has been reported (Mikuła et al. 2015). In this model, a single epidermal cell of a stipe explant differentiates into somatic embryo. The origin of the embryo (just one cell) is an advantage because each step of embryogenesis can be followed directly. Moreover, even formation of embryos at the earliest stage of development is well visible and can be tracked easily. It is worth emphasizing that the development of the globular embryo structure in majority of spermatophytes mimics that of zygotic embryo. In contrast to fern zygotic embryo, first embryogenic divisions in somatic embryogenesis of *C. delgadii* (about 10-11) are perpendicular to the polar axis of the stipe explant. Hence this model system can be an interesting system to study symplasmic communication, as a way of information exchange through plasmodesmata (PDs) between cells. Hormones, transcription factors, and different classes of RNA are able to move by PD. Thus, PDs participate in the coordination of plant growth and its development (Wróbel-Marek et al., 2015). In the case of somatic embryogenesis most of symplasmic communication studies have been performed on plants belonging to spermatophytes, and it is unexplored in ferns. Symplasmic communication during somatic embryogenesis of *C. delgadii* was examined with the use of low-molecular weight fluorochromes (HPTS, HPTSA, FDA). Explants were examined under confocal and epifluorescence microscope. Patterns of fluorochromes distribution changed during the culture and stages of somatic embryogenesis.

[1] Mikuła A., Pożoga M., Tomiczak K., Rybczyński J.J (2015) Somatic embryogenesis in ferns: a new experimental system. Plant Cell Rep. 34 (5): 783-794

[2] Wróbel-Marek J., Kulińska-Łukaszek K., Kurczyńska E.U (2015) Komunikacja symplastowa i jej rola w rozwoju roślin. Post. Biol. Kom. 42 (3): 573-594

The conducting parenchyma cells – an important element of symplasmic transport inside feather moss stems

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Feather mosses serve as important regulatory organisms for many ecological processes in boreal forests, and are generally thought to be ectohydric species capable of external transport of water and nutrients using overlapping concave leaves. However, surprisingly little is known about their ability to transport solutes internally, and there is a lack of understanding of the cells specialized in symplasmic and apoplasmic communication inside their stems.

We studied the pathways of the short- and long distance transport in stem gametophores of feather mosses, e.g. *Pleurozium schreberi* and *Hylocomium splendens* to understand the mechanism responsible for water and solute transport in these species. We showed that the stems of feather mosses are filled with vertically-orientated conducting parenchyma cells which are connected by numerous plasmodesmata. These cells further exhibit characteristic ultrastructural features that facilitate cell-to-cell transport. Through the use of loading experiments with the symplasmic tracers (e.g. the CFDA and HPTS tracers) we confirmed that the conducting parenchyma cells transport solutes efficiently via plasmodesmata. Our study provided, for the first time, direct evidence of internal solute transport in *P. schreberi* and *H. splendens* stem gametophores and demonstrated a functional role of conducting parenchyma cells for symplasmic communication in these species.

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OP2.3

3D visualization of thylakoid membrane development

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Chloroplast biogenesis is a complex process integrated with plant development, leading to fully differentiated and functionally mature plastids. At the structural level of chloroplast biogenesis, the regular network of paracrystalline prolamellar bodies (PLBs) and the flattened porous membranes of prothylakoids develop into the chloroplast thylakoids. Three-dimensional reconstruction is required to provide more complete understanding of this transformation.

We used electron tomography and confocal microscopy to reconstruct the process of structural membrane transformation during the etioplast-to-chloroplast transition in runner bean (*Phaseolus coccineus*). We point out the importance of particular chlorophyll-protein complex components in the membrane appression during subsequent stages of chloroplast biogenesis.

We provide 3D models of the bean chloroplast biogenesis allowing spatial reconstruction of the internal membranes of the developing chloroplast and visualize the transformation from the tubular arrangement to the linear system of parallel lamellae.

The results show that the transformation of PLBs consists of the untwining of tubules from the PLB structure in a continuous process, without dispersion to vesicles. The tubular structure of the PLB transforms directly into flat slats that eventually form grana. We demonstrate that grana membranes, from the beginning of their formation, associate with stroma thylakoids in a helical way. The main structural stages of chloroplast internal membrane biogenesis are presented in a movie showing time development of the chloroplast biogenesis as a theoretical dynamic model of this process.

ET analysis was performed in the Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology on High-Performance Biology Transmission electron microscope JEM 1400 (JEOL Co., Japan, 200 equipped with tomographic holder and 11 Megapixel TEM Camera MORADA G2 (EMSIS GmbH, Germany)

Chiral events in the development of *Physcomitrella* gametophores

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Apical growth and the architecture of bryophyte gametophytes for a long time have been of interest to classical plant anatomy and morphology, but recently, with genetically well defined, new model plant *Physcomitrella patens* at hand, also to experimental biology. In light of this, it is surprising how little is known, about chiral aspects of moss development. In this study we report the unknown manifestation of direction sensing in orthotropically growing gametophores of *Physcomitrella patens*. Their three dimensional (3D) growth proceeds through the activity of tetrahedral apical cell dividing parallel to the lateral three of its four faces in a chiral manner. The cell segments (merophytes) cleaved by the apical cell give rise to the leaves. Their distribution is sometimes claimed to be similar to that of higher plants but in mosses is strongly affected by clonal relationship between the leaves and merophytes – a condition nonexistent in leafy sporophytes. Chiral events associated with the development of gametophores indicate that their configuration is not selected randomly.

OP3.1

Multicolour FISH-based analysis of the origin of mutagen-induced micronuclei in *Brachypodium distachyon*

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Plant bioassays are commonly used to assess chromosome rearrangements induced by environmental pollutants and mutagens. *Brachypodium distachyon* has many qualities that can make it a useful model in mutagenesis to analyse "hot spots" of DNA damage. Among them are small nuclear genome (~350 Mb) with low number of chromosomes (x=5) and the availability of BAC-libraries of *B. distachyon* nuclear DNA. It is also characterised by a small physical stature, short life cycle, undemanding growth requirements and self-fertility.

The application of modern cytogenetic techniques, such as fluorescent in situ hybridization (FISH) permits more detailed analyses of chromosome aberrations and micronuclei. Here, we applied the fluorescence in situ hybridization (FISH) with four different DNA probes (5S rDNA, 25S rDNA, *Arabidopsis*-type (TTTAGGG)n telomeric sequence and *Brachypodium* originated centromeric BAC (Bacterial Artificial Chromosome) clone CB33J12) in order to analyse the micronuclei formation in *Brachypodium* root tip cells that were subjected to the chemical clastogenic agent – maleic hydrazide (MH). Ten different types of micronuclei characterised by the presence or absence of specific FISH signal(s) were distinguished. We demonstrated that most of the MH-induced micronuclei originated from the distal regions of chromosomes. Furthermore, for the analysis of the distribution of mutagen-induced chromosome breaks we used FISH with selected single-copy chromosome-specific BAC clones. Our findings demonstrate a promising potential of *B. distachyon* to be a valuable model to analyse the detailed effects of various genotoxic agents on the plant nuclear genome and extend the understanding the mechanisms of chromosomal aberrations.

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Aminoacids motifs responsible for activity properties of S1/P1-like nucleases involved in plant PCD

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Programmed cell death (PCD) is one of the most important cell process, plays crucial role in morphogenesis, defense responses to pathogens and other stress condtidions. Although in animals and plants, degradation of cellular DNA and RNA is a inherent step of PCD, role and mechanism of this process it's different. Previous studies have demonstrated that S1/P1 nucleases are mainly responsible of nucleic acids degradation in plants. As a result of their high homology, to well described fungus nucleases S1 and P1 with catalytic activity in low pH and in presence of zinc-ions, it was assumed that the same conditions occurs during hydrolysis of the plant's DNA and RNA. In spite of this, our research exhibits that plant S1/P1 nucleases have a high diversity of the catalytic requirements. Some of them demonstrate activity in neutral pH and in presence of calcium or manganese ions, while low pH and zinc ions inhibit their activity. These information could lead to described a exact step of PCD and when degradation of nucleic acids occurs. To better understand structures features responsible for enzymatic properties of plants S1 nucleases, we performed a directed mutagenesis of regions close to activity center of ENDONUCLEASE 4 (AT4G21585) and examined influence of this mutations synergistically or independently on activity properties of enzyme.

OP3.3

Apoplast markers of cell differentiation – spatio-temporal analysis of the autografted *Arabidopsis* seedlings

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Grafting means joining excised tissues together (from the different or the same plants) in a manner that, if successful, permits two sets of vascular tissues become one. During this process multiple changes in cell fate take place, such as dedifferentiation, redifferentiation or transdifferentiation. Differentiation occurs within tissues from both parts of a grafted plant and leads to establishment of vascular tissue between scion and stock.

The role of cell walls is to provide shape and control cell growth. They also serve as a way for intercellular transport of substances and take part in communication between cells. Cell walls are composed of polysaccharides (cellulose, hemicelluloses and pectins), structural and enzymatic proteins, phenolic and lipid substances. Most of these components is classified as constitutive, what means that they are present in walls of each type of a plant cell. However, when differentiation starts, rearrangements in the composition and/ or structure of wall occur, depending on the final function of the differentiating cell.

The main aim of the study was to verify whether selected apoplast components can be considered as markers for particular cell differentiation events, triggered by grafting procedure. The subsequent regeneration stages of autografted *Arabidopsis* seedlings, wild type and two mutants: qua2-1 (exhibiting low amount of homogalcturonan) and xxt1xxt2 (exhibiting no xyloglucan), were mounted, sectioned and immunostained. For immunohistochemical analysis we applied a broad set of monoclonal antibodies raised against pectic (LM19, LM20, LM5, LM6, LM8), hemicellulose (LM15, LM21, LM10), extensin (JIM11, JIM12, JIM20) and arabinogalactan proteins (LM2, JIM8, JIM13, JIM16) epitopes.

There were no significant differences in regeneration of wild type and xxt1xxt2 seedlings, whereas efficiency of grafting qua2-1 mutant was about 40%. Moreover, the results from immunocytochemical analysis show that epitopes of low methyl-esterified homogalacturonan, arabinogalactan proteins and extensins may be involved in establishing graft union. Particular epitopes from each group of components (pectins: LM5, LM6; hemicelluloses: LM10; arabinogalactan proteins: JIM8, JIM13, JIM16; extensins: JIM11, JIM12, JIM20) can be suggested as markes for tracheary elements differentiation. These findings provide new insights into the role of apoplast components during plant cell differentiation and, in a wider sense, during regeneration stages of grafted seedlings.

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Plant cell wall in Pb accumulation and tolerance

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Lead, one of the most abundant and hazardous trace metals affecting living organisms, has been commonly detected in plant cell walls (CWs) including some tolerant plants, mining ecotypes as well as hyperaccumulators. The aim of our studies was to examine the role of CW in plant defense strategy to this metal. Experimental objects were tip growing cells of *Funaria hygrometrica* (Hedw.) *protonemata, Populus tremula* x *tremuloides* (Michx.) and *Arabidopsis* thalana (L.) root hairs, diffuse growing cells – *Populus* and *Arabidopsis* root tips and *Lemna trisulca* (L.) fronds. The material was exposed to aqueous solution of Pb at concentration 1mM, during 4h. Control – were the plants cultured in the same time in distilled water.

In all analyzed plant objects and cells CW was the compartment which accumulated large amounts of Pb and being commonly remodeled in response to this metal. Cell wall remodeling included: (1) increase of low-methylesterified pectins level (up to 40%), essential CW compound in binding, accumulation and compartmentalization of Pb, (2) appearance of callose which forms a physical barrier for Pb migration, and Pb entering the protoplast (3) increase of CW thickness, (4) formation of local CW thickenings abundant in low-methylesterified pectins, callose and accumulating large amounts of Pb.

Simultaneously, in *Populus* root tips exposed to Pb, the activity of pectin methylesterase (PME; enzyme responsible for low-methylesterified pectins formation) activity markedly increased and higher level of PttPME1 (one of the genes encoding PME) transcript occurred. The transcript level was especially high near the sites of CW thickenings formation such as cell junctions or CW lining intercellular spaces. Elevated activity of PttPME1 and PME resulted in general increase of the low-methylesterified pectins level. How this compound is important for Pb accumulation revealed the comparison of Pb accumulation in *Populus* wild type (WT) and its 7B line which showed over-expression of PttPME1. The line 7B accumulated about 300% more Pb in their tissues than WT. Thus, we may conclude that CW remodeling is essential and widespread defense strategy of plants in response to Pb. It leads to the increase of CW capacity for Pb accumulation and compartmentalization protecting protoplast from its toxicity. Callose occurrence could additionally limit Pb migration and entering the protoplast. CW remodeling, among others, is involved in the increase of PttPME1 expression and PME activity.

This is worth to underline that obtained results revealing a natural defense strategy of plants in response to heavy metal simultaneously indicated a promising direction for enhancing plant efficiency in phytoremediation.

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OP3.5

Genome-wide association studies of *Arabidopsis thaliana* responses to disturbed iron and manganese homeostasis

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Iron (Fe) and manganese (Mn) are micronutrients essential for the normal growth and development of plants. They are cofactors of many important enzymes and are involved in basic plant processes like photosynthesis and nitrogen metabolism. The deficiency as well as the excess of Mn and Fe are harmful for plants. Moreover, many publications indicate the importance of Fe-Mn balance in plant metabolism. In our previous study, we investigated the performance of *Arabidopsis thaliana* accessions and selected mutant lines grown in various soil mixes that varied in Fe and Mn content, as well as in Fe/Mn ratio (Ihnatowicz et al. 2014). This led us to elucidate the genetic and molecular basis of observed chlorosis of nramp1 mutant, which was related to disturbed Mn and Fe homeostasis.

Here, we investigated the performance of 192 *Arabidopsis* accessions from HapMap population cultivated in previously used soil mix characterized by the Mn deficiency conditions caused by the excess of Fe. The performance of plants was tested by measuring flowering-related traits that are considered major indicators of plant ability to adapt to specific environments. We observed a significant phenotypic variation in flowering time-related traits among analyzed HapMap lines. The collected phenotypic data were further used in GWAS analysis in order to identify genes possibly involved in maintaining optimal Fe and Mn concentrations. As a result, we identified a set of candidate genes and analyzed the growth and development of selected T-DNA insertional mutant lines. Plants were grown in soil mixes and hydroponic cultures with various Mn and Fe availability. Particularly interesting was the response of cax4 mutant.

The CAX4 gene is indicated in the literature as encoding a cation/$H^{(+)}$ antiporter that plays an important function in root growth under heavy metal stress conditions (Mei et al. 2009). A detailed functional characterization of CAX4 is currently in progress.

[1] Ihnatowicz A, Siwinska J, Meharg AA, Carey M, Koornneef M, Reymond M (2014) Conserved histidine of metal transporter AtNRAMP1 is crucial for optimal plant growth under manganese deficiency at chilling temperatures. New Phytologist 202: 1173-1183.

[2] Mei H, Cheng NH, Zhao J, Park S, Escareno RA, Pittman JK, Hirschi KD (2009) Root development under metal stress in *Arabidopsis thaliana* requires the H^+/cation antiporter CAX4. New Phytologist 183: 95-105.

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Chelant-assisted phytoextraction and accumulation of Zn by *Zea mays*

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The pollution caused by heavy metals has been reported as critical environmental problem. Some metals, such as Cu, Fe, Mo, Mn and Zn, are essential mineral nutrients, however, their elevated concentrations produce toxic effects in all living organisms. Plants natural propensity to take up metals is used in phytoremediation. The low-cost, plant-based phytoextraction technique has often been described as a promising technique to remediate heavy metal contaminated agricultural land. The major issue hampering the efficiency of plant to remediate is that most of the heavy metals in soil are static and their accessibility and phytoextraction speed is confined by diffusion and solubility to roots surface. The application of chelating agents has shown positive effects in increasing the solubility of heavy metals in soil and therefore in enhancing phytoextraction. Ideally, plants used for the purpose of soil phytoremediation should grow fast, have a high biomass production, enhanced metal tolerance and high potential to accumulate metals in the aboveground parts. To fulfill the requirements it's been suggested the use of plant species with large biomass production and rapid growth (i.e. crops). These plants are typically characterized by a low translocation ratio of the metal from the roots to the overlying parts, therefore phytoextraction induced by chelator administration is one of possible developmental pathway for this technique. So far there are only a few studies on chelant-assisted phytoextraction of Zn using *Zea mays* plants. Currently, new chelates with high biodegradability are being sought and tested. One of proposed candidates is nitrilotriacetate (NTA), NTA is known to be fairly biodegradable, and has replaced EDTA in detergents, although its metal complexes are not as solid.

Its known that NTA solubilizes soil-polluting metals much more efficiently than low-molecular weight organic acids such as citrate and oxalate, and it also has the advantage of being commercially available in large quantities at reasonable costs.

A study was carried out to investigate the potential of Corn (*Zea mays*) for Zn phytoremediation. The maize (*Zea mays* L.) were cultivated in hydroponic experiments with Zn^{2+} concentrations ranging from 25 to 40 μM with and without addition of NTA. In treated plants was observed reduction in root and shoots length. Also, the concentrations of macronutrients N, P and K decreased in both shoot and root, while Fe concentration increased . The use of laser ablation LA, ICP and confocal microscopy has allowed to analyze the ability of maize to uptake, transport and accumulate Zn in individual tissues. NTA, not only has a strong potential to solubilize heavy metals in soil, but also enhances the uptake and translocation of heavy metals into the shoots of remediation plants.

OP3.7

Transcriptomic response to a salt stress and salt shock in leaves of sugar beet (*Beta vulgaris*, ssp *vulgaris*) and its halophytic ancestor, *Beta vulgaris* ssp. *maritima*

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Sugar beets display relatively high level of salt tolerance when compared to other crops, and are considered as salt-tolerant glycophytes, with traits of salt-tolerance inherited from their halophytic ancestor, *Beta vulgaris* ssp. *maritima*. Aim of the study was to distinguish between mechanisms of salt-tolerance which were preserved or lost in sugar beet during domestication. The trancriptomic response to salinity in leaves of sugar beet variety, *B. vulgaris* cv. Huzar and B. *vulgaris* ssp. *maritima* were assessed using Illumina paired-end sequencing technology. Two salinization protocols; a gradual exposure to increasing levels of salt (salt stress), and single step salt treatment (salt shock) treatment, were applied. Transcriptomic approach was complemented by estimation of transpiration and photosynthetic rates and mineral analysis of plant tissue. Moreover, several growth and morphological characteristics were determined. The study revealed that more genes were altered in their expression level in sugar beet than in halophytic beet, in response to salt stress. Whereas, 721 genes were differentially expressed in cv. Huzar, the number of 228 differentially expressed genes (DEGs) were identified in leaves of *B. vulgaris* ssp. *maritima*, after the salt concentration was gradually increased up to 300 mM. This finding suggests that the adaptation to salinity in sugar beet variety required more profound reorganization of leaf transcriptom, than in its halophyte relative. Salt shock treatment resulted in higher number of differentially expressed genes then salt stress treatment, but with smaller differences between genotypes. The single step-treatment with 300 mM NaCl resulted in 1484 and 1247 differentially expressed genes in leaves of *B. vulgaris* ssp. *maritima* and *B. vulgaris* cv. Huzar, respectively. Gene ontology (GO) enrichment analysis revealed several GO categories among salt-responsive genes in both, *B. vulgaris* ssp. *maritima* and *B. vulgaris* cv. Huzar. Some GO categories, such as oxidation-reduction process, carbohydrate metabolic process, water transport or photosynthesis represented genes which were frequently reported to alter in their expression level in plants during the response to salinity. Other GO terms revealed by the enrichment analysis, such as those related to secondary metabolism (triterpenoid biosynthetic process,camalexin biosynthetic process) or interactions with fungi and bacteria (response to chitin, pathogenesis, defence response) referred to genes which have not been regarded as involved in salt tolerance, yet. Several GO terms, referred to and the cell energy metabolism and other processes of basic metabolism. Significant number of DEGs of unknown function were detected in plants representing both genotypes. These genes might provide a link to so far uncharacterised mechanisms of salinity tolerance.

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Coupling high-throughput phenotyping and gene expression analyses to study the natural variation of morpho-physiological and molecular responses to long-term water deficit in *Arabidopsis thaliana*

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Acclimation to water deficit (WD) enables plants to maintain growth under unfavorable environmental conditions. Although plant responses to long-term water deficit were intensively studied over the past decade, the molecular mechanisms are not completely understood. Manual maintenance of constant soil water content while performing multiple measurements is highly labor-intensive and sometimes not feasible. Automatic phenotyping platforms enable researchers to conduct reproducible experiments on multiple plants in parallel. This study investigated the natural variation of response to long-term WD in *Arabidopsis thaliana*. Eighteen accessions, adapted to various environmental conditions, were grown under well-watered conditions, and were subjected to moderate (MWD) and severe (SWD) constant soil WD using the PHENOPSIS phenotyping platform (INRA, Montpellier). WD treatments were employed at an early stage of plant development and soil water content was maintained at a constant level until reproductive age was reached. Thirteen morpho-physiological traits were measured, and SWD conditions had a greater effect than MWD conditions. Multivariate analyses indicated that trait responses associated with plant size and water use drove most of the variation. Accessions with similar responses at these two levels were grouped in clusters that displayed different response strategies to WD. The expression levels of sixteen genes associated with stress response displayed large natural variation under WD conditions. Responses of morpho-physiological traits such as projected rosette area, transpiration rate, and rosette water content were correlated with changes in the expression of stress-related genes, such as NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3 and N-MYC DOWNREGULATED-LIKE 1 (NDL1) in response to WD. Interestingly, the morpho-physiological acclimation response to WD was also reflected in the gene expression levels (most notably those of NDL1, CHALCONE SYNTHASE and MYB DOMAIN PROTEIN 44) in plants cultivated under well-watered conditions. Our results may lead to the development of biomarkers and predictors of plant morpho-physiological responses based on gene expression patterns.

Access to the PHENOPSIS platform was permitted thanks to funds from the European Plant Phenotyping Network (EPPN, grant agreement no. 284443) funded by the FP7 Research Infrastructures Programme of the European Union. Research was also supported by a PRELUDIUM (2012/05/N/NZ9/01396) grant from the National Science Centre, Poland.

OP3.9

Barley brassinosteroid mutants provide an insight into phytohormonal homeostasis under control condition and during plant reaction to drought stress

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Brassinosteroids (BRs) are a class of steroid phytohormones, which regulate various processes of morphogenesis and physiology – from seed development to regulation of flowering and senescence. Many of the physiological functions of BRs are regulated by a complicated, and not fully elucidated network of interactions with metabolic pathways of other phytohormones. It is known that drought is one of the most adverse and multidimensional environmental stresses that affect plant growth and yield. Therefore, the aim of this study was to characterize phytohormonal homeostasis in barley (*Hordeum vulgare*) in reaction to drought and validate role of endogenous BRs in regulation of this process.

Material of this study included the barley cultivar 'Bowman' and five Near-Isogenic Lines (NILs) representing characterized semi-dwarf mutants of several genes encoding enzymes participating in BR biosynthesis and signaling. Analysis of endogenous BRs concentrations in these NILs confirmed that their phenotypes result from abnormalities in BR metabolism. In general, concentrations of eighteen compounds, representing various classes of phytohormones, including brassinosteroids, auxins, cytokinins, gibberellins, abscisic acid, salicylic acid and jasmonic acid were analyzed under control and drought conditions in the 'Bowman' cultivar and the BR-deficient NILs.

In reaction to drought, the semi-dwarf NILs exhibited delayed wilting when compared with the 'Bowman' cultivar. Abnormalities in BR metabolism do not seem to negatively affect transpiration rate and stomatal conductance in the semi-dwarf NILs under the control condition. In response to drought, the semi-dwarf NILs maintained the transpiration rate and stomatal conductance at a similar or even higher level when compared with the 'Bowman' cultivar. Drought induced a significant increase in accumulation of the biologically active form of BRs – castasterone in all analyzed genotypes. Another biologically active form of BRs – 24-epi-brassinolide – was identified in one, BR-insensitive NIL under normal condition, but its accumulation was drought-induced in all analyzed genotypes. Analysis of concentration profiles of several compounds representing gibberellins allowed an insight into the BR-dependent regulation of gibberellin biosynthesis. The concentration of the gibberellic acid GA7 was significantly lower in all NILs when compared with the 'Bowman' cultivar, indicating that GA7 biosynthesis represents an enzymatic step at which the stimulating effect of BRs on gibberellin biosynthesis occurs. Moreover, the accumulation of GA7 is significantly induced by drought in all the genotypes. Biosynthesis of jasmonic acid is also a BR-dependent process, as all the NILs accumulated much lower concentrations of this hormone when compared with the 'Bowman' cultivar under normal condition, however the accumulation of jasmonic acid, abscisic acid and salicylic acid were significantly stimulated by drought.

Mitochondrial response to the various water deficiency conditions in three cultivars of cauliflower

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Vast experimental data concerning plant responses to drought (or water) stress have been published up to date. Strikingly, selected ideas on the mitochondrial biogenesis in the mentioned detrimental conditions were the less investigated issue, especially between diverse *Brassica* species and cultivars. Cauliflower (*Brassica oleracea* var. *botrytis*) belongs to important agronomically vegetables with the major cultivation yield in the Middle Europe. The early generative phase of curd ripening belong to the key developmental stages with some physiological demands. In addition, due to the size of its vegetative organs it is sensitive to the low levels of water in the soil.

The main goal of our project was to estimate the impact of middle and severe water deficiency conditions on the mitochondrial proteome of three cauliflower cultivars sharing diverse ('Adelanto' and Casper' vs 'Pionier') drought tolerance.

We investigated mitochondrial proteomes of the analysed cultivars by 2D PAGE (IEF/SDS-PAGE) coupled with LC-MS/MS and identified 32 drought-responsive protein spots. Surprisingly, mitochondrial proteomes significantly varied among all cultivars (only 4 spots common for 'Adelanto' and 'Casper'), however spots specific to the given drought level prevailed. Massively downregulated spots exceeded upregulated ones. The drought responses for 'Pionier' were particularly specific. Various respiratory (ex. ATP synthase, proteins for CII and CIV biogenesis), transporter (ex. diverse VDAC isoforms and dicarboxylate antiporters) and matrix proteins (ex. HSPs, DNA-binding proteins, RNA editing and translation factors, mitochondrial thioredoxins, diverse multifunctional enzymes for aminoacid, carbohydrate, lipid and nucleotide metabolism and some novel proteins) appeared drought-responsive. The level of mitochondrial marker proteins assayed by additional immunoassays showed cultivar-specific responses. The low-molecular-weight dehydrin-like proteins (dlps; previously characterized by Rurek [2010] BMC Plant Biol, 10:181) were mainly upregulated in 'Adelanto' and 'Casper'. On the contrary, 'Pionier' showed only minor alterations within the dlps profile. Dehydrin-specific tryptic peptides in few spots from 2D gels were detected, highlighting the importance of participation of such proteins in cauliflower drought response. Results obtained from molecular analyses will be also compared with the ones from the standard physiological assays (including measurements of dark and light respiration and photorespiration rates in mature cauliflower leaves), as only limited coordination of response on diverse structural and functional levels was noticed. For instance, a lack of coordination between proteomic and transcriptomic alterations (particularly for AOX1a) was evident.

We conclude that mitochondrial biogenesis may be strongly but in diverse manner affected in various cauliflower cultivars and that such alterations coincide with drought tolerance.

OP3.11

The tonoplast intrinsic protein (TIP) gene subfamily of aquaporins in barley: structural features and expression profiles during drought stress

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Plant aquaporins are membrane channels involved in the transfer of water and small solutes across cell membranes. They are implicated in various physiological processes, including growth, development and adaptation to stress. In our study, the Tonoplast Intrinsic Protein (TIP) gene subfamily of barley, an important crop species, was investigated and characterized. TIPs are found to play a key role in controlling cell water homeostasis by rapid water transport between vacuole and cytoplasm of plant cells. In barley, the HvTIP subfamily encompasses 11 members. Survey of theirs promoters revealed the presence of cis-regulatory elements involved in abiotic and hormone responses. Gene expression studies of HvTIPs during progressive drought stress and subsequent recovery have been carried out in leaves of 'Sebastian' variety using qPCR technique. The results obtained suggest that some of investigated HvTIPs might be functionally implicated in adaptation to drought stress in barley.

Nod factor perception and signal transduction during endosymbiotic interactions of *Medicago truncatula*

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The legume *Medicago truncatula* maintains endosymbiotic interactions under phosphate and nitrate limiting conditions with respectively mycorrhizal fungi and rhizobia. Both microsymbionts produce lipochitooligosaccharides (LCOs) which are perceived by plant receptors with extracellular LysM domains and an intracellular kinase domain (LysM-RLKs). Mycorrhizal fungi in addition secrete short chain chitooligosaccharides (COs) which also function as signal molecules. Three LysM-RLKs from *M. truncatula*, LYR3, LYK3 and NFP, were studied in detail in our group. LYR3 is a high affinity binding protein for LCOs but not for COs. Swapping experiments with LysM domains from different plant species identified the crucial role of the third LysM motif in LCO binding. LYR3 from two *Lupinus* species which do not form the mycorrhizal symbiosis are deficient in high affinity binding to LCOs. LYR3 and NFP lack an active kinase domain and LYR3 is phosphorylated by the active kinase domain of LYK3. FRET experiments showed that LYR3 and LYK3 interact at the plasmamembrane and this interaction is inhibited or disrupted by addition of LCOs. Co-expression of NFP and LYK3 in tobacco leaves provokes a cell death response that is attenuated in the presence of LYR3. Thus LYR3 may play a role in regulating the functional interaction of NFP and LYK3. Low level expression of these 3 proteins hampers their visualization in roots of *M. truncatula*. Ongoing experiments to increase the detection sensitivity and thus study the distribution and regulation of these proteins during endosymbiotic interactions will be presented.

[1] Nikita Malkov, Judith Fliegmann, Charles Rosenberg, Virginie Gasciolli, Ton Timmers, Julie Cullimore, Jean-Jacques Bono (2016) Molecular Basis of Lipo-chitooligosaccharide recognition by the Lysin Motif Receptor Like Kinase LYR3 in legumes. Biochemical Journal. DOI: 10.1042/BCJ20160073.

[2] Judith Fliegmann, Alain Jauneau, Carole Pichereaux, Charles Rosenberg, Virginie Gasciolli, Antonius.

[3] C.J. Timmers, Odile Burlet-Schiltz, Julie Cullimore, Jean-Jacques Bono (2016) LYR3, a High-affinity LCO-Binding Protein of *Medicago truncatula*, interacts with LYK3, a Key Symbiotic Receptor. FEBS Letters. DOI: 10.1002/1873-3468.12191.

OP4.2

Analysis of gstu13 plants uncouples two putative signaling functions of indole glucosinolate metabolism products in flg22-triggered immune responses in *Arabidopsis*

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PENETRATION2 (PEN2)-mediated indole glucosinolate (IG) metabolism constitutes an important component of pre-invasive immunity in the model plant *Arabidopsis thaliana*. PEN2 has been also shown to be indispensable for deposition of callose upon recognition of the bacterial microbe-associated molecular pattern flg22. In addition, pen2 mutants are defective in the flg22-induced accumulation of indole-3-carboxylic acids (ICAs) whose biosynthetic pathway does not include PEN2 enzyme. Therefore it has been postulated that IG metabolism products can act as signaling molecules required to trigger callose deposition and ICA biosynthesis. In this study, we identified Glutahione S-trasferase U13 (GSTU13) as an enzymatic component of the PEN2 pathway. In accordance with this function, gstu13 mutants were defective in pre-invasive resistance against fungal pathogens and in flg22-triggered callose deposition. However, our analysis indicted that opposite to pen2 line gstu13 plants accumulated wild type-like levels of ICA in response to flg22. These findings indicate that different IG-metabolism products are required for flg22-triggered callose deposition and for activation of ICA biosynthetic pathway.

The sequence of enhanced generation of *Pisum sativum* defense signalling molecules in response to pea aphid infestation

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The recognition of aphid infestation by plants likely occurs either through the use of herbivore-associated molecular patterns (HAMPs) or, acting largely inside the cell, polymorphic nucleotide-binding site-leucine-rich repeat (NBS-LRR) protein products, encoded by a majority of resistance genes. This activation may induce defensive reactions which are the result of highly coordinated sequential changes at the cellular level, comprising, among other changes, the synthesis of signalling molecules. However, under natural conditions the effect of many stress factors acting simultaneously or successively is frequently observed. Therefore, the first aim of this study was to verify whether an enhanced generation of signalling molecules such as salicylic acid (SA), jasmonates (JA/MeJA), abscisic acid (ABA), ethylene (ET), hydrogen peroxide (H_2O_2) and nitric oxide (NO), occurs in leaves of *P. sativum* L. cv. Cysterski seedlings in response to pea aphid infestation. In turn, the second aim of study was to determine how cross-interactions of stress factors, i.e. abiotic (lead) and biotic *A. pisum*, regulate the level of the above – mentioned signalling molecules. Additionally, the third aim was to examine the influence of these stress factors on other defensive reactions of pea. Little is known about the time-dependent aspect of induced defensive reactions at the cellular and molecular levels. Unique differences were observed in responses associated with the generation of signalling molecules by *P. sativum* L. to *A. pisum* infestation. Moreover, our research results showed that exogenous administration of lead at a low concentration causing the hormesis effect and a toxic concentration of lead to the medium, on which pea seedlings were cultured stimulated generation of signalling molecules. Additionally, cross-interactions of stress factors, i.e. lead and *A. pisum*, significantly increased production of these molecules. Concentrations of SA and salicylic acid glucoside (SAG) were highest in roots and leaves of pea seedlings cultured on the medium with toxic concentrations of lead and infested by *A. pisum*. Also, the level of ABA was highest in the above – mentioned tissues. In the case of the application of these two different lead concentrations we demonstrated significant differences in the intensity of generation of signalling molecules in pea seedlings infested by the pea aphid.

Additionally, the unique dialogue of signalling molecules affected the activation of other defence responses, i.e. the generation of semiquinone radicals and the synthesis of secondary metabolites such as flavonoids. These studies are valuable as they provide insight into defence mechanisms of plants in the environment with numerous stressors.

The role of metabolic components, volatile compounds, PR proteins and mechanical strengthening in multilayer protection of cucumber plants against *Rhizoctonia solani* induced by *Trichoderma atroviride* TRS25

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In the cucumber plants, *R. solani*-induced disease symptoms included irregular lesions and rot on the roots followed by shoot and leaf dark brown blight blotches and plant collapse. Spread of the disease was limited when the plants were pretreated with *T. atroviride* TRS25. The systemic disease suppression was not related to direct inhibition of *R. solani* growth and infection by TRS25 but to cucumber resistance induction with simultaneous plant growth promotion. Biochemical protection of cucumber was related to enhanced activity of defence enzymes e.g. guaiacol peroxidase (GPX), syringaldazine peroxidase (SPX), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) as well as ortodihydroxyphenolics (oDP), phenylpropanoids (PP) and favonoids (FL) concentration increases in the conditions of hydrogen peroxide (H_2O_2) accumulation, resulting in thiobarbituric acid reactive substance (TBARS) decrease.

Moreover, the obtained results indicate that TRS25-induced resistance (TISR) against *R. solani* may depend on accumulation of salicylic acid derivatives, that is methyl salicylate (MeSA), ethylhexyl salicylate (EHS), salicylic acid glucosylated conjugates (SAGC) and β-cyclocitral as well as unsaturated fatty acid derivatives such as Z-3-Hexanal, Z-3-Hexenol and E-2-Hexenal which are volatile organic compounds (VOC). The obtained results point to important, not previously documented, roles of these VOC in TISR signaling with up-regulation of PR1 and PR5 genes characteristic of systemic acquired resistance (SAR) and of PR4 gene, marker of induced systemic resistance (ISR). The study established that TRS25 enhanced supplementary deposition of callose and lignin in specialized plant cells which mechanically protected vascular system in cucumber shoots and roots as well as assimilation cells and dermal tissues in cucumber shoots and leaves. These compounds protected cucumber organs against *R. solani* influence and made them more flexible and resilient, that contributed to better nutrition and hydration of plants. The growth promotion coupled with systemic mobilization of biochemical, molecular and mechanical strengthening might be positively involved in multilayer protection of cucumber plants against *Rhizoctonia solani* activated by TRS25. Based on the results, TRS25 strain has potential to be effective biological control agent (BCA) for *R. solani*-induced disease management in cucumber plants.

OP4.5

miRNAs and mRNA targets in potato-PVY pathosystems

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Our study demonstrates strain-specific and temperature-dependent expression of a set of stress-responsive host miRNAs and their target mRNAs in two potato-Potato virus Y (PVY) pathosystems. Potato cultivar Etola exhibits strain-specific hypersensitive resistance to PVY^{NTN} isolate PVY-3202 (Yin et al. 2016). Both the $PVY^{N\text{-}Wi}$ isolate PVY-3411 and the PVY^{Z}-NTN isolate PVY-3303 elicit local and systemic necrotic lesions. However, PVY-3411 induced severe leaf symptoms, faster systemic viral coat protein and RNA accumulation in the non-inoculated upper leaves; PVY-3303 caused mild symptoms and delayed viral spreading in cv. Etola. At 16 dpi, the tested miRNAs and targets are altered only in plants of cv. Etola infected with PVY-3411 but not those infected with PVY-3303 nor in plants that are resistant to PVY-3202. The up-regulation of stu-miR162, stu-miR168a, stu-miR172e and two members of stu-miR482, together with their target transcripts, *DCL1, AGO1-2, TOE3, Gpa2* and *CC-NBS-LRR*, respectively, in PVY-3411-infected plants correlates with high abundance of PVY HC-Pro RNA encoding an RNA-silencing suppressor and might be linked with the severe symptoms in leaves.

The diploid potato clone DG 81-68, possessing the novel gene Ny-DG on chromosome IX, confers the temperature-dependent symptomless resistance to PVY^{NTN} at 20°C; however, at 28°C the resistance was overcome and accompanied by local and systemic necrosis (Szajko et al. submitted). At 6 dpi, in the inoculated leaves of DG 81-68 at 28°C, the increased expression of stu-miR162, stu-miR168a and stu-miR482 promoted the down-regulation of their targets *DCL1, AGO1-2* and *CC-NBS-LRR*, respectively. The expression of stu-miR482 (PotatoMir1005658171_x16170) and miR172e remained unchanged, whereas their targets *Gpa2* and *TOE3* were down-regulated. However, in the inoculated leaves at 20°C, all five tested miRNAs and their targets showed parallel down-regulation. In the non-inoculated upper leaves, changes in gene expression levels, i.e., down-regulation, were only detected for the tested five miRNAs at 28°C.

In conclusion, the alteration of the tested stress-responsive host miRNAs and their targets was strain dependent and related to symptom severity in the PVY-Etola interactions. In the PVY-DG 81-68 interactions, changes in miRNAs and their targets expression levels are related to the type of reaction, which in turn are dependent on temperature, and differ between the local inoculated and the upper non-inoculated leaves.

[1] Yin Z, Xie F, Michalak M et al (2016) Potato cultivar Etola exhibits hypersensitive resistance to PVYNTN and partial resistance to PVY^{Z}-NTN and $PVY^{N\text{-}Wi}$ strains and strain-specific alterations of certain host miRNAs might correlate with symptom severity. Plant Pathol.doi: 10.1111/ppa.12599

[2] Szajko K, Yin Z, Marczewski W (Submitted) Accumulation of miRNA and mRNA targets in potato leaves displaying temperature-dependent responses to *Potato virus Y*

HD2C histone deacetylase binds to SWI/SNF chromatin remodeling complex and act together to regulate *Arabidopsis* heat stress response

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Histone acetylation is a reversible process that depends on the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Five classes of histone deacetylases exist in plants. HD2C, the most thoroughly studied member of the HD2 family, is involved in responses to salt and osmotic stresses and abscisic acid treatment. Studies in yeast and animals have revealed that HDACs often act as components of multiprotein complexes, including chromatin remodeling complexes (CRCs). However, interactions between HDACs and CRCs in plants have yet to be demonstrated. Here, we present evidence for the interaction between *Arabidopsis* HD2C deacetylase and a SWI/SNF chromatin remodeling complex containing BRM ATPase as the catalytic subunit. Moreover, we reveal a novel function of HD2C as a regulator of the heat stress response. HD2C transcript levels were strongly induced in plants subjected to heat treatment and the expression of selected heat-responsive genes was up-regulated in a hd2c mutant, suggesting that HD2C acts to down-regulate heat-activated genes. In keeping with the histone deacetylase activity of HD2C, the altered expression of HD2C-regulated genes coincided in most cases with increased histone acetylation at their loci. Microarray transcriptome analysis of brm and hd2c mutants identified a subset of commonly regulated heat-responsive genes, and the effect of the double brm hd2c mutation on the expression of these genes was non-additive. Moreover, heat treated hd2c, brm and brm hd2c mutants displayed similar rates of growth retardation. Taken together, our findings suggest that HD2C and BRM act in a common genetic pathway to regulate the *Arabidopsis* heat stress response.

OP5.2

The impact of mutations affecting NuA4 histone acetyltransferase on the life cycle of *Arabidopsis thaliana*

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Nucleosomal Acetyltransferase of histone H4 (NuA4) is a protein complex responsible for highly coordinated acetylation of nucleosomal histones which supports transcription and DNA-repair in yeast and in human chromatin. NuA4 targeting and regulation depends on transcription factors as well as multiple chromatin-level inputs interpreted by its chromatin-mark reader subunits. Although the essential catalytic core of the complex can acetylate nucleosomal histones on its own, loss of the targeting subunits compromises NuA4 functionality in both transcription and DNA repair.

I will present recent progress in our attempts to understand the role of plant NuA4 in which we focus on the targeting part of the complex. Thanks to the high evolutionary conservation of the subunit composition of this complex between yeast and plants, supported by our published proteomic data (Bieluszewski et al., 2015), we were able to investigate the physical link between the catalytic and the targeting part of the *Arabidopsis* NuA4 with genetic approaches. I will show how mutations of the putative scaffold subunits of plant NuA4 affect development of the photosynthetic apparatus and reproduction. The molecular basis of NuA4 involvement in the regulation of transcriptional activity of nuclear genes associated with photosynthesis will be discussed as well.

Our results indicate that NuA4 complex is an indispensable part of chromatin-level regulation of chloroplast function, possibly as a transcriptional coactivator, as well as reproduction, through a yet unknown mechanism.

Linker histones act as powerful lock of heterochromatin activity in *Arabidopsis*

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Linker (H1) histones comprise a family of chromatin proteins involved in both structural and regulatory mechanisms inside the nucleus. They have been extensively studied in all main eukaryotic taxa, and commonly have been found to occur in multiple redundant, non-allelic variants, as well as to be essential for proper development and post-embryonic survival. Surprisingly, we found that *Arabidopsis thaliana* which contains only three non-allelic H1 variants, remains viable, with little phenotypic effect, in the triple h1 mutants. We have examined these mutants thoroughly and found that such a mild response on the level of phenotype is strongly contrasted by drastic changes in transcriptome, small transcriptome, epigenome and chromatin structure. We found that lack of H1s renders heterochromatin constantly available to RNA polymerases and, likely, other factors, a situation of great potential danger to genome stability due to the risk of transposon mobilization and structural aberrations of centromeres. Our data show that plants can effectively cope with these dangers by mobilizing ancient RNAi and RNA degradation pathways. We hypothesize that such an ability may be a specific adaptation of plants, in which 'highly availible' H1-depleted chromatin occurs in several key developmental phases, including gametogenesis and meristem formation.

OP5.4

Progressive methylome modulation during water-deficiency conditions in barley is coupled with transcriptomic responses

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Adverse environmental conditions can strongly affect plant growth and development, what leads to huge reduction of crops productivity. One of the basic strategies underlying rapid responses of plants to stresses, is the modulation of multi-level epigenetic machinery, with DNA methylation ahead, resulting in global-wide transcriptional reprogramming and in increasing of transposons mobility. This research attempts to characterize the dynamics of drought stress-induced changes in DNA methylation in barley and its putative role in drought-related transcriptomic response.

To identify a wide set of differentially methylated sites (DMS) affected by drought stress in barley leaves we applied high-throughput Methylation Sensitive Amplification Polymorphism Sequencing (MSAP-Seq) technology, developed by our group. MSAP-Seq is a simple method that allows for parallel identification of hundreds of thousands of sites at low cost and, thus, is especially useful in multi-sample studies involving large genome crops. Detailed profiling of the barley methylome at eight consecutive time points during progressive water deficiency stress, followed by rewatering phase, revealed its dynamic modulation in terms of the quantity and localization of drought-induced changes. We identified more than two thousand of drought-related differentially methylated sites (DR-DMSs) in barley leaves. The total number of DNA methylation changes, especially those related to genes, increased gradually under drought. Interestingly, the majority of early DR-DMSs remained persistent under rewatering, whereas late DR-DMSs were rather reversible. Functional analysis of genes with DR-DMSs revealed an enrichment of processes related to photosynthesis, what corresponds to reduction of photosynthesis performance under drought observed in leaves.

Parallelly performed global-wide transcriptome profiling, carried out with mRNA-Seq at each of the time points, allowed for a comprehensive and precise characterization of transcriptomic response to drought stress. Consequently, we identified sets of early and late drought-responsive genes, which transcription could be potentially regulated by identified DR-DMSs. Altogether our analyses suggest that the dynamic nature of methylome changes can constitute to an important layer of regulatory machinery driving progressive transcriptomic response to stress in large-genome crops.

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H2A.Z impact on gene expression in plant response to stress

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H2A.Z belongs to evolutionarily highly conserved histone variants. Histone variants are histones that are specifically exchanged with canonical histone proteins in chromatin to change its properties and so affect processes on DNA. The H2A.Z distribution pattern throughout a genome is to a large extent universal for all eukaryotes, with conspicuous enrichment around transcription start sites (TSS) of genes. This distinct pattern suggests the engagement of H2A.Z in transcription regulation, which has been shown by many research groups (Billon and Cote, 2013, Subramanian et al., 2015). However, the exact manner in which H2A.Z affects gene expression remains unclear. Previous reports noticed that *Arabidopsis* H2A.Z enriches bodies of genes known for their involvement in a response to changing environmental conditions (so called 'responsive' genes) (Coleman-Derr and Zilberman, 2012). This might suggest that H2A.Z plays some role in repression of these genes in ordinary (not-restrictive) conditions. We analyzed the transcriptome of the arp6 mutant line, in which H2A.Z deposition is impaired, and indeed found stress-responsive genes overrepresented among these hyperactive in arp6. In wild-type plants these responsive genes have high levels of H2A.Z in their bodies. We also treated plants with drought stress to monitor both changes in gene expression profile and changes in H2A.Z distribution/occupancy globally, using techniques associated with high throughput sequencing (RNA-seq, ChAP [chromatin affinity purification] – seq). We demonstrated that H2A.Z is highly enriched within bodies of genes strongly responding to the stress, irrespective whether they are activated or repressed. However, H2A.Z partially disappears from activated genes and enriches even more bodies of repressed genes. Interestingly, what changes in parallel with gene expression is H2A.Z occupancy, not H2A.Z distribution. This indicates that H2A.Z in gene bodies has a repressive effect on transcription, preventing unwanted expression in non-inductive conditions. Simultaneously, decreased activity of some genes in the arp6 line seems to result from another H2A.Z role at their +1 nucleosome, where H2A.Z appears to be important to maintain transcriptional activity (Sura et al., 2017).

[1] Billon, P. and Côté, J. (2012) Precise deposition of histone H2A.Z in chromatin for genome expression and maintenance. Biochim. Biophys. Acta 1819: 290-302.

[2] Coleman-Derr, D. and Zilberman, D. (2012) Deposition of Histone Variant H2A.Z within Gene Bodies Regulates Responsive Genes. PLoS Genet. 8: e1002988.

[3] Subramanian, V., Fields, P.A., Boyer, L.A. (2015) H2A.Z: a molecular rheostat for transcriptional control. F1000 prime reports: 2015 Jan 5; 7:01.

[4] Sura, W., Kabza, M., Karlowski, W.M., Bieluszewski, T., Kuś-Slowinska, M., Pawełoszek, Ł., Sadowski, J., Ziolkowski, P.A. (2017) Dual role of the histone variant H2A.Z in transcriptional regulation of stress-response genes. Plant Cell: tpc.00573.2016.

OP6.1

Nitric oxide status in roots of soybean seedlings exposed to cadmium

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Although the formation of nitric oxide (NO) has been well documented in in vivo experiments of various plant systems challenged by cadmium (Cd), the functional role of endogenous NO during plant response to the heavy metal is still the object of extensive investigations. As we postulate peroxynitrite ($ONOO^-$) formation could provide an important regulatory loop for NO bioactivity under Cd stress conditions, since $ONOO^-$ can provoke the phenomenon of tyrosine nitration, assumed as a regulatory mechanism for protein activity. The effect of short-term (48 h) moderate (85 μM $CdCl_2$) and high (170 μM $CdCl_2$) Cd stress on endogenous $ONOO^-$ generation was investigated in the roots of 2-days old seedlings of soybean (*Glycine max* L.). The control roots showed a relatively high level of $ONOO^-$, whereas Cd- mediated $ONOO^-$ formation was detected only at more intense Cd stress. The Cd-induced $ONOO^-$ accumulation was accompanied by enhanced levels of both nitric oxide (NO) and superoxide ($O2^{\cdot-}$). The contrasting response appeared during moderate stress which provoked a huge NADPH-oxidase dependent $O2^{\cdot-}$ accumulation with slightly elevated reactive nitrogen species formation. The nitroproteome analysis revealed a proteins pool undergoing tyrosine nitration that were down-regulated during both moderate and high Cd stress conditions. The most pronounced changes within the reduced pool of 3-nitrotyrosine targets in soybean roots revealed proteins involved mainly in primary metabolism that are not implicated in plant defense strategy against heavy metal stress. Moreover, sequential treatment with $ONOO^-$ and Cd was tuned with diminished Cd toxicity and up-regulation of gene coding for peroxiredoxin (Prx).

Taken together, soybean seedlings mainly exposed to lower intensity of Cd stress, showed enhanced Pxr gene expression correlated with temperate $ONOO^-$ formation what finally affected post-translational protein modification via tyrosine nitration.

Hydrogen cyanide mimics nitric oxide cellular mode of action

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Hydrogen cyanide (HCN) is a fire gas molecule detected in the environment and in living organisms. This toxic molecule is released during biomass combustion and is present in cigarette smoke. Plants synthesize HCN as co-product of ethylene biosynthesis; liberate this compound from cyanogenic glucosides, present in more than 2500 species (Zagrobelny and Muller, 2011). Nitric oxide (NO) belongs to reactive nitrogen species (RNS) is a gaseous compound of dual physiological function, described by a nitrosative door schema (Krasuska et al. 2015). NO participate in protein postranslational modification nitration and S-nitrosylation. Nitrated proteins as stable metabolites can be considered as good markers of nitrooxidative status in cells. The aim of our work was to indicate HCN involvement in protein nitration and S-nitrosylation. We showed the presence of nitrotyrosine and S-nitrosocysteine residues in BSA treated with HCN (for 1 h, 3 h and 24 h) using specific antibodies. We demonstrated increased NO emission from the solution containing HCN using 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA) reagent, and increased of nitrite (NO -) level measured using modified Griess reagent (Sigma). We also observed differences in HPLC chromatograms of tryptophan and tyrosine treated with HCN, similar to those one obtained after NO fumigation.?

[1] Zagrobelny M., Moller B.L. 2011. Phytochemistry 72:1585-1592

[2] Krasuska et al. 2015. Reactive Oxygen and Nitrogen Species Signaling and Communication in Plants, Seria: Signaling and Communication in Plants, red: Gupta KJ, Igamberdiev AU, Springer International Publishing Switzerland 2015, 215-237

OP6.3

Proteomic analysis of S-nitrosylated proteins in wheat seedlings with different dehydration tolerances

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Reactive nitrogen species (RNS) are, as the reactive oxygen species (ROS), the natural by-products of energy metabolism which cooperate in maintaining the cell redox homeostasis. Environmental stresses, including drought, can affect theirs increased production, which interferes with redox cell homeostasis and in a consequence drive the secondary oxidative and nitrosative stress. So the changes in the cell redox potential may lead to posttranslational modifications of proteins. In case of RNS the S-nitrosylation of cysteine residues seems to play a major role in processes of plants acclimation to dehydration.

The experiments were carried out on four- and six-day-old wheat (*Triticum aestivum* L.) seedlings of *Zadra* cultivar since it was evaluated that such materials differ in sensitivity to dehydration: four–day-old are tolerant whereas six-day-old seedlings are drought sensitive. Both types were subjected to drought resulted in 70% leaf water deficit estimated as WSD. Detection of S-nitrosylated proteins was based on 'biotin switch' and an immunochemical method. Proteome maps were analysed using the Delta2D software and protein spots that differed in abundance at least two-folds in response to dehydration were identified by MALDI-TOF and LC-MS/MS.

The dehydration of drought tolerant four-day-old seedlings altered the expression patterns of 24 proteins which were S-nitrosylated: 7 increased in abundance whereas 17 decreased. Dehydration of drought sensitive six-day-old seedlings altered the expression of 20 S-nitrosylated proteins, of which 8 increased and 12 decreased in abundance. The classification of differentially S-nitrosylated proteins resulted in following summary. For four-day-old drought tolerant seedlings 25% were classified as nucleic acid and protein metabolism-related, 21% as stress-response proteins, 13% to energy metabolism, and 21% as related to other metabolic pathways. In six-day-old drought sensitive seedlings the percentage of differentially S-nitrosylated proteins differed: 30% constituted proteins related to nucleic acid and protein metabolism, 20% the stress-related proteins, and 20% the proteins related to energy metabolism. Two proteins were identified in both types of seedlings: oxygen-evolving enhancer protein 2 and calcium dependent protein kinase isoform 11. In drought tolerant seedlings the expression of those proteins increased whereas in the sensitive seedlings – decreased. The obtained results confirmed earlier findings that plant protein S-nitrosylation is a widespread posttranslational modification involved in the plant's response to unfavourable environmental conditions, although the functional role of this modification and its impact on protein function remains to be established.

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Interactions between light induced signal and reactive oxygen species during light-dependent germination of *Arabidopsis thaliana* seeds

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The germination is a complex process involving several changes at the physiological, biochemical and molecular levels in an imbibing seed. On the other side, a dormancy was defined as a failure of a viable seed to complete germination even under favorable conditions. It is known that gibberellins (GA) and abscisic acid (ABA) regulate seed germinability. It was also shown, that reactive oxygen species (ROS) regulate seed dormancy alleviation and germination i.e. by carbonylation of specific proteins. However, a role of ROS depends on the ability of cells to maintain the balance between their production and removal. The level of ROS in the cell is controlled by an antioxidative system composed of various elements including: catalase (CAT) and vitamin E. One of the source of ROS in seeds are membrane-bound NADPH oxidases (RBOH, Respiratory Burst Oxidase Homologue). The seed-related events are controlled also by many environmental factors including: light and temperature. In the case of seeds of *Arabidopsis thaliana*, their germination can be stimulated by red light and it has been established that such reaction is related to changes in GA and ABA metabolism. The signal induced by red light in *Arabidopsis* is mostly perceived by phytochromes and its further transmission involves many regulators i.e. PIF1 (Phytochrome-Interacting Factor 1) and HY5 (Elongated Hypocotyl 5). Nevertheless, the possible role of ROS in light-dependent seed germination is unknown. Therefore the aim of this study was to verify, if the mechanism of regulation of *Arabidopsis* seed germination by light is related to modulation of ROS metabolism and/or signaling. The *Arabidopsis* dormant and after-ripened seeds were used in biological assays in different light conditions, on water or in the presence of DPI (diphenylene iodonium; an inhibitor of NADPH oxidases activity). To characterize the effect of light on the relative level of transcripts of genes related to ROS metabolism and signaling (such as: RBOHB, RBOHD, CAT2, VTE1, MAPK3 and 6) and light signaling (i.e. HY5) in germinating *Arabidopsis* seeds, the qRT-PCR (quantitative Real-Time PCR) analysis was performed. In addition, in situ ROS localization in seeds germinating in different light conditions was performed using a NBT (nitroblue tetrazolium) staining and profiles of carbonylated proteins were identified by immuno-oxyblot. The obtained novel results indicated that the mechanism of light action during seed germination may require the modulation of ROS metabolism and signaling by changing the expression of certain ROS-related genes transcription and profiles of oxidized protein. This study provide significant new findings in the field of seed biology and light signaling about possible interactions between environmental and internal factors controlling processes occurring in seeds.

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Poster Presentation

8th Conference of the Polish Society of Experimental Plant Biology
Communication in plants: from cell to environment

P1.1

Ontogenetic changes in the regulation of organogenesis and vascular development in the inflorescence stem of *Arabidopsis thaliana pin1* mutant

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Plants of *Arabidopsis thaliana* with impaired polar auxin transport (PAT) due to the mutation in the *PIN1* gene have a completely blocked organogenesis during early stages of the inflorescence development. Later on, the inflorescence meristem usually forms some deformed organs, and that organogenic activity increases over time, finally leading to the formation of the fascinated stem with organ-like structures.

Specific reconstruction of the vascular system accompanies that reestablishment of the organogenic activity, what together suggests, that auxin gradually accumulates in the inflorescence meristem of the *pin1* mutant. According to the latest hypothesis, it is believed that auxin is synthesized in older organs and transported to the meristem by PAT using the plasma membrane transporter *PIN1*, where it induces organ primordia formation. We have examined, whether auxin accumulates in the meristem and what is your source, when PAT is blocked. By analyzing the expression of auxin biosynthesis genes, auxin accumulation and auxin response, we have identified potential sources of this hormone, that may be responsible for the organogenesis and vascular development in *Arabidopsis thaliana* inflorescence, under the PAT inhibited conditions.

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Methodological concerns in respect to the AtHB8 gene expression analysis

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The AtHB8 gene (*Arabidopsis thaliana* homeobox 8) is a member of the HD-ZIP III gene family and is considered to be required for the irreversible determination of cells to differentiation into vascular elements. AtHB8 is expressed during vascular tissues formation already in the preprocambial stage, therefore is often used as a marker to identify the early stages of vascularization, mainly in the studies concerning leaf development. The transgenic line most commonly used, for such analysis, is the pAtHB8::GUS, where β-glucuronidase coding gene (GUS) is under the control of the AtHB8 gene promoter. In our studies pAtHB8::GUS line showed very high variability in terms of the localization and intensity of the GUS signal, which strongly depended on the plant growth conditions and the protocol for signal detection. In addition, the comparison of the AtHB8 gene expression pattern of two lines: pAtHB8::GUS and pAtHB8::YFP, in several experimental systems, showed significant differences between them in the signal localization in plant tissues.

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P1.3

Effect of after-ripening on abscisic acid content and its biosynthesis in triticale grains

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Primary dormancy is initiated during seed maturation, and it is characterized by the inability of seeds to germinate under favorable conditions. In some species, dormancy can be broken when seeds are still on the mother plant, which leads to pre-harvest sprouting (PHS). On the other hand, seeds of some species require a long period of dry storage (the after-ripening) to break dormancy. Abscisic acid (ABA) is a plant hormone that is responsible for acquisition of primary dormancy and its maintenance in dry and in imbibed seeds. It is assumed that the decrease of ABA content during germination of seeds may result from increased catabolism of this phytohormone; however it is also known that in the imbibed seeds a synthesis of ABA takes place as well. Thus, differences in the length of dormancy in different species and cultivars may be the result of differences in the balance between these two processes. In our work, we investigate the contribution of ABA and its biosynthesis in the dormancy maintenance of the mature, freshly harvested and the after-ripened grains of two triticale cultivars, which differ in the PHS susceptibility.

Two genes of 9-cis-epoxycarotenoid dioxygenase, TsNCED1 and TsNCED2, which is considered as a key regulatory enzyme in ABA biosynthesis, were cloned in triticale. The freshly harvested grains of Leontino cultivar, which is more susceptible to PHS, germinated in almost 100% when imbibed, while grains of Fredro cultivar, less susceptible to PHS, were still dormant. These differences were correlated with ABA content. During first hours of imbibition, in the embryos of both cultivars, a decrease of ABA content was observed; however in Leontino to a lower level. In subsequent hours of imbibition, the low ABA content was maintained in Leontino, while in Fredro an increase of ABA level occurred. The higher ABA content in Fredro was probably due to an intensified ABA biosynthesis, which resulted from the higher expression of TsNCED1, but not TsNCED2. The after-ripening did not change the ABA content in dry seeds of both cultivars; however led to the dormancy breaking of Fredro grains. When the after-ripened grains of this cultivar were imbibed, ABA content declined to a lower level, than after imbibition of the freshly harvested, dormant grains. The more pronounced decrease of ABA content was due to reduced expression of TsNCED1, but not TsNCED2. Thus after-ripening may affect the dormancy of triticale grains by weakening the ABA synthesis, which results from decreased expression of TsNCED1. To confirm that TsNCED1 is involved in regulation of ABA synthesis, a functional analysis was performed. Seeds of transgenic tobacco overexpressing TsNCED1 showed delayed germination and higher ABA content compared to seeds of wild type plants. Therefore, TsNCED1 might play a key role in the regulation of ABA content in triticale grains, thus affecting grains dormancy.

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Development of plastids in pollen grains of barley cultivars differing in the level of green and albino plantlet regeneration during androgenesis

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The doubled haploid technology is widely used to shorten the development of new varieties in breeding programs. Androgenesis, which is the process of plant development from a male gametophyte, is the most effective method of doubled haploids production. During microgametogenesis, after first pollen mitosis microspores form a generative and vegetative cell. The vegetative cell contains amyloplasts supplying starch for further actions. Amyloplasts, like all plastids differentiate from colourless proplastids, which are present in microspores but eliminated from generative cell during fertilization. When applying stress treatment (e.g. cold, starvation) to microspores isolated before the first microspore division, the androgenesis may be induced through in vitro microspore embryogenesis and plantlet regeneration.

However, androgenesis is a highly genotype-depend process whose effectiveness in cereals is vastly limited by regeneration of albino plantlets that lack chlorophyll in normally green tissues. It is widely known that albino plantlets contain undeveloped plastids that cannot fulfil their role.

In this study, two barley cultivars that differ extremely in terms of green plantlet regeneration during isolated microspore culture were chosen to evaluate the expression level of genes related to plastid development, plastome copy number and plastid ultrastructure during in vivo development of pollen grains, from early microspores to mature pollen. The expression level was estimated for genes related to transcription and translation of plastid genes, protein import, plastid divisions, plastome replication and starch biosynthesis. The divergent pattern of plastid differentiation during pollen development in vivo was observed based on the expression level of analysed genes. It was noticed that cultivar, which has shown less than 5% of green regenerants (cv. 'Mercada') during in vitro culture revealed faster differentiation of proplastids into amyloplast, compared to the cultivar with 95% of green plantlets (cv. 'Jersey'). At the stage of culture induction (mid-late to late uninucleate microspores), the cultivar with the low level of green plantlet regeneration contained amyloplasts with starch grains. Additionally, the plastome copy number was twice lower than in the cultivar with a high number of green plantlet regenerated. Moreover, cultivar 'Jersey' contained undifferentiated plastids at the developmental stage of microspores used to initiate the in vitro culture. To verify the correlation between the rate of plastid differentiation and level of green plantlet regeneration the expression level of genes related to starch biosynthesis was analysed during in vivo pollen development in 10 different barley cultivars.

P1.5

Identification of miRNA precursors deferentially expressed during generative organ abscission in yellow lupine based on the RNA-seq analysis

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Yellow lupin (*Lupinus luteus* L.) is an important legume crop, in which formation and development of flowers and seeds is often associated with their abscission. In our previous analysis of transcriptomes of flowers, flower pedicels and pods of *Lupinus luteus* abscised and maintained on plant by NGS sequencing we identified 28 deferentially expressed unigenes (DEGs) common for all library comparisons. Interestingly, among common DEGs we have also found a precursor of miR169, which is the first evidence of micro RNA engaged in abscission.

Plant miRNAs are about 21-nt-long small regulatory RNAs that recognize their mRNA targets based on imperfect sequence complementarity and in that way repress expression of the target gene by guiding degradation and/or translational repression of the associated mRNA target. MiRNAs are produced from single-stranded precursors that form hairpin structures, with the mature miRNAs sequence residing in one arm of the stems.

The aim of this work was to identify other miRNA precursors deferentially expressed during generative organ abscission in mentioned cDNA libraries.

As a result of the analysis of de novo assembled transcriptome of yellow lupine, we have found 527 sequences indicating homology to known miRNAs. Among them we revealed 7, 14 and 4 DEGs in flowers, flower pedicels and pods of *Lupinus luteus* abscised and maintained on plant, respectively. Identified putative precursors are very similar to pre-miRNAs indentified in other legume species. The occurrence of three pre-miRNA has been confirmed by PCR. Presented data are the first step to study the putative miRNAs involved in generative organ abscission in yellow lupine.

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Changes in accumulation of miR167, miR169 and miR390 precursors during development of generative and vegetative organs of yellow lupine (*Lupinus luteus*)

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Yellow lupine (*Lupinus luteus*) as well as other legumes, is a plant of high economic importance, due to its low soil requirements and variety of applications. One of the most important processes that affect its productivity is the effectiveness and speed of growth, which is regulated by both environmental conditions as well as internal plant signaling mechanisms. Important part of the mentioned above signaling mechanism is micro RNA (miRNA) which is directly tied to post-transcriptional gene silencing in plants.

The aim of the study was the analysis of three miRNA precursors which could – according to our preliminary data – participate and play a important role in regulation of generative development of yellow lupine. The plant material used in the study consisted of roots, root nodules, leaves, cotyledons, leaf pedicles, stems, hypocotyls and flowers. Plants were cultivated in growth chamber with stable environmental conditions, after 27, 36 and 48 days of growth vegetative organs were harvested. From the 48 day old plants the flowers were also collected.

Our study shows that the expression of miR167, miR169 and is highly specific both in different organs, as well as in different developmental stages. The results obtained in the course of this study are in accordance with literature data as well as our previous experiments, showing that miRNAs and its targeted genes are of major importance in growth and development of yellow lupine.

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P1.7

Genome-wide association study (GWAS) in maize (*Zea mays* L.) reveals new candidate loci for controlling response to light

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Light is one of the main factors, which regulate plants life. Many protein, such as phytochomes (Phy) or cryptochromes (Cry) are involved in light sensing colors and it's intensity. It is known, that natural variation in responses to light exists .Lloss of function in protein involved in light signaling in *Arabidopsis* usually results abnormal phenotype, which indicate importance of proper light sensing for plant functioning. In maize (*Zea mays* L.), probably due to its ancient genome duplication, much less light-mutants is known. It makes difficult to identify key genes involved in light sensing.

One useful predictor of plant light sensitivity is ratio between hypocotyl (in dicotyledons) or mesocotyl (in monocots) in dark-grown and light-grown plants. During de-etiolation process mesocotyl growth is inhibited so smaller mesocotyl length ratio between light and dark growth plants indicate higher light sensitivity. Decreasing cost of massive parallel sequencing has allowed re-sequencing of hundreds of maize genomes and identifying millions of polymorphism which can be used as markers in genome-wide association study (GWAS). The toolis promising approach for quantitative trait locus (QTL) mapping.

To explore natural variation in light response of maize we have measured mesocotyl length in ca 300 inbread lines under five different light treatments: white, red, far-red, blue and dark. We have found high correlation between responses to each light color, which confirm previous reports. Moreover we have found significant correlation between ratio of mesocotyl length in each light condition and dark and flowering time. This may indicate that light sensitivity of plant contribute to regulate this agronomic important trait.

In order to identify locus underlying maize response to different light we have performed GWAS based on ratio of mesocotyl length in each light condition and dark in population ~ 300 inbred lines and more than 20 mln single nucleotide polymorphisms (SNP). We have found more than 60 SNPs associated with response to each light with small and moderate effect. This indicates that light sensing in maize is polygenic and complex trait. These SNPs can be used as target for genome editing with CRISPR/Cas9 to better understand their role in controlling maize light response or used as marker in genomic prediction (GP) in breading program.

The involvement of chloroplast protein AtDeg2 in chronological progression of ontogenesis stages in *Arabidopsis thaliana*

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Chloroplast protein AtDeg2 is an ATP-independent serine endopeptidase containing a protease domain with a trypsin type catalytic triad (HDS) and two PDZ domains marked PDZ1 and PDZ2. Recombinant AtDeg2 was shown to catalyze in vitro hydrolysis of various artificial protein substrates thereby demonstrating to be bona fide proteolytic enzyme. Additionally, AtDeg2 might act as chaperone as well, as judged by its ability to inhibit aggregation of denatured lysozyme in vitro. Therefore, the regulatory functions of AtDeg2 in vivo are fulfilled due to the interplay between its protease and chaperone activity. Our studies are focused on the role of AtDeg2 in progression of ontogenetic stages in *Arabidopsis thaliana*, namely we investigate whether AtDeg2 chaperone activity is associated with the process. To achieve this goal transgenic plants expressing truncated AtDeg2 variant deprived of its chaperone activity were generated. Here we present the results of comparative phenotypic analysis of the wild type and the mutant plants.

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P1.9

Formation of the exocyst complex in plant cells

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Exocytosis is a fundamental process in all eukaryotic cells and is indispensible for cell growth and development that require delivery of components to the plasma membrane or to the extracellular environment. Exocytosis is localized process that occur preferentially at specific domains. Exocytotic vesicles are tethered at the plasma membrane by the octameric exocyst complex. All proteins in the complex have been conserved during evolution and and exocyst complex is functional in plants. Here we show localization of exocyst subunits in living plant cells and unravel some of protein-protein interactions between exocyst subunits and proteins interacting with exocyst using microscopy approaches. Using FLIM-FRET assay we show the potential spatial orientation of the subunits within the complex and distinguish the major and periferial subunits.

The enigmatic class II trehalose-phosphate synthase family

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There are 11 TPS (trehalose phosphate synthase) genes in *Arabidopsis thaliana*, and phylogenetic analysis shows that these form two distinct clades, class I (AtTPS1-AtTPS4) and class II (AtTPS5-AtTPS11). Both classes of TPS protein contain a glucosyltransferase domain similar to the TPS enzymes from *Saccharomyces cerevisiae* and *Escherichia coli*, and a C-terminal region that resembles the phosphatase domain of the yeast trehalose-6-phosphate phosphatase (TPP) but is catalytically inactive. Only class I TPSs have TPS activity and are responsible for synthesis of trehalose-6-phosphate (Tre6P), which is an essential sugar signalling metabolite in plants. The function of the class II TPS proteins is unknown. Expression of many class II TPSs is restricted to specific cell types, often in meristematic regions, and dependent on the plant's developmental stage and transcript levels are highly dynamic.

There is also evidence of post-translational modification of the class II TPS proteins. Analogous, non-catalytic proteins in yeast (ScTPS3 and ScTSL1) form a complex with ScTPS1 (TPS) and ScTPS2 (TPP), and influence the synthesis of Tre6P and trehalose. Furthermore yeast two-hybrid and bimolecular-fluorescence-complementation assays show that some of the class II TPS isoforms in rice can associate with OsTPS1, and co-immunoprecipitation experiments show that AtTPS1 can interact with AtTPS5 and AtTPS7, suggesting a possible role for these class II proteins in regulation of OsTPS1 and AtTPS1 activity. Conservation of Tre6P binding site residues in the TPP domain of the class II TPS proteins as well as suggests that they might also act as Tre6P binding proteins, with potential to play a role in sugar signalling pathways downstream of Tre6P.

Due to the strong likelihood of functional redundancy, double, triple and quadruple knock out mutants have been generated, in combinations based on phylogenetic relationship (clade I: AtTPS5-AtTPS7 and clade II: AtTPS8-AtTPS11) and expression patterns. Flowering is slightly delayedi n single mutants lacking TPS7, but not in the tps5 or tps6 mutants. However, flowering is further delayed in the tps5/tps7 and tps6/tps7 double mutants and most severely affected in the tps5/tps6/tps7 triple mutant. Loss of clade II TPS has little effect on flowering time, with only a 2-4 day delay even in the tps8/tps9/tps10/tps11 quadruple mutant. The tps7 and combinatorial clade I mutants have elevated levels of sucrose, Tre6P, trehalose, starch, several amino acids (glycine, serine, homoserine, proline and threonine), but decreased anthocyanin content. The metabolic phenotype is progressively more severe in double and triple mutants lacking TPS7, and is stronger in plants grown in high light conditions compared to low light. The quadruple tps8/9/10/11 mutant also has moderately increased levels of Tre6P and trehalose, but unlike the clade I mutants, has a higher anthocyanin content than wild-type plants.

P1.11

Blue light-controlled chloroplast movements in the model plant *Brachypodium distachyon*

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Chloroplast translocations are ubiquitous in photosynthetic organisms. On the one hand, they serve to optimize energy capture under limiting light, on the other hand, they minimize a potential photodamage of photosynthetic apparatus in excess light. In higher plants chloroplast movements are mediated by phototropins. These blue light receptors control also other light acclimation responses, phototropism and stomatal opening. So far, *Arabidopsis thaliana* was .the main model plant to study the mechanism of blue light signaling to chloroplast translocations in terrestrial plants.

Here, we propose *Brachypodium distachyon* as a model plant to study chloroplast movements in temperate cereals. Chloroplasts of *Brachypodium* respond to light analogously to those in *Arabidopsis*. According to BLAST analysis, the amino acid sequence of *Brachypodium* phot1 is 65% identical, and that of phot2 is 71% identical to the sequence of the respective phototropin in *Arabidopsis*. Both phototropin1 and 2 are expressed in *Brachypodium*, as shown using quantitative real-time PCR.

We investigated also chloroplast movements in leaves of four major crop grasses, wheat, rye, barley and rice. Chloroplasts respond only to blue light in all these species. Fluence rate-response curves for the investigated C3 grasses are very similar to those measured for *Arabidopsis*, which points to a similar mechanism of chloroplast redistribution in both groups.

Taking into consideration the conserved synteny and similarity of responses to light, *B. distachyon* appears to be a valuable model plant to study the molecular mechanism of chloroplast movements in cereals.

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Annexin 5 affects reproductive development in *Arabidopsis*

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Plant annexins comprise a family of multifunctional calcium and membrane binding proteins however, a detailed biological role of each family member remains poorly elucidated. Here we show that *Arabidopsis* annexin 5 (ANN5) is expressed in organ specific manner. Manipulation in expression level of ANN5 affects multiple aspects of plant development.

Analysis of ANN5 expression profile in *Arabidopsis* revealed the highest mRNA level in pollen grains. Consistently, RNAi-mediated suppression of ANN5 results in formation of smaller pollen grains, enhanced pollen lethality and delayed pollen tube growth in pistils. Moreover, ANN5 knockdowns display aberrant development during transition from the vegetative to generative phase and seed development that is reflected by delayed bolting time and reduced embryo size, respectively.

Live cell imaging of ANN5-GFP has shown that ANN5 is localized to the nucleus and transiently associates with plastid nucleoids. This finding together with the fact that ANN5 combines membrane and calcium ion binding capacities suggests that ANN5 may be implicated in the crosstalk between the nucleus and plastids. We hypothesize that ANN5 may contribute to changes in the plastid function during transition to the reproductive development, pollen formation and embryogenesis.

P1.13

Signalling and metabolic responses of *Arabidopsis* hormonal mutants to wounding of rosette leaves

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Wounding is a common stress factor in natural environment caused by abiotic factors (e.g. strong wind, hail) and biotic factors (e.g. herbivores, insect feeding, human activity). Wounding induces defense mechanisms in the place of injury (local response), and in undamaged tissues (systemic response). In both systemic and local responses many signalling molecules are involved, e.g. jasmonic acid (JA), abscisic acid, ethylene or some reactive oxygen species. Wounding induce defense mechanisms as a combination of biochemical and molecular processes which involve different enzymes and metabolites, although we are still far from understanding this complexity. The aim of the research was to verify, how mechanical injury of leaves of *Arabidopsis thaliana* L. influence physiological processes and overall metabolism including secondary metabolites content, oxidative stress parameters and antioxidant enzymes activity. Moreover the purpose of study was to check if hormonal imbalance in mutants could modify elements of signal transduction pathway and metabolism in response to wounding. We used *Arabidopsis thaliana* L., wild type (wt) and hormonal mutants: ein4 (ethylene insensitive), aos (deficit of JA, disrupted signal transduction of wounding), rcd1-1 (reduced sensitivity to ABA, ethylene, MeJA).

Measurements were made 2 and 24 hours after injury of rosette leaves. We observed that after wounding the strong response occur in the place of injury. Mechanical wounding of leaves caused fast deposition of callose and strong accumulation of H_2O_2 in the area of injured tissues. High level of H_2O_2 was observed also in vascular tissues of wt, ein4 and rcd1-1 mutants. However, in aos plants accumulation of H_2O_2 was lower, what indicate that low JA content in mutant can influence production of hydrogen peroxide.

Activities of catalase and superoxide dismutase were not significantly affected after injury in leaves of studied plants. In plants with ethylene insensitivity high level of polyphenols was observed and in rcd1-1 mutants significantly increased tannins content. These results indicate that mechanical wounding and changes in hormones sensitivity and/or biosynthesis have an influence on *Arabidopsis* metabolism. Our results also indicate, that in wounding signalling important role play H_2O_2 and JA. Increase of H_2O_2 after wounding in different tissues suggest both protective and signalling function of this compound.

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Profiling the expression of gibberellin metabolism genes and GA3 localization in the floral abscission zone of *Lupinus luteus*

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Yellow lupine (*Lupinus luteus* L.) is a pro-ecological species mainly due to the ability of symbiosis with Bradyrhizobium bacteria that fix nitrogen. Thus this species is a perfect forecrop and reduces the need for fertilizers. In addition, because of its high protein content in seeds, it is of great use. On the other hand the limiting factor in lupine yield is the premature and excessive abscission of flowers. This process occurs in the abscission zone (AZ) located at the base of these organs. Many studies indicate that the key elements which coordinate AZ functioning are phytohormones.

In this paper, we identified the cDNAs of genes involved in gibberellins (GAs) biosynthesis and catabolism. We have shown that their expression changes during functioning of AZ and under the influence of abscisic acid (ABA) or 1-aminocyclopropane-1-carboxylic acid (ACC) – the main modulators of flower's abscission in *L. luteus*. It was also confirmed by immunolocalization studies of gibberellic acid (GA3) which is accumulated in active AZ cells, as well as after ACC application. That effect was not observed after ABA treatment. Obtained results show that GAs interacts with other phytohormones may regulate the functioning of AZ in *L. luteus* flowers.

P1.15

Cloning, expression and kinetic analysis of 1-O-(indole-3-acetyl)-β-D-glucose: myo-inositol acyltransferase (IAInos synthase) from rice (*Oryza sativa*)

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Indole-3-acetic acid (IAA) regulates various physiological processes in plants thus precise control of concentration of this phytohormone is crucial for plant homeostasis. One mechanism responsible for regulation of the auxin level is conjugation of IAA to various molecules through ester or amide bond. Monocotyledonous plants are rich sources of IAA-ester conjugates (e.g. IA-myo-inositol, IAInos) although their biosynthesis pathway has only been partial characterized in *Zea mays*. Conjugation of IAA to myo-inositol is catalyzed by 1-O-(indole-3-acetyl)-β-D-glucose (1-O-IAGlc) : myo-inositol acyltransferase (IAInos synthase) according to the equation:

1-O-IAGlc + myo-inositol → IAInos + glucose

Based on alignment of amino acid sequences, IAInos synthase has been classified to the serine carboxypeptidase-like acyltransferase family (SCPL). SCPL acyltransferases are homologous to serine carboxypeptidases and they catalyze wide range of plant secondary metabolism reactions. SCPL acyltransferases utilize energy-rich esters of 1-O-β-glucose as acyl donors instead of coenzyme A thioesters which are substrates of more abundant BAHD acyltransferases.

In our previous studies we have identified IAInos synthase in extracts of rice seedling (Ciarkowska et al. 2016). Thus, in current study we have cloned gene encoding this enzyme to bacterial expression vector pET-28a(+) and produced recombinant protein in *Escherichia coli* strain BL21-CodonPlus(DE3)-RIL. IAInos synthase accumulated as insoluble inclusion bodies. Lowering induction temperature from 37°C to18°C did not result in increased solubility of recombinant protein but addition of 2% (v/v) glycerol led to its higher yield. Recombinant IAInos synthase was purified by preparative SDS-PAGE. Catalytically active IAInos synthase has been obtained by refolding procedure.

We have determined that optimal pH for recombinant IAInos synthase activity is 7.4. This enzyme exhibited Michaelis-Menten kinetics for myo-inositol with K_m of 0.83 mM and V_{max} of 18.5 nmol min-1 mg protein-1. Phenylmethylsulfonyl fluoride (PMSF) that is known inhibitor of serine carboxypeptidases, inhibits the IAInos synthase.

[1] Ciarkowska A, Ostrowski M, Jakubowska A (2016) Abiotic stress and phytohormones affect enzymic activity of 1-O-(indole-3-acetyl)-β-D-glucose : myo-inositol indoleacetyltransferase from rice (Oryza sativa)." Journal of Plant Physiology 205: 93-96

Induction of secondary dormancy in *Rosa canina* seeds – proteomic approach

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Secondary dormancy is induced by environmental condition e.g. under influence of warm temperature, in seeds which primary dormancy was fully broken. It is undesirable trait in agriculture, horticulture and forestry because require much more effort to overcome it than primary dormancy. Investigation of proteins, product of genes activated during a complex process as is secondary dormancy induction was the aim of presented research.

Seeds of wide rose (*Rosa canina* L.) were collected from individual trees in October 2015 and were desiccated to 9% of water content. Afterwards seeds were subjected to warm/cold stratification (16 weeks at 25°C followed by 22 weeks at 3°C) which leads to dormancy breaking and germination. The secondary dormancy was induced by using 20°C for 8 weeks after stratification. After warm/cold stratification cold treatment at 3°C caused seedling emergence at 51-75%, besides warm treatment at 20°C induced secondary dormancy in 94-97% of seeds. Only 3-6% of seeds germinated.

The protein extraction was done for fresh after drying seeds, seeds after warm/cold stratification (when dormancy was already broken), and after inducing secondary dormancy. Proteome maps (2D-IEF/SDS-PAGE) were established, which displayed on average 425 Coomassie blue stained spots. A total set of 19 spots showing significant changes in volume (Image Master 7, ANOVA and Tukey-Kramer test) were identified by ESI-MS/MS. For all spots the homologies were found in NCBI data base. Identified protein were classified into four class (KEGG database): metabolism (7 proteins), genetic information processing (5), cellular processes (4), unclassified (3).

It can be concluded that secondary dormancy is characterized by quantitative and qualitative changes in proteome. For secondary dormancy induction the most abundantly variable proteins were classified as involved in energy metabolism pathways and genetic information processing.

P1.17

Calcium-dependent protein kinase (CDPK) gene family in potato: genome-wide identification, expression in organs and involvement in disease resistance

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Plants are constantly exposed to different stresses and because of that, they have evolved a complex defence strategy providing a protection against stress factors. One of the response to different stimuli is change in concentration of intracellular Ca^{2+}. Calcium-Dependent Protein Kinases (CDPKs), unique for plants, are both important sensors and effectors of Ca^{2+} flux in plants. They are involved in growth and developmental processes as well as in defence strategy against different environmental stresses. CDPKs are encoded by multi-gene families. Despite extensive studies of CDPKs in many species, knowledge concerning the specific expression patterns and evolutionary history of the CDPK family in potato (*Solanum tuberosum*) remains very limited.

Presently, we have identified the whole CDPK family in potato using bioinformatics methods. The phylogenetic relationships and expression profiles of the CDPK genes identified in the potato genome were also estimated. Identified CDPKs were divided into four subfamilies based on a phylogenetic tree and gene structures. Next, detailed QPCR expression analysis were carried out for the CDPK genes in different organs such as young and mature leaves, stems, young shoots, roots, stolons, swollen stolons, flowers and tubers. The expression of all CDPKs was observed in all organs analysed, although the level of their expression varied greatly.

Furthermore, changes in CDPKs activities as well as their expression profiles were investigated in leaves of *Solanum tuberosum* cv Bzura, *S. tuberosum* clone H-8105 and *S. scabrum* that exhibited field resistance, susceptibility and non-host resistance, respectively, in response to the *Phytophthora infestans*. *P. infestans* is the pathogenic oomycete that causes late blight, the most destructive potato disease. Leaves of *Solanum* species were treated with elicitor (culture filtrate of *P. infestans*, CF).

CDPKs activities in *Solanum* leaves increased after elicitor treatment, were positively correlated with plant resistance, however varied with respect to intensity and timing. The expression of CDPKs in studied *Solanum* species were differentiated, also dependent upon the level of resistance. The obtained results provide a perspective on the evolutionary history and general biological roles of the potato CDPK family.

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Expression of PIN genes in root nodules of fabacean model plants

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Despite auxin is postulated as one of the pivotal factors in nodulation, the role of auxin polar transporters in this process remains unclear. Fabaceans can form either determinate-type root nodules, which exhibit temporary meristematic activity or indeterminate-type root nodules with persistent meristematic zone.

Auxin distribution can vary depending on the nodules type. Therefore, our study aimed to compare the expression level and tissue specificity of genes encoding auxin efflux carriers – PIN-formed proteins (PINs) in two species, *Medicago truncatula*, and *Lotus japonicus*, forming indeterminate-type or determinate root nodules, respectively.

Our previous qPCR results described expression profiles of PINs in *M. truncatula* root nodules. High expression level was a feature mostly of those, encoding orthologs of *Arabidopsis thaliana* ER-localized PIN5 subclade. Based on those data, three PIN genes were selected and their expression was analyzed within root nodule tissues using GUS reporter system. Their expression pattern varied depending on the nodule developmental stage. MtPIN4 expression was observed within the whole nodule primordium with the exception of outermost cortical cells, whereas the expression of MtPIN9 and MtPIN11 was limited to the apical, meristematic part of the nodule and to the vascular connection with root stele. During the nodule development, the gradual restriction of MtPINs expression to the nodule apical part occurred. The oldest root nodules, i.e. 8 weeks post inoculation, demonstrated strong expression of MtPIN4 and MtPIN9 in vascular bundle apices at the nodule meristematic zone. In mature *M. truncatula* root nodules, the expression of PIN11 was visible in the cells located directly below meristematic zone. However, it was hardly visible in the oldest examined root nodules.

Our study on determinate-type root nodules in *L. japonicus* enabled to identify eight LjPIN sequences. In order to indicate which of them play a role in the development of root nodules, we compared their expression levels in developing and mature root nodules. The gene encoding LjPIN6, which is a direct ortholog of ER-localized AtPIN6, demonstrated high expression in both examined stages. In developing root nodules, compared to mature ones, we also observed relatively high expression level of genes encoding LjPIN4 and other LjPINs orthologous to plasma membrane localized AtPINs. Taken together, our results suggest the importance of both, polar auxins transport and auxins intracellular homeostasis during *L. japonicus* root nodule differentiation.

Our results further support statement that auxins, along with their polar transporters, are pivotal in root nodules development regardless of their type. Moreover, we postulate that intracellular auxin homeostasis, dependent on ER-localized PINs, is essential for nodulation in both tested model fabaceans.

P1.19

Polyamines in dark-induced leaf senescence

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Leaf senescence is a terminal step in plant growth and development. Considerable information on processes and signals involved in this process has been obtained, although comparatively little is known about leaf senescence in monocotyledonous plants. In particular, little is known about players involved in leaf senescence imposed by a prolonged dark treatment. New information has now been unveiled on dark-induced leaf senescence in a monocot, barley. A close association has been found between ubiquitous polyamines, reactive oxygen species (ROS), and senescence of barley leaves during prolonged darkness. Although polyamines (putrescine, spermidine and spermine) are absolutely essential for critical cellular functions, including regulation of nucleic acids and protein synthesis, macromolecular structural integrity and signalling, a strong link between polyamines and dark-induced leaf senescence has been found using barley plant as a model of monocts. The poster summarizes the recent molecular, physiological and biochemical evidence implicating polyamines in dark-induced leaf senescence, broadening our knowledge on the mechanistic events involved in this important plant death process.

The data presented is a recent work conducted in our lab that was supported by grants from the Polish National Science Centre to ES-N (No. N N303 418236)

The use of RNA-seq for identification of genes associated with fertility restoration in sugar beet

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In sugar beet, hybrid seed production is based on cytoplasmic male sterility (CMS) which results from the presence of the so called sterilizing (S-) cytoplasm (carrying the mitochondrially encoded sterility determinant) as well as recessive alleles in two nuclear loci – X/x (Rf1/rf1) and Z/z (Rf2/rf2). The corresponding dominant alleles, referred to as restorers, bring at least a certain level of pollen fertility to plants carrying the S-cytoplasm. In the reported experiment we compared transcriptomes of five male-sterile and five male-fertile plants from a population in which male fertility was conditioned by the presence of the Z (Rf2) restorer. Sequencing data were generated with the Illumina platform employing the variant of PE100. Ca. 34 million reads were obtained per analyzed plant. Of those, over 95 % passed the quality control, and ca. 80 % were mapped to the reference genome. For 158 genes differences in transcript accumulation, which were observed between the sterile and fertile plants, were at least 4-fold. Of those genes, for 113 transcript accumulation was higher in the restored (male-fertile) plants and for 58 transcript accumulation was observed exclusively in such plants. Special attention was directed to chromosome 4 in which the Z (Rf2) restorer is located. At least 4-fold differences in transcript accumulation were observed for 45 genes from this chromosome, and for the majority of them (39) higher transcript accumulation was noted in the restored plants. The genes, which are upregulated in the male-fertile plants, may participate in nucleo-mitochondrial signaling and pollen production. Moreover, among such genes from chromosome 4, one can expect the presence of the Z (Rf2) restorer. Therefore, in the future research sequence polymorphisms found in the candidate genes from chromosome 4 will be examined with respect to co-segregation with fertility restoration.

P1.21

The effect of 24-epibrassinolide on the green alga *Acutodesmus obliquus* (Chlorophyceae)

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Brassinosteroids, a group of phytohormones, play an important role in the plant growth and development as well as in the adaptation of plants to environmental stresses. Studies explain the effect of 24-epibrassinolide (EBL) in the range of concentrations 0.0001-10 μM on the green unicellular alga *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata (SAG strain No. 276-6) (Chlorophyceae) during 7 days of cultivation. EBL is an effective stimulator for algal development, causing an increase of the number of cells and the contents of selected metabolites such as proteins, monosaccharides and photosynthetic pigments (chlorophyll a and b, carotenoids). Furthermore, EBL limits the formation of reactive oxygen species (i.e. hydrogen peroxide) and oxidative damage by inhibition of lipid peroxidation. The positive effect of EBL can be alleviating by antioxidants such as ascorbate peroxidase, catalase, superoxide dismutase and ascorbate. EBL is the most active at the concentration of 1 μM stimulating the growth and the content of metabolites and the activity of enzymatic antioxidants in the green alga at the 5th day of cultivation.

GUS reporter gene expression level in transgenic callus obtained from DR5rev::GUS, AtPIN1::GUS and AtPIN7::GUS *Arabidopsis thaliana*

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The mechanism of auxin transport and the formation of its gradient in the callus tissue is very important for the growth and differentiation of the callus cells in vitro conditions. The localization of the auxin gradient and selected AtPINs genes expression during the callus formation in vitro conditions was determined using the GUS (β-glucuronidase) reporter protein. Blue product of the GUS activity, providing a higher level of auxin, was observed only in the four outer layers, intensively proliferating of DR5rev::GUS callus cells. The localization of AtPIN1 gene expression included more external layers of callus cells than DR5rev::GUS. In contrast, the localization of the AtPIN7 gene expression was identified only in cells that most likely will come into being conductive vascular tissue cells in the future.

The aim of the study was to determine the level of the reporter protein GUS in transgenic callus obtained from DR5rev::GUS, AtPIN1::GUS and AtPIN7::GUS *Arabidopsis thaliana*. GUS fluorometric assays were carried out according to the method described by Jefferson et al. (1987) using microtitre well plates, 1–2.5 mg of protein, and 1 mM 4-methyl-umbellifereryl glucuronide as a substrate. GUS activity was determined by continuous assays using Fluorolite1000 microtiter-plate reader (Dynatech Laboratories) and software. Successive fluorescence readings were determined at a wavelength of 365/460 nm. For all the results shown in this work, samples were extracted twice and each extraction was assayed in duplicate.

It has been shown that the level of GUS reporter gene expression in transgenic callus obtained from DR5rev::GUS was an approximate 30 % less than in AtPIN1::GUS callus. On the other hand, it was found that GUS activity level was at AtPIN1::GUS callus three times higher than in AtPIN7::GUS callus, which confirms earlier results that the AtPIN7gene plays an important role in the formation of callus in vitro conditions.

P2.1

Luffa cylindrical L. as a model for phloem study in symplastic loaders

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The Cucurbitaceae species (cucurbits) are widely used as model plants for research, especially in studies of phloem physiology. These species are unusual in that, they possess a dual phloem transport system, that includes fascicular phloem (FP) located in vascular bundles and extrafascicular phloem (EFP) located peripheral to the vascular bundles. Cucurbits are known as symplastic phloem loaders that transport α-galactosyl-sucrose oligosaccharides. Despite intensive studies, many aspects of phloem biology of cucurbit plants have not been yet explained.

Most cucurbit species are considered to be relatively difficult to genetically modify. However our previous results suggest that the leaves of *Luffa cylindrica* L. (luffa), belonging to Cucurbitaceae family, can be transiently transformed in a relatively simple manner and used as plant expression system. We are convinced that use of cucurbit expression systems could greatly improve our knowledge of physiological and biochemical processes in these plants. The loose anatomical structure of luffa leaves allows to use the leaves as a tool for studies requiring the application of liquids into their tissue, e.g. for monitoring of macromolecules long distance trafficking in the phloem. In contrast to other cucurbits species, the anatomical structure of luffa leaves allows for an effective application of *Agrobacterium tumefaciens* to transient transformation.

Here we present the results of epifluorescence microscopy and morphometric analysis of sieve element systems in selected Cucurbitaceae species. The purpose of these comparative analyses was to determine whether luffa could be used as a representative model for phloem biology studies in Cucurbitaceae. In the later part of our work, we analysed the efficiency of the ectopic gene expression in luffa leaves compared to *Nicotiana benthamiana* – the most popular transient plant expression system.

The relative expression level of analysed reporter genes in agroinfiltrated luffa leaves was lower than in *Nicotiana benthamiana*. However, due to the greater leaf biomass produced by luffa, can these plants in many cases appeared to be a better alternative to the production of recombinant proteins than *N. benthamiana*. Moreover, we observed that in contrast to *N. benthamiana*, the efficiency of luffa leaves agrotransformation was largely independent of the growth phase of these plants.

Dystribution of cell wall components in *Daucus* protoplast cultures

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Re-synthesis of the cell wall is one of the most important key steps in protoplast development preceding mitotic divisions and initiating establishment of a protoplast-to-plant system. Over the years many systems of protoplast culture including different variants of chemical nursing have been applied to over 400 species. Recently, phytosulfokine (PSK) – a peptidyl plant growth factor, has been recognized as a promising intercellular signaling molecule involved in cellular proliferation and dedifferentiation. It was shown that PSK stimulated and enhanced cell divisions in protoplast cultures of several species leading to callus and proembryogenic mass formation. The aim of the present study was to investigate composition of the reconstituted cell wall in protoplast-derived cells of two carrot subspecies to evaluate the effect of PSK on the distribution of cell wall components.

The analyzed tissues were obtained from protoplast cultures of *Daucus carota* subsp. *sativus* and *Daucus carota* subsp. *gadecaei*. Protoplast were isolated from 2-week-old shoot cultures, embedded in alginate solutions and cultured in CPP medium (Maćkowska et al. 2014) or CPP medium supplemented with 100 nM of PSK. The samples from protoplast-derived tissues were collected after 10, 20, 30 and 60 days of cultures, released from alginate matrix by its depolymerization in sodium citrate solution and positioned in 1% Seaplaque Agarose. For immunohistological analysis samples were prepared according to Sala et al. (2017), cut with the Zeiss HYRAX M40 rotary microtome and collected on microscopic slides covered with poly-L-lysine. For immunolabelling sections were incubated with primary monoclonal antibodies (pectic epitopes: LM20, LM19; AGP epitopes: JIM4, JIM8, JIM13; extensin epitope: JIM12), and then with the secondary antibody (AlexaFluor 488 goat anti-rat IgG, Sala et al. 2017).

In *Daucus carota* subsp. *gadecaei*, cells derived from PSK-treated cultures in comparison to control cultures were characterized by: a/ lower presence of pectic epitope recognized by LM19 antibodies, b/ changes in the presence of pectic epitope recognized by LM20 antibodies in respect to culture duration, c/ presence of AGP epitopes and extensins recognized by JIM12 antibodies.

Cells of *Daucus carota* subsp. *sativus* derived from PSK-treated cultures in comparison to control showed: a/ lack of any differences in pectic and AGP epitopes after 10 days of culture, b/ differences in AGP epitope localization recognized by JIM4 antibody after 20 days of the culture, c/abundant presence of extensins after 60 days of the culture.

Obtained results suggests: 1) diverse response of used subspecies to PSK in terms of culture development, 2) diversity in chemical composition of cell walls in control and PSK-treated cultures.

[1] Maćkowska et al., (2014). Plant Cell Tiss Organ Culture 117: 241-252 Sala et al., (2017), BMC Plant Biol 17:25

P2.3

Gamma-secretase complex from *A. thaliana* as an endocytosis contributor?

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Gamma-secretase is a complex of four proteins, each with at least one transmembrane domain. In most cells, the complex includes: presenilin (PS1 or PS2), nicastrin (NCT), APH-1 (anterior pharynx-defective 1) and PEN-2 (presenilin enhancer 2) – and are present in 1:1:1:1 ratio. Over the years, presenilins and γ-secretase have been very extensively investigated in animals, particularly in humans, due to participation in the regulation of the Notch signaling pathway, which controls embryogenesis, and in the processing of amyloid precursor protein (APP). Mutations in the γ-secretase complex may lead to abnormal cleavage of APP – what is directly correlated with an etiology of Alzheimer's disease. γ-secretase is also involved in the regulation of Ca2+ flux in cells. Complex may be involved in the regulation of endocytosis, recycling and degradation of selected membrane receptors. In animal cells mutations of any presenilin result in reduced endocytosis of membrane receptors, such as transferrin, Notch or EGFR. The roles of gamma-secretase complex in plants are almost completely unknown, available results are derived from studies of the moss *Physcomitrella patens* and the mouse-ear cress *Arabidopsis thaliana*. In *A. thaliana* protoplasts, the existing data confirm localization of γ-secretase subunit homologs within endomembrane system. Interestingly, however, none of known major substrates of the animal γ-secretase complex, has been identified in plants. Presenilin and the whole complex of γ-secretase has been hypothetically considered as complex regulating vesicular transport.

Here we show contribution of gamma-secretase complex in endocytosis in *A. thaliana* cells. Impact of presenilins on endocytosis process was analyzed by observation of FM4-64 dye endocytosis in *A. thaliana* seedlings, respectively in wild type and ps1/ps2 mutant plants. Interactions during endocytosis between γ-secretase complex and receptor proteins – FLS2 (flagellin-sensitive 2) and BRI1 (brassinosteroid insensitive 1) was examined. Furthermore the capability of complex to taking activity towards animals receptors: hTFR (human transferrin receptor) and EGFR (epidermal growth factor receptor) was analyzed. These proteins are not the direct substrates of the complex, but their correct endocytosis depends on presenilins activity.

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Deciphering cell-to-cell communication in wood

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Studies concerning communication processes over long- and short-distances within two distinct systems of symplasm and apoplasm are crucial for understanding of tree functioning. In wood, the major secondary tissue of trees, symplasmic and apoplasmic transport proceeds via different cell types. The vessel elements are responsible for fast and long-distance transport of water within the apoplasm system, whereas the symplasm system is comprised of long-living parenchyma cells.

In our project we visualise transporting pathways between parenchyma cells of secondary xylem, by the application of various fluorescent tracers, in the presence or absence of different metabolic inhibitors, to three species of trees – *Acer pseudoplatanus* (sycamore maple), *Fraxinus excelsior* (European ash) and *Populus tremula* x *tremuloides* (hybrid aspen). Concomitantly, the cellular and subcellular localisation of the tracers is analysed with the fluorescence and confocal laser scanning microscopes. Our study revealed that the cells of the xylem parenchyma are involved in 1) an efficient symplasmic exchange of nutrients with use of plasmodesmata and 2) solute transport from the vessel elements using the routes of the vesicular transport and the transcellular exchange via anion transporters.

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P2.5

Localization of selected primary cell wall proteins and polysaccharides during somatic embryogenesis of Dendrobium berry „Oda”

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The cell walls are very dynamic structures enclosing each plant cell and still allowing transfer of solutes and signalling molecules between the cells themselves and the cells and environment, control of cells and the whole plant form, growth and development; they play also a significant role in plant defence and their responses to environmental stresses. To fulfil these functions plant cell walls must be a tightly regulated dynamic system in charge of sensing, processing and responding to internal and external cellular signals, functioning as an “intelligent frontier” capable to co-ordinate growth of the whole-plant by optimizing growth and differentiation of individual cells. Thus, it is believed that its components and the way they are arranged in the cell wall could have an effect on some developmental processes, including somatic embryogenesis.

In this work an attempt was made to localize the selected proteins and polysaccharides of the primary cell wall during somatic embryogenesis of Dendrobium berry ‘Oda’ on different steps of development, including embryogenic cells, somatic embryos, young plantlets and leaf of mature plants. To localize arabinogalactan proteins, arabinans, xyloglucans, β-1,4-mannans, and esterified and unesterified pectins specific primary rat IgM monoclonal antibodies LM14, LM13, LM25, LM22, LM20, LM19, respectively, were used. The AlexaFluor 488 goat anti-rat IgG was used as secondary antibody. All images were taken using a confocal microscope NIKON A1R MP (Nikon) equipped with a digital camera DS-5Mc (Nikon).

Distribution and content of studied cell wall components differed greatly in cells during induction and development of somatic embryos of Dendrobium. Arabinogalactan proteins were localized mainly in embryogenic cells and mature plants. They were distributed either intracellularly or in the cell walls. Xyloglucans were localized uniformly in cell walls and their amount was increasing during embryo development in contrary to mannans and arabinans which were widely distributed in cell walls on every developmental stage, but their content was decreasing with plant maturation. Pectins, both esterified and unesterified, were present in cell walls during all stages of Dendrobium development. Esterified pectins were most abundant in cell walls of young embryos whereas the content of unesterified ones increased with the embryo development.

Our data showed that somatic embryogenesis of Dendrobium orchid involves a number of changes within the cell wall macromolecules including many proteins and polysaccharides. A dynamic reorganization of the cell wall components is essential because of a variety of important biological functions they perform. A more precise understanding of their functions during the somatic embryo development is essential.

Phytotoxicity of diclofenac: a study with the green alga *Chlamydomonas reinhardtii*

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The occurrence of pharmaceuticals and personal care products in the environment is an emerging problem nowadays and the non-steroidal anti-inflammatory drug diclofenac (DF) belongs to the most ubiquitous pharmaceuticals in aquatic ecosystems. Diclofenac, specifically designed to elicit a biological response in humans and animals might have negative effects on non-target organisms. To date, little is known about potential detrimental effects of DF on plants and algae. Thus, the aim of this study was to investigate the effect of DF on the green alga *Chlamydomonas reinhardtii*, regarded as a model organism in physiological, biochemical and toxicological studies at both cellular and molecular levels.

C. reinhardtii wild type cc-1690 (Chlamydomonas Resource Center, USA) was grown in intensive batch cultures (30°C, 2.5% CO_2, ~125 μmol×m^{-2}×s^{-1} PAR). DF inhibited the growth of algae population in a concentration dependent manner (EC50 = 426 μmol/dm^3).

DF at EC_{50} concentration caused diminishment of the chlorophylls content in the cells (60-80% of control) while the carotenoids level increased (~150% of control). Chlorophyll a fluorescence in vivo analysis (OJIP test) showed that DF inhibited photosynthesis performance (P.I. ~50% of control) mainly due to lowering of the active PS II reaction centres fraction (RCm ~60% of control). The yield of primary photochemistry (φPo) was also lower (~80% of control) in DF-treated cells while the non-photochemical energy dissipation parameter (DI0/RC) was much higher (~150% of control). The most pronounced change in DF-treated algae was the two-fold increase of H_2O_2 production which indicates strong oxidative stress in the cells.

The data suggest that inhibition of population growth caused by DF results from its negative effect on photosynthesis and from oxidative stress induction in algal cells. We suppose that contamination of the water environment by anti-inflammatory drugs may potentially cause adverse effects on non-target organisms, including algae populations.

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P3.2

An ATP-binding cassette subfamily G full transporter is potentially involved in lipid derivatives translocation in *Medicago truncatula*

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Members of the so-called G subfamily of ABC transporters have been shown to be involved in numerous physiological processes associated with plant response to biotic and abiotic stresses. There are at least 30 full-size ABCGs identified in a model legume *Medicago truncatula*. Phylogenetic analyses of them revealed a clade consisting of homologs of transporters involved in functional cuticle formation in various plants namely: *Arabidopsis* (AtABCG32), Rice (OsABCG31) and Barley (HvABCG31). Among *Medicago* members of this clade, we focused on the ABCG19 protein. Performed phenotypic tests on mtabcg19 mutants indicated higher water loss and increased permeability of the cuticle thus suggesting also functional homology within this clade. Since MtABCG19 is expressed not only in the aboveground tissues but also in root therefore, we raised a question about its role in this organ. We have tested the level of MtABCG19 mRNA accumulation in roots upon mild water shortage mimicked by PEG/ABA. Applied drought stress strongly induced MtABCG19 expression alongside with MtGPAT5 gene from suberin biosynthesis pathway. The spatiotemporal expression pattern of MtABCG19 is consistent with the deposition place of suberin, a lipophilic macromolecule, similar to cutin in structure but specific to roots (suberin, usually differs from cutin, which has C16 and C18 fatty acids, by a higher content of C20–C24 aliphatics and aromatics). Further phenotypic analyses by fluorescent imaging of mtabcg19 mutant roots has shown differences in suberin deposition pattern between WT and mtabcg19 mutants. Obtained results, together with the observation that MtABCG19 is a plasma membrane protein, suggest that it is possibly involved in translocation of different cutin and suberin monomers in a spatial manner.

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The influence of mineral nitrogen on the *Medicago truncatula* root nodules

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Legume species are unique among cultivated plants for their ability to carry out endosymbiotic nitrogen fixation with rhizobial bacteria, a process that takes place in a specialized structure known as a nodule. In order to investigate this symbiotic process, *Medicago truncatula* Gaertn. was chosen as a model plant. The developmentally structured organization of *M. truncatula* nodules makes them an ideal system to study the different stages of nodule and symbiosome development. Although many issues connected with symbiosis are well documented the influence of mineral nitrogen on the nodulation and structure of the root nodules remains an open question. It is known that low dose of nitrogen (0,5 mM) may stimulate the inoculation whereas the excess (above 10mM) suppress the nodulation and impair the nitrogen fixation. The main goal of our project was to characterize the influence of mineral nitrogen on the process of nodulation. We focused on the disturbances in the structure and ultrastructure. Firstly, we verified how the nodule are growing in the presence of different concentrations of nitrogen in the substrate. Secondly, we investigated the structure and ultrastructure of root nodules using light and transmission electron microscopy. Although the nitrogen may inhibit formation of root nodules we showed that even high nitrogen concentration (20 mM) do not disturb the nodulation. However, the root nodules are smaller and the alterations in the cell structure, especially in cortical layers are visible. Our study is the first step in the description of the effects of mineral nitrogen excess on the root nodules.

P3.4

Railroad tracks – "research training ground" to study microevolutionary processes of plants

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Microevolutionary processes of plants occuring on the anthropogenic habitats play a special role in the era of progressive environmental pollution. One of the areas where microevolutionary processes may take place are railway tracks. There are difficult conditions for plants growth, among others: insolation, and poor in nutrients substrate, which consists of crushed stone mixed with river sand. In addition, railway tracks are the areas of contamination with heavy metals, PHA and PCB, as well as regular herbicide treatment. The intensification of stress factors occurring on railway tracks leads to strong natural selection, which favors the microevolutionary processes.

The aim of the research was to verify, whether the microevolutionary processes have occurred on railway tracks, which could lead to creation of a new form of the plant *Geranium robertianum* L., adapted to occurrence on railway tracks. *G. robertianum* is a herbaceous plant which prefers shaded, fertile and moist forest habitats, with a high content of phosphorus and nitrogen in the soil. It occurs commonly in Poland and Europe. Apart from forest areas, it also can be found on railway areas. An interesting question is why the railway embankments are widely settled by the *G. robertianum* plants, despite the conditions prevailing there are completely different from those that this species prefers.

In frame of the research two populations of plants were compared: "railway" population from Waliły-Stacja village and "forest" population from woods near Zajezierce village (North-eastern Poland). During morphological observations and physiological studies it was shown that plants of the railway population were smaller, had a smaller assimilation surface and showed better adaptation to high intensity of light exposure, than the forest population. Plants from the railway population were characterized by increased levels of anthocyanins, which indicates better protection of photosynthetic apparatus against the insolation. Therefore, plants of the railway population showed adaptation to unfavorable conditions on the railway tracks. Further studies have shown that the *G. robertianum* track population from Waliły-Stacja also stands out in comparison to other populations of this species, both track and forest. Studies have shown formation of separate forms of *G. robertianum* on railroad tracks in Waliły-Station. This is an excellent example of occurence a microevolutionary processes in plants on anthropogenic habitats.

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The role of gibberellin and zearalenone in the mechanism of vernalization

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Wheat is an annual crop that requires the exposure to cold temperatures and long days in order to undergo transition from the vegetative to the reproductive phase. The physiological mechanism of vernalization is interesting in that sense that the perception and initial transduction of the environmental stimulus occur during the chilling period, but the developmental consequences are expressed after vernalization. Since GAs are involved in the regulation of both stem elongation and flowering in numerous plants, it has repeatedly been proposed that GAs are involved in the regulation of events following vernalization. Although previous works showed contribution of GAs as a mobile signal transmitting flowering stimuli in grasses, they are not the sole factor in determining transition into flowering. Their concentrations are modulated by integrating various endogenous and external signals. The mechanisms of vernalization underlying the process are not yet fully understood. Plant regulators may partially substitute vernalization; zearalenone (ZEN) showed high activity in shortening flowering induction period during vernalization of winter wheat (Biesaga-Kościelniak and Filek 2010). The mechanisms of ZEN action remains unknown. Our aim was to verify the hypothesis: ZEN-induced stimulation of wheat generative development is via regulation of GA production.

During short vernalization of isolated wheat embryos in in vitro conditions (MS0 and medium with ZEN) levels of GA1, GA3, GA4, GA5, GA6, GA7 were monitored by UHPLC-MS/MS (Dziurka et al., 2016). GA3ox transcript accumulation was analyzed by qPCR. Samples were collected after 0, 3, 6, 9, 12 and 15 days of vernalization at 5°C fallowed by 10 day acclimation at 10°C. It was found that ZEN significantly modifies GA levels through the changes in accumulation of transcripts of genes coding enzymes involved in GA synthesis. This effect could explain the acceleration of wheat development induced by ZEN.

[1] Biesaga-Kościelniak J., Filek M. (2010). In E. Lichtfouse (Ed.), Sustainable agriculture reviews 3. Sociology, organic farming, climate change and soil science (pp. 419–435). Berlin: Springer

[2] Dziurka M., Janeczko A., Juhasz C., Gullner G., Okleskova J., Novak O., Saja D., Skoczowski A., Tobias I., Barna B. (2016). Plant Physiology and Biochemistry

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P3.6

Cadmium affects root growth and polypeptides pattern of *Vicia faba* seedlings

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Cadmium (Cd) has become a widespread non-essential heavy metal and one of the most toxic and dangerous environmental pollutants, with relative high mobility in the soil-plant system. The roots are well established as a main site of Cd action leading to numerous disorders including inhibition of root growth, alterations in their morphogenesis and causing changes in global gene expression as well as modifications of protein metabolism. In the present study, we examined the growth rate of roots, polypeptides pattern and expression of selected genes in root tips of broad bean (*Vicia faba* L.) seedlings treated with 70 μM and 140 μM Cd for 24 and 48 hours. The applied concentrations of Cd reduced root growth by 12% in the case of 70 μM and by 40% in the case 140 μM Cd as compared to control seedlings. Denaturing gradient gel electrophoresis of protein extracted from root tips demonstrated Cd dependent changes in accumulation of some polypeptides. The most pronounced included polypeptides with molecular weight of about 20, 66 and 116 kDa. The mass spectroscopy analysis of selected polypeptides revealed proteins involved in primary metabolism and plant defense strategy including DNA replication, protein synthesis, ubiquitination of proteins and response to biotic (bacteria and fungus) and abiotic (heavy metals, cold) stresses. The level of transcripts encoding selected proteins will be evaluated to confirm their involvement in response to Cd stress.

Phytoextraction of arsenite in young tree species – hydroponic experiment

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Arsenic (As) originating from anthropogenic and natural sources is a significant environment problem, especially when As levels exceed the safe amount for human health (in soil, water and air). One of the most promising biological method is phytoremediation and mainly phytoextraction, where contaminants are accumulated from substrates by roots and can be transported to the harvestable plant organs. The phytoextraction of As is dependent on numerous factors, e.g. the specific interaction between As and other elements but also the possible interactions between this metalloid forms.

The aim of studies was to investigate the uptake and accumulation capabilities one of As form, arsenite [As(III)], by one-year-old tree species (*Acer platanoides* L., *Betula pendula* Roth., *Quercus robur* L., *Ulmus laevis* Pall) in hydroponic culture. A further aim was to analyse the influence of As(III) on the content of selected nutritional minerals, such as B, Ca, K, Mg, Na and Si. The highest accumulation of As(III) in *A. platanoides* (BCF = 2.16) and Q. robur (BCF = 2.69) was observed. In *A. platanoides* 80% of total As(III) of the whole plant was accumulated in its stem (TF=10.8). TF for *Q. robur* was 0.1 and this tree species accumulated As(III) mainly in its roots – 85% of total As(III) of whole the plant. The high As(III) accumulation capabilities of *A. platanoides* and its location predominantly in the stem classify this tree species as a hyperaccumulator and a good candidate for As(III) phytoextraction, while Q. robur would be more suitable for phytostabilization and eco-restoration.

As(III) uptake also caused alteration in the content of nutritional minerals. Decreased levels of B and Si were detected in the roots of most trees exposed to As(III) indicating the competition between these elements in the uptake process. In *A. platanoides* stems a high accumulation of As(III) resulted in a significant decrease of Si content in all the studied organs, suggesting that As(III) was transported from root to shoot predominantly by Si transporters. As(III) also led to a general alteration in the level of other macronutrients – e.g. an increase in the level of Na and K. As(III) uptake and accumulation results in a deterioration of cation balance and homeostasis, which could be one of the factors affecting the vitality of the examined trees.

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P3.8

Changes in apoplastic metabolism of reactive oxygen species in RBOHD and RBOHF mutants of *Arabidopsis thaliana* during ammonium treatment

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The use of ammonium (NH4) as the only source of nitrogen in the soil solution leads to the growth inhibition and development disorders („ammonium toxicity"). One of the reasons of plant growth limitations may be the changes of reactive oxygen species (ROS) metabolism in the apoplastic space. Recently we demonstrated that the treatment of *Arabidopsis thaliana* plants with the nutrition containing NH4 resulted in the oxidative stress and increased apoplastic ROS production (Podgórska et al. 2015). The homeostasis of apoplastic ROS is crucial for maintaining the proper plant development. It is known that apoplastic ROS are involved in the regulation of plant cell elongation and division. The main amount of apoplastic ROS is derived from NADPH oxidases (RBOHs). RBOHs are plasma membrane located enzymes encoded by 10 genes in *Arabidopsis thaliana* (rbohA-rbohJ). The purpose of this study was to determine the influence of decreased expression of RBOHD and RBOHF isoforms on the metabolism of apoplastic ROS during ammonium nutrition.

The content of H_2O_2 and concentration of low molecular mass antioxidants in apoplast were analyzed. Obtained results showed that the dysfunction of RBOH isozymes does not have major affect on apoplastic ROS metabolism under long-term NH4 nutrition. Our results suggest that other enzymes rather than RBOH D and F are involved in the modification of apoplastic ROS metabolism during ammonium treatment.

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Photosynthetic apparatus arrangement of *Arabidopsis thaliana* mutants with reduced lutein content under light stress conditions

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Lutein is the most abundant carotenoid in higher plant chloroplasts. This carotenoid is extremely important for the photosynthesis process being both an antennae complex component and a lipid phase component. As a membrane component it influences the membrane rigidity due to lutein vertical and horizontal orientation in lipid bilayer.

The aim of this investigation was to demonstrate the structural role of lutein in the thylakoid membrane arrangement and the influence of its reduced content on the condition and composition of the photosynthetic apparatus in plants cultivated in the optimal light condition and in light stress conditions.

A. thaliana mutants with reduced different lutein contents (ccr1-1, ccr2-1, lut2) were used for experiments. Structural investigations were performed with the help of transmission electron microscopy and also confocal microscopy for 3D modeling. Obtained structures were correlated with the photosynthetic complexes (CP) composition measured by the 77K fluorescence, immunodetection and with photosynthetic apparatus functionality detected by the chlorophyll a fluorescence in vivo. Differences in the chloroplast grana structure, especially in their height and in stroma lamellae in particular, were demonstrated. Structural changes were accompanied with different arrangement of CP complexes and modified functionality of the photosynthetic apparatus. This points out the importance of lutein in the formation and function of thylakoid membranes. These results will provide a basis for the analysis of the lutein role in the rearrangement of plastid membranes in the early stages of chloroplast biogenesis in *Arabidopsis thaliana* cotyledons.

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TEM images were performed in the Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology on High-Performance Biology Transmission electron microscope JEM 1400 (JEOL Co., Japan, 200 equipped with tomographic holder and 11 Megapixel TEM Camera MORADA G2 (EMSIS GmbH, Germany)

P3.10

Biomarkers of oxidative stress in *Salix eriocephala* exposed to heavy metals

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The increasing level of heavy metals in the environment may affect plant growth and metabolism. A consequences of the presence of toxic metals in plant tissues is the formation of reactive oxygen species (ROS), responsible for initiation of oxidative damage. The premise of this study was to examine whether and to what degree was induced the oxidative stress in willow (*Salix*) growing in hydroponic cultures, exposed to cadmium and zinc ions. We evaluate the response of one-year-old cuttings of *Salix eriocephala*. Differences in generation of free radicals, i.e. semiquinone (being organic radicals) and ROS (superoxide anion and hydrogen peroxide) was observed. The level of lipid peroxidation was used as an indicator of ROS mediated damage of cell membranes. Increased peroxidation of lipids was associated with enhanced lipoxygenase (LOX) activity.

Sexual reproduction efficiency of relict stands of *Betula nana* L.

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The dwarf birch *Betula nana* L. is one of the climatic relict plants in central Europe. The main objective of this study was to assess resources of genetic diversity and to investigate the effectiveness of generative reproduction of the dwarf birches in relict and central populations. Amplified fragment length polymorphism (AFLP) method revealed that the relict stands from Poland and Belarus were genetically different but not genetically depauperate compared with the widespread localities from Finland and Russia. The matrix incompatibility counts implied that most genotypes were formed by meiotic recombination, which is associated with the sexual mode of reproduction. Comparisons of reproductive parameters among relict and central stands of *B. nana* revealed that the number of male flowers and seed mass were higher in relict populations, whereas the number of seeds germinated after scarification was higher in central localities. Based on the last parameter, which is more important in determining the generative reproduction efficiency, we assume that the effectiveness of sexual reproduction of *B. nana* can be higher in the central populations compared with the relict stands.

P3.12

Desiccation and cold stress induce accumulation of sucrose and RFOs, but not cyclitols, in seedlings of fenugreek (*Trigonella foenum-graecum* L.), alfalfa (*Medicago sativa* L.) and buckwheat (*Fagopyrum esculentum* Moench.)

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Sucrose, raffinose family oligosaccharides (RFOs) and cyclitols are considered to be low molecular weight metabolites involved in the plants tissues response to the abiotic stresses such as desiccation, cold and osmotic stress. In 7-days old seedlings of fenugreek, alfalfa and buckwheat both desiccation and cold stress induce drastic changes in the composition and content of soluble carbohydrates. Tissues of roots, hypocotyls and cotyledons accumulate a large amounts of sucrose (up to 130 mg g-1 of dry mass), which coincides with the reduction of the levels of fructose and glucose. Both stresses induce biosynthesis and accumulation of galactinol and RFOs (raffinose and stachyose), but not species-specific cyclitols: D-pinitol (in fenugreek and alfalfa) and D-chiro-inositol (in buckwheat). Tissues accumulate more sugars in response to desiccation, in comparison to the cold stress. The concentrations of raffinose and stachyose are highest in hypocotyl (up to 12 and 4 mg g-1 DW, in buckwheat), and lowest in roots.

The concentration of RFOs in seedlings of buckwheat is several-fold higher than that in fenugreek and alfalfa. Our results confirm the involvement of sucrose and RFOs in response of vegetative tissues to desiccation and cold stress. However, the protective role of cyclitols seems to be questionable in regard to negligible changes in their concentrations.

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The role of acid phosphatases in oat plants (*Avena sativa* L.) acclimation to Pi deficiency

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Deficiency of inorganic phosphate (Pi) is a common feature in the environment, including cultivated soils. Plants acclimate to Pi deficiency by developing a number of mechanisms increasing Pi uptake and transport or Pi mobilization/recycling processes. Acid phosphatases (EC 3.1.3.2, APase) are believed to enhance Pi uptake and are important components of many plants responses to Pi starvation. The main purpose of this study was to investigate the mechanisms that enable oat plants (*Avena sativa* L.) to grow under low Pi conditions. In particular, we focused on the activity and localization of APases to evaluate their role in oat response to Pi deficiency. Oat plants have been usually grown on poor soils, with a low Pi content, but their adaptive responses to such conditions are not fully understood. Four oat cultivars (*A. sativa*, cv. Arab, Krezus, Rajtar and Szakal) were grown for three weeks in nutrient media with various phosphorus sources: inorganic – KH_2PO_4 (+P, control), organic – phytic acid (PA) and with no phosphate (-P). Plants grown on -P nutrient media had significantly decreased phosphorus content in the tissues. Pi deficiency caused inhibition of shoot growth, root elongation was not affected and root/shoot ratios of -P plants increased, whereas PA plants showed a similar growth to control. Photosynthesis rate as well as productivity parameters were decreased under low Pi nutrition. Generally, pH changes in the growth media or rhizosphere were not observed. Extracellular APases activity was determined in root exudates and by in vivo stain; APases are mainly located in the epidermis of young, growing roots, and may be secreted to the soil and hydrolyse different forms of organic phosphorus. Another mechanism of oat cultivars acclimation to phosphate deficiency is Pi remobilization from internal sources, which involves intracellular enzymes, thus intracellular APases activity in shoots and roots were also investigated. In addition, protein extracts from tissues were run on native discontinuous PAGE to determine APases isoforms. Pi starvation significantly increased the activity of extracellular and intracellular acid phosphatases in comparison to the control plants. However, both secreted and internal APases activity in plants grown on phytate was more similar to control than to -P plants. Generally, acid phosphatases played important role in acclimation to Pi deficiency of all studied oat cultivars, especially extracellular enzymes involved in Pi acquisition. All oat plants grew equally well both on medium with Pi and where Pi was replaced with phytate, although phytate was considered not available for other plants. The studied oat cultivars generally demonstrated similar acclimation mechanisms to Pi deficiency, however, they can used different pools of acid phosphatases.

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P3.14

Global analysis and comparison of transcriptomic changes in *Medicago truncatula* and *Lotus japonicus* root nodules during drought stress

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Fabacean plants evolved the ability of symbiotic interaction with nitrogen-fixing bacteria collectively termed rhizobia. This symbiosis manifests itself in the formation of special organs, root nodules, in which bacteria differentiate into bacteroids, that are able to assimilate atmospheric nitrogen. This ability makes fabaceans species ideal crops since their requirement for nitrogen fertilization is reduced. Generally, fabaceans form two types of root nodules: determinate, that are characterized by a spherical shape and definite nature of meristem, or indeterminate, that have cylindrical shape and persistent meristem.

Despite the developmental mechanisms of these two types of root nodules are well described, the specific signaling pathways that control their meristematic activities are not yet fully explained. It is very important to reveal the molecular processes that maintain meristematic activity in root nodules because they are crucial for nitrogen fixation efficiency, especially when plants are exposed to unfavorable environmental conditions.

The aim of our study was to compare metabolic processes and signaling pathways in root nodules of two fabacean model species, *Medicago truncatula*, forming indeterminate nodules, and *Lotus japonicus*, forming determinate nodules. Using next-generation RNA sequencing we analyzed and compared the transcriptomic changes in root nodules of both species at two different stages of drought stress.

Our results demonstrated that in *M. truncatula* after two and four days of drought stress, in comparison to well-watered plants, there was a difference in the expression level of 371 and 787 genes, respectively. In *L. japonicus*, after two and four days from the last watering, we found 122 and 1225 differentially expressed genes, respectively. Genes with significantly changed expression level encoded broad range of enzymes engaged in signal perception, phytohormones signaling, synthesis of osmoprotectants and secondary metabolites, cell wall remodeling and cell cycle.

Our study presents the first such broad comparison of transcriptomic changes in indeterminate versus determinate root nodules and reveals similarities and differences in their response to drought stress.

Housekeeping gene identification and selection for real-time RT-PCR normalization in rhododenrons during cold stress

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Freezing temperature is one of the main and deciding environmental factor limiting growth and development of rhododendrons. Within the genus *Rhododendron* the ability to survive very cold temperatures varies widely. Plant stress studies are more and more based on gene expression using real-time RT-PCR that is at present the most sensitive method for the detection of low abundance mRNA. The accurate quantification of the expression of genes involved in cold stress in rhododendrons requires reliable internal controls with highly stable expression independent of cold stress conditions, so the selection of suitable candidate genes is highly desired. Here, five housekeeping genes i.e. actin (act2), elongation factor 1-α (ef1α), heat shock 70 kDa protein (hsp70), α-tubulin (tua4) and β-tubulin (tub9) during cold stress were tested on 4 rhododendron taxa i.e. *R. aureum, R. brachycarpum, R. purdomii* and *R. yakushimanum* 'Koichiro Wada' in order to assess their value as internal controls in expression studies. We measured the expression profile of cold-related gene – dehydrin (dhn-5) using the most stable and one least stable reference genes in all *Rhodendron* taxa. The search of reference genes in 4 rhododendrons was based on the identification of orthologues of genes stably expressed in *Arabidopsis thaliana*. Consensus sequences based on Multiply Sequence Alignment were used for designing PCR primers which products were sequenced using Beckman Coulter CEQ 8000. The expression of these genes was determined using NormFinder, BestKeeper and ΔCt approach. The results showed that actin (act2) was the most stable reference gene among the five tested. We propose that act2 should be the preferred reference gene for normalization and quantification of transcript levels in gene expression studies in rhododendron species under cold stress conditions.

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P3.16

Studies of zearalenone interaction with native and model plant membranes – protective effect of 24-epibrassinolide

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Zearalenone is considered as one of the Fusarium mycotoxin which reveals the negative impact on plant growth and yielding. The mechanisms of zearalenone (ZEA) absorption by cells and the possibility of protection against this substance are intensively studied. In this work the effect of ZEA action directly on the plant membranes and the influence of 24-epibrassinolide (EBR) were investigated. EBR belongs to the brassinosteroids, relatively new recognized group of hormones, used also as the anti-stress factor (Janeczko et al. 2003). Two wheat genotypes: Parabola (tolerant) and Raweta (sensitive) were chosen to undergo experiments. Plants were cultured to the phase of the grains appearance. The non-matured germs were used to obtain the calli cells. The action of ZEA (30 μM) and EBR (0.05 μM) was studied after the application of these molecules (separately and in mixture) into the culture media. After 7 days, calli were collected to the biochemical analyses (antioxidative enzymes) and the membrane lipids isolation. The ZEA accumulation was detected by HPLC-MS technique. It was found that in spite of the lower uptake of ZEA in cells of the sensitive genotype, the increase of SOD, CAT, POD and APX activity indicated the stimulation of the oxidative stress in higher degree, than in tolerant ones. EBR presence in the media with ZEA decreased both the ZEA absorption and the stimulation the activity of enzymes. The phospholipids (main lipid fractions) were obtained from cell membranes. For this lipids, the monolayers were formed, using the Langmuir technique. Monolayers' properties were characterized by surface pressure vs. molecular area isotherms. The short-time impact of both substances was investigated by simultaneous addition ZEA and EBR into the phospholipids obtained from non-treated (control) calli. It was found that irrespectively of the treatment (short and 7-days) ZEA interacts with lipid monolayers was causing their expansion. The EBR added together with ZEA, reduce the effect of this mycotoxin on surface properties of lipid films. The more significant surface changes of lipid monolayers were observed after short-term treatment.

[1] Janeczko A., Filek W., Biesaga-Kościelniak J., Marcińska I., Janeczko Z. 2003. Plant Cell Tiss. Org. Cult.. 72: 147-151.

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Role of triticale glutamate dehydrogenase under salt stress

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Glutamate dehydrogenase (GDH; EC 1.4.1.2) enzyme catalyzes the reversible reaction involving assimilation of ammonia into glutamate and deamination of glutamate to form 2-oksoglutarate and ammonium. In some plants species, GDH is a hexamer and can be separated into seven isoenzymes that are composed of two distinct subunits: α and β. Those subunits are encoded by two separate nuclear genes GDH1, encoding β subunit, and GDH2, encoding α subunit. In triticale, a common Polish cereal two genes encoding GDH: TsGDH1 (β subunit) and TsGDH2 (α subunit) were cloned.

To investigate the function of the two GDH subunits, transgenic *Arabidopsis* plants were obtained by introducing the coding region of TsGDH1 or TsGDH2 linked to the 35S CaMV promoter, via *Agrobacterium tumefaciens* transformation. We generated transgenic *Arabidopsis thaliana* lines with increased GDH activity via alternation of β and α subunits levels. No phenotypic changes were observed between the transgenic and control plants under standard nutrient conditions, however GDH activity, both the aminating and deaminating, was higher in transgenic plants compared with the wild type plants. In order to obtain a better understanding of the physiological function of triticale GDH, the transgenic and WT *Arabidopsis* plants were grown in the presence of 50 mM NaCl. A greater shoot and root biomass, an increased GDH activity and the chlorophyll content were observed in the overexpressing lines following growth under the salinity stress, compared with the untransformed plants. These results indicate the importance of triticale glutamate dehydrogenase into abiotic stress response such as salinity.

P3.18

The search for native protein substrates for chaperone activity of chloroplast protein AtDeg

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The chloroplast protein AtDeg2 is a non-ATP hydrolizing protease peripherally connected with stromal side of thylakoid membrane. Besides proteolytic function AtDeg2 has chaperone activity as well, consisting in an ability to prevent aggregation of DTT-denatured lysozyme in vitro and this implies that the regulatory functions of AtDeg2 in vivo is fulfilled due to the interplay between its protease and chaperone activity. A primary structure of AtDeg2 molecule consists of a catalytic triad (H-159 D-190 S-268) – containing protease domain as well as two PDZ domains, marked PDZ1 and PDZ2. We show hereby that the deletion of PDZ1 domain abolished completely chaperone activity of AtDeg2 having no impact on protease activity. To search for native protein substrates for chaperone activity of AtDeg2 disclosed under high irradiance conditions the plants expressing AtDeg2 devoid of PDZ1 domain with a C-terminal GFP tag were generated (AtDEG2 ΔPDZ1-GFP). The search was accomplished by the application of diagonal electrophoresis, namely the native protein substrates were identified as the spots migrating below the diagonal of the gel when chloroplast proteins of high irradiance exposed plants expressing AtDEG2 ΔPDZ1-GFP were analyzed, but not in the case of chloroplast proteins of high irradiance exposed wild type plants. The spots represent proteins which are part of intermolecular protein aggregates held by disulphide bridges, formed as a result of exposition of transgenic plants to high irradiance, due their inability to suppress the aggregation and/or to re-solubilize the aggregates.

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The role of the diamine oxidase in modification of the plasma membrane proton pump activity under cadmium stress

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In plants, polyamines (PA) are involved in different physiological processes and responses to abiotic stresses. PA are oxidatively deaminated by amine oxidases: diamine oxidase – DAO and polyamine oxidase – PAO. In cucumber (*Cucumis sativus* L.) roots, under physiological conditions, much higher DAO than PAO activity was observed. The aim of our study was to determine the role of the diamine oxidase in modification of the PM proton pump activity in cucumber roots under cadmium stress. Plants have been treated with 10 μM $CdCl_2$ for three days and next were moved to the control conditions for another three days – post-stressed plants. Treatment of plants with Cd stimulated DAO activity, measured both as transporting and hydrolytic activity. Moreover, enhanced expression of three of PM H^+-ATPase genes (CsHA2, CsHA4 and CsHA8) was observed. In order to find the correlation between the stimulation of DAO and PM H+-ATPase activities, the assay with 0,1 mM aminoguanidine, the DAO inhibitor, was performed. The aminoguanidine application eliminated the stimulating effect of cadmium on PM H^+-ATPase activity and CsHA2, CsHA4 and CsHA8 genes expression. The increase of DAO activity under cadmium stress could contribute to H_2O_2 and/or NO generation. We suggest that DAO plays a role in the adaptation of plants to cadmium stress by oxidation of putrescine, contributing to the increased production of NO and H_2O_2, which may modify the activity of PM-H^+-ATPase, the key enzyme in adaptation to abiotic stress.

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P3.20

Acclimation of brassinosteroid-deficient barley mutants to low and high temperature

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Brassinosteroid are plant steroid hormones involved in plant growth and stress response but mechanisms of their action are only partly explained. The aim of our experiment was to answer the following question: is the tolerance to low and high temperature lower in brassinosteroid-deficient barley mutant then in wild type (Delisa). It was assumed that if BR are involved in regulation of acclimation processes to low and high temperature – plants with disorders in BR synthesis (mutant 522DK) would be less tolerant of low and high temperatures compared to wild type. Plants after acclimation at 27°C (7 days) were subjected to high temperature stress (45°C). Likewise, the plants after acclimation at 5°C (21 days) were subjected to frost (-8°C). Prior to acclimation (20°C) and on the last day of acclimation, fast chlorophyll a fluorescence kinetic measurements were made as well as accumulation of transcripts of protective heat shock protein (HSP90) and membrane associated proteins (ATPase and HvPIP) was measured. At the end of stress, thermal damage was assessed by analyzing changes in permeability of the cell membranes and based on the regrowth test (modified Larsen method). Surprisingly, the wild type Delisa was less resistant to high temperature (45°C) than the BR-mutant 522DK. Already during acclimation at 27°C, mutant leaves was characterized by higher PSII efficiency. At the same time, during acclimation at 27°C, the accumulation of the HSP90 transcript in the mutant increased while in the wild type decreased. No significant differences were found between the mutant and Delisa in frost tolerance (based on leave regrowth). The importance of changes in the accumulation of transcript HSP90 and also changes in the accumulation of transcripts of ATPase and HvPIP in mutant and wild type during acclimation to high and low temperatures will be discussed.

Toward the isolation of barley mutants in a violaxanthin de-epoxidase-related gene via TILLING approach

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Violaxanthin de-epoxidase (VDE) is an enzyme that catalyzes the two-step mono de-epoxidation reaction converting violaxantine to zeaxantin. It is involved in dissipation of excess energy in the chlorophyll of the light-harvesting protein complex of photosystem II (PSII) in a process of non-photochemical quenching. It was shown that environmental stresses such as drought induce the expression of violaxanthin de-epoxidase encoding gene, making it one of the important elements of plant protection against the excess light and heat. Importantly, the process of non-photochemical quenching prevents photoinhibition, but its activation reduces the efficiency of photosynthetic energy conversion. Thus, the identification of new allelic variants of VDE gene that encodes faster VDE enzyme may allow more rapid relaxation of heat dissipation at PSII and increase assimilation efficiency. Here, we report the first step of the isolation of mutants in violaxanthin de-epoxidase-related gene (HvVDE) in barley – the identification of mutated barley lines using TILLING strategy. The HvVDE genomic sequence is 2770 bp long, composed of six exons. For TILLING analysis the sequence was divided into two fragments, where the more distal one represents most of the region of higher sequence conservation. The analysis of this fragment was performed for 6144 M2 plants from HorTILLUS TILLING population developed in the Department of Genetics, University of Silesia in Katowice. Twelve mutations were found in this region: eight of them were missense, two were silent and other two located in the intron. Half of the mutations were in homozygous stage. An in silico analysis showed that these mutations may have an impact on the secondary structure of protein encoded by HvVDE gene. The identified mutant lines will be subjected to the phenotypic evaluation in order to evaluate their influence on the efficiency of photosystem II performance.

P3.22

Analysis of the mechanisms of genotoxic effect of aluminum in *Hordeum vulgare* cells

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Aluminum toxicity is one of the major factors, which is responsible for limitation to crop productivity on acid soils. *Hordeum vulgare* is a model crop plant, which is very sensitive to aluminum. The aim of this study was to analyze cyto-molecular aspects of $AlCl_3$ treatment in barley: DNA damage and disturbances of the cell cycle. The optimalization of the procedure of the treatment of *H. vulgare* Sebastian var. seedlings with $AlCl_3$ was previously applied using analyzes of root growth as well as cyto- and genotoxic effects. The treatment of barley seedlings was performed in hydroponics. The following $AlCl_3$ doses were selected to experiments: 20 μM, 30 μM and 40 μM, using 1 and 7 day treatment. For analyzing DNA damage after treatment with $AlCl_3$ TUNEL test was used. $AlCl_3$ treatment led to DNA fragmentation – the effect depended on dose of aluminum and time of treatment. The influence of aluminum treatment on cell cycle profile was investigated using flow cytometry. It was observed that after aluminum treatment the frequency of cells in G1 and S phase is decreasing, whereas the frequency of cells in G2/M phase is increasing. These observations suggest that $AlCl_3$ has impact on cell cycle profile. Taking into account the changes of cell cycle profile, the analysis of the effect of $AlCl_3$ on disturbances of S phase using analogue of thymidine 5-ethynyl-2′-deoxyuridine (EdU) was also performed. The incorporation of EdU allowed cytogenetic analysis and estimation the frequency of nuclei in S phase. It was observed that $AlCl_3$ decreased the frequencies of the S phase cells. The use of reaction with 5-ethynyl-2`-deoxyuridine confirmed the results of cell cycle analysis with flow cytometry. In the future it will be of particular interest to get knowledge on the effect of aluminum on DNA damage and cell cycle selected *H. vulgare* mutant lines with altered Al tolerance.

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Functional foods – enrichment of crop plants in the selenium compounds with anticancer activity

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Selenium is an important element for the proper functioning of the human body. Its compounds exhibit antitumor effects as well as contribute to delaying the aging process. The low content of selenium in Polish soils results in insufficient quantities of this element supplied to our organisms. Dietary supplementation with selenium may take place through the so-called functional foods, as exemplified by plants with increased selenium content. On the other hand, the toxicity of excessive amounts of selenium in the diet makes the dietary enrichment of this element should be preceded by research and carried out in a thoughtful manner. Moreover, the most beneficial effect on the human body is shown only by selected selenium compounds, including selenium amino acids. The purpose of the research was to achieve a state in which the plant will take selenium to a sufficiently high level, this element will be located mainly in the edible part of the plant and will be transformed from an inorganic forms to an organic ones which are most beneficial to human health. Studies on the uptake and metabolism of selenium compounds by plants were carried out on edible onions (*Allium cepa*). The purpose of the study was to investigate the effect of different levels of macroelements (phosphorus and sulfur) on selenium uptake by plants from liquid medium and its metabolism and degree of translocation to the aboveground part of the plant. The study analyzed the degree of selenium uptake by the plants, its location and, above all, the chemical form under which selenium was in the plants.

P3.24

Jasmonic acid and abscisic acid in cold storage of *Taraxacum pieninicum* – comparison of plant growth regulators

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Taraxacum pieninicum is probably the oldest endemite of the Pieniny Mountains (Western Carpathians, Poland). It occurs in the upper parts of the Trzy Korony massif, on Okrąglica Mt. The present population consists of two clusters of a small number of individuals, therefore this species is listed as critically endangered (CR), and even as declining – critically endangered (E) on Polish red lists. Protection of *T. pieninicum* involves constant monitoring of its natural habitat as well as protection of the gene pool in the Seed Bank of the Polish Academy of Sciences Botanical Garden in Powsin, Warsaw.

However, the availability of seeds is very limited.

In vitro culture provides possibilities to conserve species by its storage in slow growth conditions and synthetic seeds technology protects plant tissue during cold treatment. However, during prolonged storage time, explants overgrow the coat thus synseeds structure is no longer functional.

The aim of this study is twofold: (a) to analyze the effect of the growth regulators: jasmonic acid (JA) and abscisic acid (ABA) on shoots proliferation in optimal growth conditions and after cold storage of synthetic seeds; (b) to inhibit the growth of shoot tips so that they would not overgrow the synseed structure during storage.

Results show that increasing ABA concentration decreases proliferation rate of shoots in optimal condition, whereas JA strongly inhibits growth of shoots without effect on proliferation rate, what can be used during synseeds storage.

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PP2A-C subunit and other parameters under hypoxia stress in tomato leaves and roots

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Hypoxia is a state of oxygen deprivation. During this stress conditions increase production of reactive oxygen species is possible. We observed higher formation of H2O2 in plants treated with hypoxia stress what proved generation of oxidative stress. There is some information that PP2A phosphatase might be involved in plant reaction to oxidative stress. The aim of the study was to investigate the PP2A – C subunit under hypoxia stress in tomato plants. Tomato plants 'Faworyt' were cultivated in hydroponics with aeration until the stage of 2-3 true leaves. Afterwards plants were divided in two treatments: control (solution with aeration) and hypoxia (without aeration). Plants were analyzed after 7 days of hypoxia (some analyzes were made also after 24 hours). Roots and leaves were analyzed separately. Total content of PP2A-C subunit as well as unmethylated ones were investigated by western-blot analyses.

Total PP2A-C subunit was higher in roots treated with hypoxia stress than in control ones. In contrast, total PP2A-C content in leaves decreased under low oxygen level stress. Lower level of total PP2A-C was observed in control roots than in control leaves. It is worth to notice that the amount of unmethylated protein was lower in leaves of hypoxia treated plants than control tomato. We observed very weak signal connected with unmethylated PP2A-C in roots. Observed oxidative stress induced also other changes in tested plants. For example we observed changes in nitrate reductase activity after 7 days of treatment in roots of tomato but not in leaves. Also decrease in photosynthesis intensity was observed in the case of stressed plants. Results suggest that hypoxia stress induced changes in C subunit of PP2A in tested tomato plants.

P3.26

Photosynthesis-related functions of vasculature-associated chlorenchymatous cells

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In most biochemical, molecular and genetic studies, a leaf is regarded as a uniformly responding unit, however leaves are not homogeneous in structure and function. Leaf venation is in continuity with the vascular system within leaf petiols and stems. Leaf veins are typically encircled by bundle sheath (BS) cells containing chloroplasts and photosynthetic cells adjacent to the vasculature are also found in petiols and stems.

The vasculature-adjacent and the corticular chlorenchymatous cells operate under conditions which differ significantly from those experienced by the mesophyll cells. Accordingly, it has been suggested that their role in plants is related rather to specific biological processes than to the photosynthetic CO_2 assimilation contributing to biomass yield. It has been suggested that these chlorenchymatous cells could be involved in long distance signaling mediated by phytohormones and H_2O_2, and in processes which allow plants to acclimatize to changes in the environment, especially to fluctuating light conditions, at the whole-organism level. However, understanding the specific physiological functions they exert at their destinations remains a challenge for the future.

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NTZIP1-like and NTZIP11 – proteins involved in the regulation of zinc homeostasis in tobacco

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Zinc (Zn) is a micronutrient essential for plant growth. Nevertheless, its high concentrations might be toxic and cause several changes in plant morphology including development of necrotic regions over the leaf blade. The one of the factors determining plant tolerance for zinc is the ability to store its high concentrations in organs, i.e. in leaves, without symptoms of toxicity. Considering tobacco, the existence of "Zn-storage cells" within leaves mesophyll was proved [1]. These cells possessed the ability to accumulate Zn in high concentration, which protects neighbouring non-accumulating cells from Zn toxicity. Accordingly, a large amount of total zinc is stored in the leaf without causing harmful effects on the organ as a whole.

Finding molecular mechanisms which differentiate mesophyll cells with respect of their ability to accumulate high concentrations of zinc is the aim of this study. On the basis of bioinformatic analysis of tobacco genome, several gene sequences encoding proteins potentially involved in Zn transport were identified. In this part of the study, protein families which participate in the transport of metal ions in plant cells (e.g. CAX, HMA, MTP, Nramp, ZIP) were considered. Then, expression of candidate genes in the tissues of plants grown at the control medium were compared to those exposed to high zinc. Significant differences in the expression level compared with control conditions were determinants for selection of transporter genes potentially involved in Zn loading/accumulation in the mesophyll "Zn-storage cells".

The second part of the study included yeast growth assay which proved that two tobacco proteins NtZIP1-like and NtZIP11 are involved in zinc uptake to the cell. Such statement was also reaffirmed by determination of the sub-cellular localization of these proteins by transient expression of the constructs 35S::NtZIP1-like::GFP and 35S::NtZIP11::GFP in tobacco leaves. It was shown that both proteins are located in the plasma membrane. Thus, NtZIP1-like and NtZIP11 were identified as the ones likely responsible for zinc accumulation in "Zn-storage cells" by enhanced zinc uptake.

[1] Siemianowski et. al., Development of Zn-related necrosis in tobacco is enhanced by expressing AtHMA4 and depends on the apoplastic Zn levels, Plant, Cell & Environment 36 (2013) 1093-1104

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P3.28

Ascorbate enhances antioxidative ability of *Chlorella vulgaris* cells grown under Pi starvation

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The alterations of photosynthetic carbon metabolism in plant cells, under conditions of plant insufficient inorganic phosphate (Pi) supply, cause an imbalance between absorption and energy consumption of the absorbed radiation. These disturbances induce the production of reactive oxygen species that lead to oxidative damage in the photosynthetic apparatus and other metabolic disorders in plant cells. The common antioxidant in plant cell is ascorbic acid; its increased production may be of great importance in plant acclimation to unfavorable stress conditions. The aim of this study was determine the changes in antioxidant capacity of *Chlorella vulgaris* under phosphate (Pi) deficiency and ascorbate participation in defense against oxidative stress. The effect of phosphorus deficiency on the level of oxidative stress and activity of enzymes involved in ascorbate metabolism was estimated. We used as experimental material unicellular algae, *Chlorella vulgaris* Beijer., grown in buffered, sterile media with different content of phosphorus: complete (control, +P), with lowered phosphate content (1/4P) and without phosphate (-P). It has been shown that Pi deficiency causes the limitation of cultures growth and the increase of photosynthetic pigments content; in addition clear symptoms of oxidative stress (lipid peroxidation and hydrogen peroxide content increase) were observed. It was also found that the level of total ascorbate increased in Pi-deficient *Chlorella*. It has been shown that Pi deficiency leads also to increased activity of ascorbate peroxidase, monodehydroascorbate reductase (in cultures of five day growth of cells ¼ P and dehydroascorbate reductase (especially in older -P cultures). Exogenously applied ascorbic acid caused reduction in lipid peroxidation products and increased capacity of hydrogen peroxide removing. The obtained results indicated that ascorbate has a significant role in reducing the effects of oxidative stress and improving antioxidant capacity of *Chlorella* cells grown under low Pi supply.

Responses of succulents to water stress: comparative analysis of four *Sedum* species (Crassulaceae)

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Drought tolerance was evaluated in four succulent species belonging to *Sedum* genus. The stress treatment was performed by stopping watering of plants maintained in controlled greenhouse conditions. After four weeks, several plant growth parameters (aerial length, fresh weight and water content), photosynthetic pigments (chlorophylls and carotenoids), osmolytes (proline and total soluble sugars), oxidative stress maker (malondialdehyde MDA) and antioxidants (total phenolic compounds TPC and flavonoids) were measured. Considerable differences in the evaluated traits were found among the control and drought-stressed plants, and between studied species as well. *Sedum ochroleucum* and *S. spurium* proved to be more tolerant to drought than *S. album* or *S. sediforme*. Drought stress caused a marked reduction in plant growth (36% and 30% reduced stem length in *S. album* and *S. sediforme*, respectively, in comparison with their control variants), and photosynthetic pigments content (60% decrease in *S. album*). It also stimulated accumulation of soluble solutes (50% higher proline concentration in sensitive S. album and S. sediforme, compared with 8% and 16% ($P>0.05$) in tolerant *S. spurium* and *S. ocholeucum* respectively) and non-enzymatic antioxidants (3-fold increase of TPC in *S. album*). In the most sensitive species *S. album* lipid peroxidation was intensified as a result of drought, as reflected by 2-fold increase of MDA content. The obtained results suggest that injures of water stress to *Sedum* species, as manifested by decline in stem length, total fresh weight and photosynthetic pigment content, may be associated with the peroxidation of membrane lipids. Additionally, in stressful conditions synthesis of non-enzymatic antioxidants is stimulated. Overall, results indicate that drought tolerant *Sedum* species can be identified using a combination of plant growth and biochemical markers.

P3.30

Silicon ions – stress factor or stimulator for green algae?

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Silicon (Si) is a microelement necessary for the life of all organisms. It is well known that it has a positive effect on the course of many physiological processes in plants related to their growth and development. In the algae, the largest amounts of silicon are collected by diatoms, which require it mainly for the production of cell covers. The deficiency of this element causes the inhibition of cell division, protein synthesis, nucleic acids, chlorophyll and fatty acids, and further disturbance of photosynthesis.

Therefore, it is suggested that supplementation with silicon may have a positive impact on the increased productivity of other strain than diatoms.

The aim of the study was to evaluate the changes in the morphology, biomass growth, photodynamic efficiency, content of chlorophyll, carotenoids, soluble sugars and starch of the green algae *Chlorella vulgaris* and *Scenedesmus armatus*, which grew on medium enriched in silicon ions of different origin (silica waste and orthosilicic acid). Both species are characterized by high resistance to stress factors and rapid biomass growth. The strains of *C. vulgaris* and *S. armatus* have been cultured on the medium Kessler and BBM respectively and modified medium in which a solution of the study sample was used silicon ions instead of distilled water.

The results showed differences in the morphology of the algal cells tested between cultures on control medium and medium supplemented with silicon ions. In both strains was found that the silicon ions agglutinated the cells. Growth dynamics of algae biomass was determined by optical density changes (OD, spectrometry). The OD value of the biomass cells of control culture of *C. vulgaris* and *S. armatus* was significantly lower than the biomass of cells growing in medium supplemented with silicon ions. The OJIP-test curves present the chlorophyll a fluorescence intensity of *C. vulgaris* and *S. armatus* were similar in size and shape between control and medium with silicon ions. However, the parameters of the photosynthetic apparatus have shown that silicon ion supplementation of the medium causes a reduction in varying degrees PSII efficiency in both tested algae strains. In addition, there was a decrease in the content of carbohydrates and photosynthetic pigments in *C. vulgaris* and *S. armatus* growing in silicon ion enriched media.

Despite the decline in light yields (including the reduction of photosynthetic pigments) and the reduction in carbohydrate accumulation, silicon ions stimulate algal biomass growth. Possible mechanisms of this phenomenon will be discussed.

The SnRK2.10 kinase positively regulates resistance of *Arabidopsis thaliana* to drought and salt stress

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In the plant kingdom the survival and reproduction abilities are conditioned by very fast detection and adequate response to changing environmental conditions. Among others salinity and water deficit are the most commonly occurring stress factors for plants all over the globe. Both stresses cause strong inhibition of plant growth and losses in cultivars of many species. The emergence of water regime triggers signaling events in cells, where in the central point are located cascades of numerous protein kinases undergoing rapid and transient phosphorylation and dephosphorylation events. In plants, SNF1-related protein kinases 2 (SnRK2s) are key regulators of responses to salt and osmotic stress. Based on a phylogenetic analysis SnRK2s are divided into three groups; group 1 consists SnRK2s non-activated in response to ABA, group 2, kinases non-activated or weakly activated (depending on the plant species) by ABA treatment, and group 3, ABA-activated kinases (Boudsocq and Lauriere, 2005). Here, we present our current research on SnRK2.10 kinase, member of ABA non-activated SnRK2s.

The kinase studied positively regulates plant resistance to salt and osmotic stress at multiple stages of plant development. As previously reported it is rapidly activated in roots and is a positive regulator of lateral root forming under salt treatment (McLoughlin et al., 2012). We showed that in response to salt stress the SnRK2.10 is activated also in rosettes of hydroponically grown *Arabidopsis thaliana* plants exposed to salinity stress. Activity of kinase studied is observed already in 5 min after stress application and reaches the maximum after 1h. Our research revealed that SnRK2.10 is needed also in seed germination in media containing NaCl. Next, we exposed plants to prolonged salt stress, which has been proved to cause considerable inhibition of photosynthesis. In snrk2.10 knockout mutants after few days of salinity stress qP, qN, Y(II) and Fv/Fm parameters were more strongly affected than those in wild type plants. Observed disorders in photosynthesis efficiency were correlated with lower levels of few main photosynthesis related proteins. As a consequence of photosynthesis inhibition, we observed reduced accumulation of dry mass of snrk2.10 mutants in comparison to wild type plants grown in salt stress conditions. Moreover, the air-dried rosettes of snrk2.10 mutants' loss water significantly faster than wild type plants. Summing up we present new physiological and biochemical data showing the SnRK2.10 role in plant resistance to drought and long-term salt stress, most probably by influencing the photosynthesis efficiency in *Arabidopsis* leaves.

[1] Boudsocq M, Lauriere C (2005). Plant Physiol 138: 1185-1194

[2] McLoughlin et al., (2012). Plant J 72:436-449

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P3.32

Peroxidases from plant waste material – biochemical characteristics

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With the increasing interest in using plant enzymes to detoxify pollutants or utilizing them in antibodies conjugation and enzyme immunoassays, the need to characterize less known peroxidases arose. The main peroxidase used nowadays is horseradish peroxidase (HRP), widely available commercially, but its cost is still quite high. Cheaper alternatives with comparable characteristics are sought for.

Potato pulp is a starch production waste product with high peroxidase activity, that was already demonstrated to detoxify phenol and 2,4-dichlorophenol solutions with satisfactory results (over 95% of pollutant removal efficiency). To characterize peroxidases present in this material, the crude extract from potato pulp were concentrated and subjected to protein electrophoresis under reducing and denaturing conditions, as well as under non-reducing and non-denaturing ones. The electrophoresis under native conditions followed by staining for peroxidase activity revealed seven putative peroxidase isoenzymes in potato pulp. Twelve protein bands were identified by means of SDS-PAGE. The enzyme extracts were then subjected to ion exchange chromatography analysis using NGC Chromatography System (BioRad) to isolate individual fractions with peroxidase activity. The fractions were further analysed by the means of isoelectric focusing (Protean i12 IEF Cell, BioRad).

Separate and combined effects of Cd and Pb on growth, medium ph, membrane potential and content of both metals in maize (*Zea mays* L.) coleoptile cells

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Among the heavy metals, cadmium and lead are elements that are very often studied due to their toxicity. In the environment, these metals are usually present together, thus causing a combined effect that may differ significantly from the one induced by each metal separately. Despite the abundant literature on single Cd or Pb exposure, the interaction between Cd and Pb in binary metal combinations on plant growth, membrane potential and the accumulation of these metals have rarely been reported. Therefore, the effects of a combination of cadmium (Cd) (chloride, 0.1 mM) and lead (Pb) (chloride, 0.1 mM) on growth, medium pH, membrane potential (Em) and the accumulation of both metals in maize coleoptile cells were studied. It was found that Cd and Pb, either individually or in combination, caused a significant reduction in the IAA-induced growth of coleoptile cells. The effect was greater when both metals were present together in the growth medium. No recovery from the inhibitory effect of Pb on cell elongation was observed when Pb was removed from the incubation medium, in contrast to the inhibitory effect of Cd. In contrast to Pb, after 360 min, Cd alone or in combination with IAA accelerated the acidification of the external medium. Within 30 min, the Cd that was added to the incubation medium caused the depolarization of the membrane potential (Em) of parenchymal coleoptile cells, whereas when Pb was added at the same time protocol, it caused membrane hyperpolarization. Interestingly, exposure to the combined metals induced the depolarization of the Em, which was similar to that induced by Cd only.

When Pb was added to the medium containing coleoptile segments that had been pretreated with Cd, a recovery of Em was observed, whereas when Cd was added to the coleoptile segments that had been pretreated with Pb suppressed Pb-induced membrane hyperpolarization. When IAA was added together with Pb, it increased the accumulation of the metal, whereas when it was added together with Cd, it did not change the content of the metal in the coleoptile tissue. However, when Cd and Pb were present in the incubation medium together, their content in the coleoptile segments was not statistically different compared to the content when the metals were present separately, thereby suggesting that both metals can enter a cell through different pathways.

P3.34

The impact of seed hydropriming on antioxidative defense system of *Brassica napus* during germination and growth under saline conditions

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Seed priming is a pre-sowing treatment which leads to seeds invigoration and alleviation of the environmental stresses during germination and further plant development. Several methods of seed priming have been developed. Hydropriming is the simplest and low-cost method preserving the high efficacy.

The aim of the study was to examine the influence of hydropriming on *Brassica napus* seed germination and seedling establishment under saline conditions. Germination tests of unprimed and primed seeds under optimal (H_2O) and stress conditions (100 mM NaCl) were performed. In order to verify the effect of seed hydropriming on the performance of rape antioxidative system, the level of non-enzymatic antioxidants (ascorbate, glutathione), the activity and gene expresion levels of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase) were analyzed in dry seeds and after exposure to salt stress. Moreover, the malondialdehyde (a lipid peroxidation marker) and hydrogen peroxide contents were determined.

Germination tests showed that hydropriming increased germination rate and maximum germination potential of seeds under saline conditions. Analyses of antioxidant machinery indicated that hydropriming triggers antioxidative defense response to salt stress by increasing activity of enzymatic antioxidants – particularly catalase and ascorbate peroxidase as well as by generating higher ratio of reduced to oxidized form of ascorbate. Summarizing, activation of antioxidant metabolism by seed hydropriming may be one of the mechanisms promoting salinity tolerance of *Brasscia napus* during germination and early seedling growth.

Plants reactions incubated with naphthoquinones

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Naphthoquinones are a large group of plants, lichens, fungi and animals secondary metabolites. They are class of organic compounds derived from naphthalene. Naphthoquinones are characterized by a series of different properties such as: antioxidative, oxidative-reducing, anti-inflammatory, anti-cancer, bactericidal, antifungal, antiviral and allelopathic. Many of these effects are related to the interaction of naphthoquinones with DNA, topoisomerase inhibition and the production of reactive oxygen species (Hook et al., 2014; Chen and Jin, 2015; Sánchez-Calvo et al., 2016).

In recent years, Karcz et all investigated the effects of various naphthoquinones on plants reactions such as: growth, growth rate, proton extrusion, redox activity, production of reactive oxygen species, catalase activity and MDA production (Rudnicka et al., 2014; Kurtyka et al., 2016; Rudnicka et al. 2017 in review; Rudnicka et al., 2017 in preparation). As a model we used 10 mm-long coleoptile segments of maize (*Zea mays* L.) cut from 96 h old etiolated seedlings. Intact coleoptile segments, with the first leaves removed, were excised 3 mm below the tip. We tested four naphthoquinones: juglone, lawsone, 1,4-naphthoquinone and naphthazarin.

The relationship between oxidative stress and redox signalling in exposed cells of naphthoquinones has recently been demonstrated (Klotz et al., 2014). Currently we are looking for dependencies (correlations) between measured parameters for different naphthoquinones.

[1] Chen J., Jin B. 2015. Investigation on redox mechanism of 1,4-naphthoquinone by in situ FT-IR spectroelectrochemistry. Journal of Electroanalytical Chemistry 756: 36-42.

[2] Hook I., Mills C., Sheridan H. 2014. Bioactive Naphthoquinones from Higher Plants. Studies in Natural Products Chemistry, Vol. 41.

[3] Klotz L-O., Hou X., Jacob C. 2014. 1,4-Naptthoquinones: From Oxidative Damage to Cellular and Inter-Cellular Signalling. Molecules 19: 14902-14918.

[4] Kurtyka R., Pokora W., Tukaj Z., Karcz W. 2016. Effects of juglone and lawsone on oxidative stress in maize coleoptile cells treated with IAA. AoB PLANTS 8: plw073.

[5] Rudnicka M., Ludynia M., Karcz W. 2017. Cellular responses to naphthoquinones: naphthazarin – in preparation.

[6] Rudnicka M., Ludynia M., Karcz W. 2017. Naphthoquinones – a Comparison of the Effects of 1,4-naphthoquinone and 2 hydroxy-1,4-naphthoquinone (Lawsone) on Auxin (IAA)-Induced Growth of Maize Coleoptile CellsPlant Growth Regulation – in review.

[7] Rudnicka M., Polak M., Karcz W. 2014. Cellular responses to naphthoquinones: juglone as a case study. Plant Growth Regulation 72(3): 239-248.

[8] Sánchez-Calvo J., Barbero G., Guerrero-Vásquez G., Durán A., Macías M., Rodríguez-Iglesias M., Molinillo J., Macías F. 2016. Synthesis, antibacterial and antifungal activities of naphthoquinone derivatives: a structure-activity relationship study. Medicinal Chemistry Research 25: 1274-1285.

P3.36

Effect of selenium on contents of selected micronutrients and activities of antioxidant enzymes in pea leaves

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Selenium (Se) is an essential micronutrient for animals and humans, but whether it is essential for plant remain controversial. Selenium can exert beneficial effects at lower concentrations, but the margin between the beneficial and harmful levels of selenium is narrow. The beneficial effects of selenium on antioxidant activity in plants were observed. Introduction of selenium in the environment of plant roots affects the activity of superoxide dismutase (SOD) which is one of the enzymes of antioxidant systems. Other enzyme involved in antioxidant system is ascorbate peroxidase (APX). Selected micronutrients are important for the activity these enzymes. SOD required Cu, Zn, Fe and Mn as cofactors. APX contains a high-covalently linked Fe.

The aim of the study was to verify effect of selenium on contents of Cu, Fe, Mn and Zn and activities of antioxidant enzymes – SOD and APX in pea leaves. Pea plants grown under glasshouse conditions on Hoagland's nutrient solutions were the object of the study. When pea seedlings were 7-day old sodium selenate and sodium selenite were added to nutrient solutions at the concentrations of 10 and 20 μM. Control seedlings were grown in nutrient solution without selenium. After 4 days of selenium exposure, the plants were harvested. The contents of Cu, Fe, Mn and Zn as well SOD and APX activities were determined in leaves of pea seedlings.

The effect of selenium on the contents of Cu, Fe, Mn and Zn has been shown. Treatment with Se decreased the contents of Zn, Mn and Fe at both forms and concentrations of compound. Cu content increased only at 20 μM concentration. The effects of selenium on activities SOD and APX have also been demonstrated. In the pea leaves treated with Se SOD activity decreased compared to the control. The addition of selenium to the nutrient solutions resulted significantly increased activity of APX compared to the control. While the largest activity was found at 10 μM sodium selenite. There were no significant differences in the impact of the two forms of selenium.

Cucumber Metal Tolerance Protein 6 (CsMTP6) is a mitochondrial Fe2+/Mn2+ exporter

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Members of the Cation Diffusion Facilitator family have been identified in all kingdoms of life and divided into three subgroups: Zn-CDF, Fe/Zn-CDF and Mn-CDF, based on their putative specificity to transported metal ions. The plant MTP6 proteins fall into Fe/Zn-CDF subgroup, however their function in the iron/zinc transport has not been confirmed yet. Here we characterize the MTP6 protein from cucumber plants. When expressed in yeast and protoplasts isolated from *A. thaliana* cells, CsMTP6 localized in mitochondria and contributed to the efflux of iron and manganese from mitochondria. The imunolocalization of CsMTP6 in cucumber membranes and tissues confirmed that CsMTP9 is associated with mitochondria and revealed a ubiquitous distribution of the protein in root tissues. The root expression and protein level of CsMTP6 was significantly up-regulated in conditions of iron-deficiency and iron excess but it was not affected by Mn availability. These results indicate that plant MTP6 proteins contribute to the distribution of iron and manganese between cytosol and mitochondria of plant cells and are regulated by Fe to maintain the mitochondrial and cytosolic iron homeostasis under various Fe availability.

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P3.38

Strigolactones are involved in barley response to drought stress

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Strigolactones (SLs) are a recently described group of phytohormones. They are involved in many developmental processes. It was shown that SLs play a crucial role in plant adaptation to the nutrient stress conditions, mainly to nitrogen and phosphorus deficiency, via modification of root/shoot architecture and promotion the symbiosis with N-fixing rhizobial bacteria and arbuscular mycorrhizal fungi (AMF). The function of SLs in plant response to other biotic stresses such as drought or salt stress was also disclosed.

Recently, the barley HvD14 gene encoding α/β hydrolase that is involved in strigolactone signalling was identified. The TILLING strategy was used to perform a functional analysis of the identified gene in order to obtain a series of HvD14 alleles. The phenotype of the plants that carry one of the identified alleles, hvd14.d, corresponded to the phenotype of the SL mutants that have been described in other species. Plants with the hvd14.d allele were also insensitive to treatment with GR24, which is the synthetic analogue of SL.

In presented studies the response to water deficiency of barley SL signalling mutant was investigated. Obtained results indicated that hvd14.d mutant is hypersensitive to drought. In agreement with the drought-sensitive phenotype, mutant exhibited increased leaf stomatal number and density relative to the wild type variety Sebastian. Additionally the stomata in hvd14.d were open for the longer time during drought and mutant loses water faster than Sebastian. Finally the activity of photosystem II after drought stress was more affected in SL mutant, in comparison to wild type. All data together indicated the positive role of SLs in plant response to drought stress.

Phosphorylation of dehydrins by SnRK2s regulates their interactions with phospholipids and subcellular localization

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SNF1-related protein kinases (SnRK2s) are involved in plant defense against abiotic stresses, especially water deficit. They are activated in plant response to osmotic stress and some of them additionally in response to abscisic acid (ABA). SnRK2s activated by ABA are key elements of ABA signaling regulating plant responses to harmful environmental conditions and plant development. Much less is known on SnRK2s not activated by ABA, however there are several examples showing that these kinases are also involved in plant defense against osmotic stress. Herein, we present data on the function of one of *Arabidopsis* ABA-non-activated SnRK2s, namely SnRK2.10. Using a simplified phosphoproteomic approach we identified several potential SnRK2.10 substrates phosphorylated by this kinase in response to salinity. Among them were dehydrins ERD10 and ERD14. SnRK2.10 interacts with them in planta and phosphorylates both dehydrins in vitro and in vivo. Our results showed that the phosphorylation of ERD14 has an impact on its subcellular localization.Additionally, we showed that SnRK2.10 is involved not only in salinity stress responses, but also in plant defense against dehydration; the water loss was significantly higher in rosettes of snrk2.10 mutants, than those of wild type plants or mutants deficient in SnRK2.4 (a kinase closely related to SnRK2.10).

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P3.40

Ethylene and its crosstalk with cadmium-induced retrograde signaling in *Arabidopsis thaliana* plants

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Cadmium (Cd) is one of the most toxic compounds that is released into our environment by historical and present-day industry and agriculture. Even trace amounts of this non-essential metal are harmful to human health, urging the need to remediate Cd-polluted soils. To this end, it is important to increase our insight into the molecular mechanisms underlying Cd stress responses in plants. Different players such as phytohormones and antioxidants are suggested to be involved in stress perception as well as mounting a signaling response, finally underlying plant acclimation to Cd. Within this framework, crucial roles were separately assigned to the phytohormone ethylene (Schellingen et al. 2014) and mitochondria (Keunen et al. 2013) in regulating acute responses of *Arabidopsis thaliana* plants to environmentally realistic Cd exposure. The aim of the current study is (1) to further characterize these Cd-induced signaling pathways and (2) to investigate potential crosstalk between them using a reverse genetic approach under acute (24 h) and more prolonged (72 h and 10 days) exposure to 5 µM Cd. We demonstrated that the mitochondrial DNA (mtDNA) content, an innovative biomarker for mitochondrial function, significantly increased after 72 h and 10 days of Cd exposure in the leaves. Expression levels of genes involved in mitochondrial biogenesis were generally upregulated by Cd, showing the highest induction after 24 h. Next to mitochondria, the endoplasmic reticulum (ER) can also regulate Cd stress responses. This was supported by a transcriptional induction of ER stress marker genes, reaching a maximum after 24 h but still present after 72 h and 10 days of Cd exposure. In addition, expression levels of transcription factors *ANAC013* and *ANAC017* increased in Cd-exposed plants. These ANACs are located at the ER membrane and mediate the induction of nuclear genes encoding mitochondrial proteins that mark retrograde signaling such as alternative oxidase 1a (AOX1a) (De Clercq et al. 2013; Ng et al. 2013). Our results therefore support a link between mitochondria and the ER in mediating Cd-induced signaling responses.

Furthermore, we show that ethylene (at least partly) mediates both mitochondrial as well as ER stress signaling. In contrast to WT plants, the mtDNA content did not significantly increase in leaves of Cdexposed *ethylene insensitive 2-1 (ein2-1)* mutants after 72 h. Furthermore, mitochondrial biogenesis as well as ER stress marker genes were not or less upregulated in *ein2-1* mutants as compared to WT plants. Also, *ANAC013* and *ANAC017* were not induced by Cd in leaves of *ein2-1* mutants. Therefore, ethylene is intertwined in the regulation of mitochondrial retrograde signaling, whether or not via ER stress, in Cd-exposed *A. thaliana*. This response deserves further investigation, including analyses at other biological levels besides gene expression.

Effect of heavy metals on selected morphological, ultrastructural and physiological parameters in two contrasting ecotypes of *Alyssum montanum*

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The aim of undertaken experiment was to determine the morphogenetic, anatomic and physiologic response of *Alyssum montanum* L. (Brassicaceae) to elevated levels of heavy metals. This pseudometallophyte is characteristic to calamine flora which spontaneously develop on old waste heap deposited as a result of Zn-Pb ores exploitation in the Olkusz Ore – bearing Region (Southern Poland) as well as on the areas unpolluted with metallic elements. The comprehensive analyses of both ecotypes were performed under in vitro conditions. The organ culture was established by placing 5 mm long shoot explants on modified MS medium (Murashige and Skoog 1962) supplemented with the combinations of zinc, lead and cadmium salts at the concentrations corresponding to the amounts of soluble forms of these elements in the calamine substrate, i.e. ZnSO4 (0.7 mM), Pb(NO3)2 (3.0 μM) and CdCl2 (16.4 μM) (Muszyńska et al. 2013). Medium without any addition of heavy metals was used as the control. The influence of tested treatment on plant growth parameters was evaluated biometrically and the Growth Tolerance Index (GTI) was ascertained for both ecotypes. Moreover, the antioxidant response of plants to heavy metal ions was determined at the cellular level, and the localization and composition of both phenolic compounds and antioxidant enzymes activities were examined under a light microscope (Olympus-Provis, Japan), an FEI 268D transmission electron microscope 'Morgagni' (FEI Company, Hillsboro, OR, USA) and an inverted confocal laser scanning microscope (Leica TCS SP5 II, Germany). To ensure better understanding of A. montanum adaptation to grow in the presence of heavy metals, spectrophotometric measurements of total phenols, phenylpropanoids (cinnamic acid derivatives) and flavonoids (flavonols and anthocyanins) were carried out in plant samples. The activity of the catalase and peroxidase was measured as well. Additionally, histochemical method based on staining with dithizone was used for detection of zinc, lead and cadmium ions in plant tissues.

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P3.42

New insight into possible role of LSU proteins family from *Arabidopsis thaliana*

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The plant response to sulfur deficiency includes extensive metabolic changes which can be monitored at various levels even before the first visible symptoms of sulfur starvation appear. Four members of the plant-specific LSU (response to Low SUlfur) gene family occur in *Arabidopsis thaliana* (LSU1-4). Three of them are induced by sulfur deficiency. LSU-like proteins do not have characteristic domains that provide clues to their function. Despite having only moderate primary sequence conservation they share several common features including small size, a coiled–coil secondary structure and short conserved motifs in specific positions. Although the precise function of LSU-like proteins is still unknown there is some evidence that members of the LSU family are involved in plant responses to environmental challenges and possibly in plant immune responses. Various bioinformatic approaches have identified LSU-like proteins as important hubs for integration of signals from environmental stimuli. It is possible that LSU/UP9 proteins modulate degradation of some specific "strategic" targets (such as TFs) in response to environmental stresses or are (directly or indirectly) involved in regulation of cellular degradation machinery. Although there is no clear evidence that LSU-like family members play such roles their interactions with presumed E3 ubiquitin ligases, chaperons (DnaJ-domain, Hsp60) and particularly with NBR1 (a selective autophagy cargo receptor) make the hypothesis plausible.

To obtain and verify the comprehensive and unbiased lists of candidates for LSU interacting proteins we conducted a tandem affinity purification coupled with mass spectrometry analysis (TAP-MS). Plants producing recombinant (TAP-tagged) LSU proteins were grown in S deficient or S sufficient conditions for 4 or 11 days. The complexes of proteins co-purifying with LSU2-TAP, LSU3-TAP and LSU4-TAP in each of mentioned conditions (tandem affinity purification) were identified by mass spectrometry. Some of the candidates for the LSU partners were tested for their direct interaction with individual LSU (LSU1-4) using additional techniques. The results of the experiments and conclusions will be presented.

The low ratio of red/far-red in the light spectrum accelerates senescence in the nest leaves of *Platycerium bifurcatum*

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The fern *Platycerium bifurcatum* is a valuable component of the flora of tropical forests, where degradation of local ecosystems and changes in lighting conditions occur due to the increasing anthropogenic pressure. The ratio of red/far-red (R/FR), in the spectrum of light reaching the plants, modifies the processes of morphogenesis and leaf senescence. In ferns, phytochrome mechanism responsible for response to changes in the value of R/FR differs from the mechanism observed in spermatophytes.

This study analyzed the course of ontogenesis of the nest leaves in *P. bifurcatum* at two values of the R/FR ratio, corresponding to shadow conditions (low R/FR) and intense insolation (high R/FR). The work used only non-destructive research methods, such as the analysis of the reflectance of radiation from the leaves, their blue-green and red fluorescence, and the measurement of chlorophyll a fluorescence kinetics. This allowed tracing the development and aging processes in the same leaves.

Nest leaves are characterized by short, intense growth and rapid senescence. The study identified four stages of development of the studied leaves related to morphological and anatomical structure and changing photochemical efficiency of PSII. Under the high R/FR ratio, the rate of ontogenesis of the leaf lamina was much slower than under the low R/FR value. As shown, the rapid aging of the leaves was correlated with faster decline of the chlorophyll content. It was shown that leaf senescence was accompanied by the accumulation of polyphenols, anthocyanins and carotenoids on the basis of reflectance and fluorescence measurements in the blue-green range.

P3.44

NtZIP1, a new Zn and Cd transporter from *Nicotiana tabacum*

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Zinc (Zn) is an essential micronutrient for all organisms. Cadmium (Cd) as a highly toxic heavy metal, reduces growth and disturbs development of plants. It was noted that the efficiency of Zn and Cd uptake and their translocation to shoots (thus root/shoot partitioning) depend on mutual concentrations of both metals in the growth medium. Recent study suggested that NtZIP1 might be involved in the regulation of this phenomenon at the molecular level in tobacco.

The aim of this research was to clone and achieve basic information on the NtZIP1: structure, membrane localization and substrate specificity. NtZIP1 belongs to the ZIP family (ZRT-, IRT-like Proteins) of Zn transmembrane transporters. In addition to Zn, some members of this family mediate translocation also other substrates including toxic Cd. ZIP proteins consist of 309-476 amino acid residues with eight putative transmembrane (TM) domains.

Membrane topology is similar, in which the amino- and carboxyl-terminals of the protein are located on the outside of the plasma membrane. There is a variable region between the TM domains III and IV, that contains histidine residues a potential metal binding domain.

With the use of BLAST programme, the amino acid sequence of NtZIP1 from *Nicotiana tabacum* was identified in the Gene Bank (based on the homology to the ZIP sequence of *A. thaliana*). Obtained incomplete sequence of NtZIP1 was used to get the full length sequence of an RNA by RACE PCR (Rapid amplification of cDNA ends). Complete ORF (open reading frame) of NtZIP1 allowed to predict a model for the membrane topology of the NtZIP1 protein by comparison of the amino acid sequence with its homologues from other plant species.

To identify a membrane in which the NtZIP1 is located, the ZIP1:GFP (green fluorescent protein) fusion protein was transiently expressed in tobacco leaves under the 2x35S CaMV (cauliflower mosaic virus) promoter. It was demonstrated that the NtZIP1 is localized in the plasma membrane.

The system of heterologous expression in yeast has been used to indicate which metals are transported by the NtZIP1 protein. Two yeast strains (*Saccharomyces cerevisiae*) were used: wild type (WT) DY1457 and the double mutant zrt1zrt2 (ZHY3), defective in high and low affinity Zn uptake. The yeast growth assay indicated that NtZIP1 transports Zn and Cd.

To conclude, NtZIP1 localized in the plasma membrane mediates uptake of Zn and Cd in tobacco cells.

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Medicago truncatula ABCG proteins between ABA transport and root morphogenesis

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Abscisic acid (ABA) movement within plant determines its mode of action and subsequently triggers adequate responses under unfavorable conditions. In *Arabidopsis thaliana*, members of the ATP-binding cassette (ABC) family have been shown to be involved in ABA transport. Intriguingly, almost nothing is known about the ABA translocation and ABA transporters in legumes. Based inter alia on phylogenetic analyses of the half-size ABCG transporters in *Medicago truncatula* we have selected four proteins potentially involved in ABA transport. We have shown that expression of the corresponding genes is strongly induced upon drought stress, mimicked by PEG and ABA. A spatial expression pattern analysis with GUS reporter gene revealed that among of them MtABCG20 and MtABCG26 are expressed mostly within vascular bundles where ABA is predominantly biosynthesized. Two others MtABCG27 and MtABCG29 are expressed in meristems and in cortex root elongation zone respectively, where ABA is necessary for lateral root growth. The corresponding proteins are located in the plasma membrane. The Bimolecular Fluorescence Complementation assays confirmed that MtABCG20 and MtABCG26 have the ability to make homodimers. Interestingly enough, MtABCG20 is also expressed in the embryo of germinating seeds. This is in contrast to its closest homologue from *Arabidopsis* AtABCG25 which has been found in the seed endosperm. Performed transport experiments in a heterologous expression system (BY2) have shown that MtABCG20 contributes to ABA extrusion. All together points to MtABCG20 as a plasma membrane ABA transporter controlling *Medicago* seed germination by ABA extrusion from embryo.

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P3.46

The toxicity and detoxification of cadmium in the green alga *Acutodesmus obliquus* (Chlorophyceae)

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The phytotoxicity of cadmium (Cd) at the range of concentrations 0.1-100 μM and defense strategies in *Acutodesmus obliquus* were studied. The biosorption of Cd by algal cells was found to be increased in a concentration dependent manner. Accumulated metal inhibits cell division, accelerates chlorosis, reduces the contents of proteins as well as induces oxidative stress. However, the activation of defense mechanism alleviating Cd toxicity was observed in algal cells. The increase in the level of non-enzymatic antioxidants and the activity of superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase were detected in cells treated with Cd. External metal stimulated the synthesis of phytochelatins (PC2-5) responsible for Cd binding and detoxification. The enhancement in xanthophyll cycle indicates its significant role in algal adaptation to adverse environmental conditions. One of the most interesting aspects of defense mechanisms against metal stress is the stimulation of the synthesis of abscisic acid which adjust algal metabolism to abiotic stress.

Different mechanism affecting frost tolerance are activated during cold temperature flooding in two winter rye lines contrasting in snow mould resistance

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The aspects of winter hardiness that have become important due to climatic changes and global warming involve excess water from melting snow and/or rain that create anoxic conditions for plant roots. High soil moisture increases in turn a risk of pathogen infection and may influence frost tolerance.

The aim of work was to investigate how flooding of roots at low temperature during cold acclimation may affect frost tolerance of two winter rye lines contrasting in snow mould resistance. Special attention was devoted to the accumulation of pathogenesis related (PR) proteins and glutathione metabolism in organs subjected to stress (roots) as well as in crowns and leaves. Expression of genes accompanying cold acclimation such as CBFIVa-2A (C-repeat/DRE-Binding Factor) and sucrose-1-fructosyltransferase (1-SST) were also analyzed.

Ten days of flooding resulted in increased frost tolerance but only in line more tolerant to snow mould. The investigated lines differed in their capability for accumulation and activity of the β-1,3-glucanases in leaves, crowns and roots. Prolonged cold flooding in less tolerant line led to loss in one of the glucanase isoform in roots and crowns, but not in leaves. The highest glutathione content was recorded in leaves while the lowest in roots. Flooding upregulated the expression 1-SST and CBF genes in a greater extend in less tolerant line.

Cold flooding induced defense reaction, including accumulation of PR proteins and glutathione, occurred particularly in leaves. Flooding treatment was also capable of achieving higher transcript level of CBF and 1-SST genes, as compared to cold treatment alone. The involvement of investigated traits in increase of frost tolerance of winter rye will be discussed.

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P3.48

Brassinosteroid regulates protective mechanisms in cucumber (*Cucumis sativus* L.) seedlings exposed to lead stress

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Brassinosteroids (BRs) are steroidal plant hormones which play a crucial role in the regulation of growth and development of various plant species. Their metabolism ensures the proper course of embryogenesis, xylogenesis, and the development of shoot and root meristems. In addition, they demonstrate protective activity in plants which are exposed to various stresses. Due to the quick development of industry, metals have become one of the most important stressor. Accumulation of metals beyond critical levels leads to oxidative stress in plants. However, BRs take part in protecting plants from toxic effect of metals. Therefore, the effect of the optimum concentration (10^{-8} M) of 24-epibrassinolide (EBL) on cucumber (*Cucumis sativus* L.) seedlings has been studied in the situation of exposure to lead in the range of concentration 10^{-6}-10^{-3} M. The study also involved the effect of EBL on the activity/content of enzymatic and non-enzymatic antioxidants such as: superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, ascorbate and proline in metal-stressed cucumber. The level of phytochelatins (PCs) and the activity of PC synthase were also studied. The research shows that EBL increases the activity/content of the above-mentioned antioxidants in cucumber exposed to metal stress. The increase is the most intensive in the lowest lead concentration (10^{-6} M) combined with EBL. In cucumber, as a response to the activity of lead ions, PC synthesis is also intensified through higher activity of PC synthase. The activity of that enzyme and the PC level are the highest in plant treated with 10^{-3} M lead. Thus, we can conclude that EBL protects plants from the toxic effect of lead. We know that EBL can modify some agronomic traits of plants, which encourages the study of application of this compound in phytoremediation.

Interactions between LHCII and PSI in M and BS chloroplasts of maize in different light conditions

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Maize (*Zea mays* L.) is a plant that represents the C4 type of photosynthesis. In these plants, assimilation and CO2 reduction reactions are spatially separated between two types of cells: mesophyll (M) and bundle sheath (BS). Both, M and BS chloroplasts differ structurally and functionally. The structure of M cells chloroplasts is similar to that of the C3 plants chloroplasts, while the chloroplasts of BS lack grana. Due to the heterogeneous distribution of photosynthetic complexes in the thylakoid membrane, photosystem I (PSI) dominates in the BS chloroplasts. The photosystem II (PSII) content is at about 20% of M chloroplasts. Although the PSII content is low in the BS chloroplasts, it is characterized by high abundance of the external antenna systems (LHCII). The LHCII proteins: Lhcb1, Lhcb2, and Lhcb3 are assembled into trimers, forming three types of antenna characterized by the various affinity of binding to the PSII core. Loosely bound trimers (L-LHCII) are a mobile pool of LHCII that can be phosphorylated and in this form, they can migrate to the outer regions of the grana or unstacked regions, where PSI is located. Therefore the LHCII complexes can be linked to either PSII or PSI and in dependence on the intensity of light they may alter their association partner, in a process called "state transitions".

Phosphorylation of Lhcb2 plays a crucial role during the transition from state 1 to state 2.

Our studies showed that under low light intensity (LL, 80 μmol photons m-2 s-1) phosphorylation of LHCII trimers is very strong in both M and BS chloroplasts, but only in BS chloroplasts, strong phosphorylation of LHCII monomers was also observed. After 1 hour exposure to high-intensity irradiation of far-red light (FR, 720nm, 200 μmol photons m-2 s-1) thylakoid proteins were dephosphorylated only in M chloroplasts. Similar results were obtained for native separation of thylakoid complexes by Blue Native PAGE (BN-PAGE). The amount of PSI-LHCI-LHCII band didn't change significantly in BS after FR treatment, while in M they disappear completely. The presence of the PSI-LHCI-LHCII complex was also confirmed by sucrose gradient ultracentrifugation of chloroplasts solubilized with digitonin and a-DDM and by low-temperature fluorescence analysis. Emission of fluorescence at 733 nm (characteristic for PSI), indicated that only in M chloroplasts exhibited classical "state transitions". In BS chloroplasts the energy absorbed by LHCII was transferred to PSI independently of light conditions.

The aim of the project is to demonstrate that the PSI-LHCI-LHCII complex (state 2) in BS chloroplasts retains its functional form permanently, independent of light conditions, ensuring efficient utilization of light energy for cyclic electron transport and ATP production.

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P3.50

The toxic effect of Cd and Zn on photosynthetic apparatus of two pseudometallophytes: *A. arenosa* and *A. halleri*

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The accumulation and tolerance of HM in plants have developed in the course of their evolution as a response to abiotic stress. The hyperaccumulation and hypertolerance of heavy metals, which is manifested as the accumulation of unusually high concentrations of metals in plant shoots without any visible symptoms of toxicity, are extreme examples of these traits. *Arabidopsis halleri* is considered as a model hyperaccumulator of Cd and Zn. *Arabidopsis* arenosa, closely related to *A. halleri*, on the basis of research which have been conducted in our laboratory also seems to be a hyperaccumulator of Cd and Zn.

The seeds of *A. arenosa* and *A. halleri* were collected from the metalliferous site located in Piekary Śląskie, vernalized and germinated on vermiculite for one month. Seedlings were cultivated in hydroponic culture on Hoagland medium by 8 weeks, then Cd or Zn was added at the concentration of 1.0 or 5.0 mM respectively. Measurements of chlorophyll a fluorescence (Pocket PEA, Hansatech Instruments Ltd, England) and pigments content (Dualex Scientific+, Force-A, France) were performed every 12 hours for 120 hours of treatment. The accumulation of Cd and Zn in shoots was measured at the end of treatment. The toxic effect of Cd was observed as a decrease of chlorophyll content by 25 and 15% in leaves of *A. arenosa* and *A. halleri* respectively. Simultaneously Cd caused three-fold increase of anthocyanins content in epidermis of leaves of both species. Higher dose of Zn caused a decrease of chlorophyll content by 20% of initial value for both species. The content of flavonols in *A. halleri* leaves was diminished by 25% under Zn treatment. By contrast, content of anthocyanins increased two-fold in epidermis of leaves of both species. Cd mainly damaged the electron transport chain of *A. halleri* at the pool of reduced QA. For *A. arenosa* all protein complexes (e.g. Oxygen Evolving Complex and Ferredoxin-NADP+ Reductase) were considerably damaged, however, the decrease of yield of photosystem II (PSII) was comparable after Cd treatment for both species (about 90% of initial value). Zn caused higher damage to photosynthetic apparatus of *A. halleri* than Cd, as a result yield of PSII was lowered by 10% compared to initial value. Treatment of *A. arenosa* with Zn caused insignificant inhibition of electron transport and decrease of yield of PSII. *A. arenosa* was characterized by accumulation of Cd or Zn in shoots at the concentration of 6000 or 5600 mg/kg DW respectively, whereas *A. halleri* accumulated 2100 or 9500 mg/kg DW of Cd or Zn respectively. Cd and Zn caused a decrease of chlorophyll and increase of anthocyanins content in leaves of investigated plant species. Both metals damaged various parts of electron transport chain and the level of damage in both species seems be correlated with concentration of metal accumulated in tissues.

High temperature changes the photosynthetic activity of *Botryococcus braunii* cells

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One of the stress factors during the cultivation of algae is the temperature, which affects the growth rate, chemical composition of biomass and physiological properties of the cells. Depending on algae species, the temperature should oscillate between 20-30°C. A temperature below 16°C slows down the growth, and higher than 35°C is lethal to many algae strains. The aim of this study was to determine, on the basis of photosynthetic activity parameters, the optimum temperature range that allows rapid biomass increase in the *Botryococcus braunii* cultures. The study used a *B. braunii* strain (SAG 807-1) was purchased from the SAG collection in Gottingen and cultured on BG11 medium. Algae culture was carried out in parallel in two SARTORIUS BIOSTAT® PBR 2S bioreactors with a capacity of 3 dm^3. The culture temperature ranged from 25 to 35°C. Duration of the culture was 8 days with a photoperiod of 16/8 hours (day/night) under white light from fluorescent lamps (OSRAM DULUXL) mounted in the photobioreactor by the manufacturer around the bioreactor tank. The performance of PSII photochemical efficiency was determined by measuring chlorophyll a fluorescence parameters using a Handy Plant Efficiency Analyzer with an attachment for liquid samples. The intensity of the excitation light was set to 3 mmol m^{-2} s^{-1}. The measurement was performed, at room temperature, after 6-minute adaptation of the sample to darkness. Adaptation time was optimized experimentally before the start of the experiments. Fluorescence kinetic parameters were recorded in the range from 10 μs to 1 s.

The analysis of the relationship between the structure and function of the photosynthetic apparatus and the assessment of algae vitality was based on the OJIP test. Subsequently, the following parameters were analyzed: F0, Fm, Fv/Fm, Fv/F0, TR0/RC, ET0/RC, DI0/RC and PI. Temperature of *B. braunii* cultures changed the efficiency of the photosynthetic apparatus during the biomass growth and could be divided into four ranges: suboptimal (18°C–20°C), optimal (23°C–30°C), supraoptimal (32°C) and lethal (33°C–35°C).

P3.52

Improving drought tolerance and productivity in barley with ABA, putrescine and hydrogen peroxide

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Drought is the major abiotic factor that triggers stress in plant cells. In addition, according to the expertise presented by climatologists global surface air temperatures are going to rise and this trend will be preserve till new, higher atmospheric energy equilibrium, changing intensity and frequency of precipitation, rate of soil moisture evaporation and water balance. Drought limits the intensity of photosynthesis, increases generation of reactive oxygen species (ROS) and disturbs lots of biochemical processes related to the plant growth and development.

We selected two varieties of barley (*Hordeum vulgare* L.), Carina and Lomerit, based on their different crop yield. Previous studies have confirmed that tested plants differ with the onset of the flag leaf senescence and gained yield. In addition measured net photosynthesis and effective PSII quantum yield confirmed that the high-yielding Lomerit is also characterized by higher photosynthetic efficiency. Four-week-old barley plants, grown under controlled conditions (16/8 h day/night, 130 µmol m^{-2} s^{-1} RGB LED light, 22-24°C; RH=55%), exposed to 7-days water deprivation, were sprayed twice (just before water limitation and in the middle of the treatment) with 100 µM abscisic acid (ABA), 100 µM 1,4-diaminobutane (putrescine) and 5 mM hydrogen peroxide (H_2O_2) to initiate and sustain drought stress response.

The treatment scheme used to initiate and then maintain the immune response measured by changes in photosynthetic efficiency (Fv/Fm, ΦPSII, ΦNPQ, ΦNO). In the case of the Lomerit variety, short-term drought no statistically affected on changes of the measured parameters, confirmed that one of the determinants of productivity yield is the high tolerance to drought stress. The intensity of stress, in more sensitive variant, was measured by the concentration of chlorophyll, relative water content (RWC), rate of membrane lipid peroxidation (MDA), endogenous hydrogen peroxide content, activities of enzymes namely superoxide dismutase (SOD) and Rubisco, and changes in the characteristic of photosystem II (PSII) (PAM). At the same time, soil moisture changes were measured to monitor the progressive water deficit.

One of the most important challenges of modern agriculture is to guarantee sustainable agricultural production, by selecting new, more drought-tolerant varieties. An alternative may be the use of compounds able to limit the negative effects of its occurrence and significantly improve productivity of crops.

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The use of CRISP/Cas9 system for targeted mutagensis of AtCPK11 involved in salt tolerance in *Arabidopsis thaliana*

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We have used CRISPR/Cas9 system to knock-out the AtCPK11 in *Arabidopsis thaliana*. The kinase is involved in signal transduction pathway in salt stress. Calcium-dependent protein kinases (CDPKs) are essential sensor-responders in Ca^{2+} signaling, modulating various of plant growth, development and response to environmental changes. In stress sygnaling networks CDPKs play roles including activation and inhibition of enzymes, ion channels and transcription factors.

T-DNA contains, beside selection genes, cmCas9 gene coding Cas9 endonuclease – core element of CRISPR/Cas9 genome editing system, a technology popular in recent years in genetic transformation research. The CRISPR (clustered regularly interspaced short palindromic repeats) genome editing system consists of the Cas9 endonuclease and a cognate single-guide RNA component. A strong expression of Cas9 is required to produce an adequate number of double-strand breaks at designated chromosomal loci – the initial step in the editing process. This can be accomplished by providing appropriate regulatory elements and optimizing the Cas9 coding sequence from *Streptococcus pyogenes*.

The preliminary work of the group concerned construction of CRISPR/Cas9 system for editing the AtCPK11 gene in *Arabidopsis*. The construct based on plasmid pDE-Cas9 (with Cas9 optimized for *Arabidopsis*), it was obtained from prof. Holger Puchta, Karlsruher Institute für Technologie. SgRNA dedicated to *Arabidopsis* was designed in our group. The aim of preliminary project with AtCPK11 is to test procedures of *Agrobacterium*-mediated transformation, regeneration of transformants, genotyping and phenotyping of genetically modified plants.

P3.54

Influence of ozone on photosynthetic activity and selected physiological parameters of three species of the genus *Clusia*

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No reports concerning the effects of ozone on the species of the genus *Clusia* are known. However, such studies appear to be important because of the reported increase of tropospheric ozone concentrations over tropical rainforests. The aim of the study was to examine whether ozone stress has a similar effect on photosynthetic activity and selected physiological parameters of plants of the genus *Clusia*, which show different physiotype of photosynthesis: *Clusia multiflora* (constitutive C3), *Clusia rosea* (constitutive CAM) and *Clusia minor* (facultative CAM). The plants were fumigated by ozone (150 ppb) in a greenhouse for 21 days at the natural photoperiod (June), temperature 25/15°C – day/night, respectively. Photochemical efficiency of Photosystem II (PSII) was evaluated on the basis of the parameters of the chlorophyll fluorescence kinetics (OJIP test). The general physiological condition of the plants was determined using fluorescence indicators of their leaves (F450/F520, F450/F690, F450/F735, F690/F735) and foliar reflectance indexes (ARI, CRI, and PRI). Ozone caused a decline in the value of Fv/Fm in *C. minor* and *C. multiflora*, reducing the amount of chlorophylls in the leaves of *C. multiflora* and *C. rosea*, while the increase of the amounts of these pigments in *C. minor* was observed. Additionally, the increase in the concentration of phenolic compounds in the epidermis of *C. rosea* and *C. multiflora* has also been observed. The values of the reflection indexes did not indicate the stress response in the tested species.

Differentiation in the pattern of osmoprotectants, phytohormones, plasma membrane and cell wall properties of *Arabidopsis* growing at low light intensity and NaCl stress

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Exposure of plants to salinity causes ionic imbalances, osmotic and oxidative (excess of AOS, active oxygen species) stresses and perturb plant metabolism (Flowers and Colmer 2015). Moreover, salt stress causes a reduction in plant cell turgor pressure and leads to ultrastructural changes at the plasma membrane (Hock and Elstner 2004, Pottosin and Shabala, 2016). Tocopherols (TOCs) can quench AOS compounds like singlet oxygen ($^1O_2$), reduce hydroxyl radical (OH) and limit the extent of lipid peroxidation. The changes in α- or/and γ-TOC content cause alteration in $^1O_2$ concentration in the cell and/or influence on membrane disorganization (Rastogi et al. 2014). *Arabidopsis thaliana* WT (wild type, Columbia) plants and three mutants: with defects in VTE1 gene (vte1), VTE4 gene (vte4) and the line overexpressing γ-TMT methyltransferase (tmt) under under 200 mM NaCl were studied.

Our results pointed out that the lack or diminished level of α-TOC under salinity lead to the stronger decrease of turgor pressure parameters like e (the elastic modulus representing the change in turgor pressure (DP) of the cell caused by a change of the cell volume (DV), P (the turgor pressure of the cell) and Lp (the hydraulic conductivity) in VTE1 and VTE4 mutants, when compared to WT and TMT plants. In parallel, the content of osmoprotectants such as proline betaine, glycine betaine, putrescine, glycerol and maltose was also lower in VTE1 and VTE4 mutants under salinity, while the level of proline and sucrose increased. Additionally, the ACC (ethylene precursor) and salicylic acid (SA) showed the opposite pattern of changes; as decrease in ACC concentration was associated with increase in SA content in VTE1 and VTE4 mutants. These changes were accompanied by the cell-specific localisation of some arabinogalactan protein epitopes and pectic epitopes that possibly were induced as a part of compensation/defence mechanisms. We suppose that lack of, at least, one TC form even at low light condition causes probably changes in the relationship of AOS, leads to the induction of different signal pathways involving active oxygen species and affect in different ways plasma membranes, their osmolarity and turgor pressure level and structure. We suggest that tocopherols regulate, at least partly, processes leading to salinity adaptation and/or senescence.

P3.56

Expression of Calcium Dependent Protein Kinase (ZmCPK11) improves salt tolerance in transgenic *Arabidopsis thaliana* leaves by regulating sodium and potassium homeostasis

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Increased enzymatic activity and transcript level of ZmCPK11 upon salinity, one of *Zea mays* Calcium Dependent Protein Kinases (CDPKs), led us to formulate a hypothesis that ZmCPK11 is involved in response to salinity and may contribute to salinity tolerance. For elucidation of the role of ZmCPK11 in response to salinity, the transgenic *Arabidopsis thaliana* plants expressing ZmCPK11 were generated. Expression of ZmCPK11 in *Arabidopsis* plants did not affect their phenotypes under control conditions. However, under salinity the transgenic plants were more tolerant to stress compare to Wild Type (WT) plants and plants expressing empty vector. The rosette leaves of control plants became chlorotic and showed aging symptoms, while the leaves of transgenic plants expressing ZmCPK11 were less affected and more green.

Sodium is one of the most toxic ions to plants. High concentration of NaCl causes osmotic stress, water deficit, disruption of K^+ uptake, inhibition of many cellular processes resulting in growth inhibition and decreased crop productivity. One mechanism of salt tolerance in plants is detoxification. It was well documented that Na^+ transporters such as AtHKT1 (high-affinity K^+ transporter 1), AtSOS1 (salt overly sensitive 1, Na^+/H^+ antiporter) and AtNHK1(a vacuolar membrane-bound Na^+/H^+ antiporter) play an important role in removing excess sodium from cytoplasm in *Arabidopsis*. Overexpressing of these proteins enhanced salt tolerance in plants. Here, we have shown that expression of AtHKT1, AtSOS1 and AtNHK1 genes was significantly higher in roots of ZmCPK11 transgenic plants compared to WT plants, both in control conditions and upon salinity stress. Because these genes play essential role in salt tolerance by regulating Na^+ and K^+ content, the accumulation and distribution of Na^+ and K^+ in roots and leaves were studied. The results showed that under salinity stress the content of Na^+ increased in all analyzed plants, but the transgenic plants had a lower Na^+ content in roots and leaves than the WT plants. The content of K^+ was slightly higher in leaves, but lower in roots of transgenic plants compared to WT plants. Maintenance of a low cytosolic Na^+/K^+ ratio is critical for the function of cells and salt tolerance. The Na^+/K^+ ratio was lower in leaves and higher in roots of three ZmCPK11 transgenic lines compared to WT plants. These results are consistent with those previously obtained by us showing that rosette of transgenic ZmCPK11 plants were more tolerant to salinity than the WT plants and root growth in transgenic plants is much more inhibited.

These results show that improved salt tolerance in transgenic ZmCPK11 plants could be achieved by upregulation of genes involved in limiting Na+ accumulation in plant cells.

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Participation of calpain and phytocystatins in the response of cereals to aluminum ions toxicity

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Poland has a large acreage of acid soils (approx. 80%) in agricultural fields. One of the main factors limiting the productivity of acid soils is the toxicity of aluminum ions. In plants, the inhibition of root elongation is important sign of phytotoxic effects of aluminum ions. This is the result of reduction of ion absorption i.e. Ca^{+2}, Mg^{+2} and K + .. Ca^{+2} ions participate as integral components of many signal transduction pathways. The calpains, intracellular cysteine proteases, which are activated by Ca^{+2} ions, probably are involved in modulation of signal transduction processes and control of cell-cell interaction. The tuning of proteolytic processes driven by the calpains is not just a consequence of their differential expression levels but it is also dependent on the presence or absence of their specific inhibitors called (in animals) calpastatins. Although, no plant calpastatin homologue sequences have been identified, the family of phytocystatins (papain-like cysteine protease inhibitors) could play the same role in plants as calpastatin in animals.

The aim of our research is to know if calpain and phytocystatins are involved in modulation of signal transduction processes in response of cereals to the toxicity of aluminum ions. The physiological test of aluminum tolerance showed a significant difference in the length of the regrowth of roots between more tolerant triticale cultivar or rye and less tolerant triticale cultivar to the aluminium toxicity. The identification of calpain gene in rye and triticale roots was performed and next the expression of this calapin and phytocystatins (previously identified in triticale) was measured. We observed different expression profiles of examined genes which indicate that they are involved in response of cereal plants to aluminium stress.

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P3.58

The effect of light on the activity of group I SNF1-related kinases 2

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Light is a key environmental factor for plants. It is a source of energy for photosynthesis, but it also provides information about surroundings. Light exerts its action upon plant cells via photosynthesis-related processes, e.g. changes in redox status or reactive oxygen species production. Light is also absorbed by photoreceptors-specialized proteins that translate the changes in the light into a signal that can be processed by the cell and lead to physiological reactions.

SNF1-related kinases 2 (SnRK2s) are plant specific group of protein kinases regulating responses to adverse environmental conditions and ABA-dependent development. They can be divided in three groups, based on phylogenetic relations and activating factors. Almost all SnRK2s are activated by osmotic stress but those belonging to group III are also strongly activated by ABA. Group II kinases are only weakly activated by ABA, whereas group I kinases are not ABA responsive. The activation of SnRK2s is rapid and leads to phosphorylation of downstream targets, such as transcription factors and RNA binding proteins.

Here we examined the activity of two group I kinases, SnRK2.4 and SnRK2.10, in adult leaves. First, the changes in activity during the day, upon turning on and off the light, were assessed. We observed light-dependent increase in the basal activity of the kinases. Light conditions have also an effect on the activation of the kinases during salt stress. In leaves of plants exposed to NaCl in light, the activation of kinases was more rapid and intense than when plants were in darkness. Possible ways of light action are discussed. Light-dependency of kinase activation agrees with the picture of light as an important environmental cue modulating responses to different stimuli.

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In search of the cause of ammonium syndrome: can it be the toxicity of excess methylglyoxal or intermediates of its catabolic pathway?

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Plants can take up two different inorganic forms of nitrogen (N), nitrate (NO_3^-) and ammonium (NH_4^+). Although NH_4^+ should be the preferred form of N, because its assimilation is associated with a lower energy demand on the cell, surprisingly cultivation using NH_4 as the sole N source for many plants leads to severe growth disorders known as 'ammonium syndrome'. In our research we assume that the cause of ammonium toxicity may be increased production of methylglyoxal (MG) or intermediates of its catabolic pathway, D-lactate or S-D-lactoylglutathione (SLG). MG is mainly released as a by-product of glycolysis and when accumulated is highly cytotoxic. To keep MG levels in check, exists the two-step reduced glutathione-dependent glyoxalase pathway comprising glyoxalase I (GLXI) and glyoxalase II (GLXII), which act sequentially to convert MG first into SLG and subsequently into D-lactate. Recently was described a novel type of glyoxalase III in plants (GLXIII), which coverts MG into D-lactate in a single step without the help of any cofactor. The produced D-lactate is then metabolized in mitochondria. The purpose of our work was to analyze MG metabolism in NH_4^+-treated plants (*Arabidopsis thaliana* ecotype Col-0) in comparison with NO_3 fed plants (control). Our results demonstrate that MG production and degradation are enhanced in response to ammonium nutrition, while the metabolism of D-lactate is diminished. Our results confirm that MG metabolism may be one of the factors leading to the development of ammonium toxicity symptoms.

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P3.60

How altered apoplastic ROS metabolism can alleviate growth cessation of the frostbite1 mutant under ammonium nutrition?

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Plants are capable of utilizing different inorganic nitrogen sources, mainly nitrate (NO_3^-) and ammonium (NH_4^+). Most crop plants are sensitive to prolonged application of NH_4^+ based fertilizers, causing severe growth suppression, which are together commonly referred as the "ammonium syndrome". However, despite many years of research, the mechanism of plant sensitivity to ammonium is still unclear.

An exceptional case is the frostbite1 (fro1) mutant, which lack mitochondrial respiratory chain complex I. These plants are not sensitive to ammonium application compared to control plants (Podgórska et al. 2015).

Plant cells grow by expanding their cell walls through loosening of wall polymers. In this process are involved reactive oxygen species (ROS), present in the extracellular space (apoplast). To understand changes in growth of fro1 under ammonium feeding, apoplastic ROS/antioxidant metabolism was analyzed.

The apoplastic ROS metabolism in fro1 under ammonium nutrition is significantly altered compared to control plants. There are changes in the capacity of the extracellular antioxidant system (e.g. glutathione levels) and in activity of ROS detoxifying enzymes, located in the apoplast (superoxide dismutases, peroxidases). We demonstrate different levels of OZF-1 gene expression, which is a marker of an oxidative stress of the extracellular space. Moreover, fro1 plants have altered cellulose content and pectin methylesterases activity.

Our results indicate that the better growth of fro1 plants in the presence of NH_4^+ might be related to the apoplastic ROS metabolism. Because these plants are characterized by mitochondrial dysfunction the possible connection between plant mitochondria functioning and the apoplastic metabolism is discussed.

[1] Podgórska et al. (2015) Plant Cell Environ, 38: 224-237

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Photosynthetic apparatus acclimatization of *Anthyllis vulneraria* L. to Pb toxicity in *in vitro* conditions

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Anthyllis vulneraria L. is a herbaceous leguminous plant which is known as a Zn hyperaccumulator. While kidney vetch response to Zn toxicity are relatively known, there is a lack of literature knowledge of *Anthyllis vulneraria* reaction to Pb.

The aim of presented research was to determine the acclimatization strategy of kidney vetch photosynthetic apparatus under high lead concentration.

Shoot explants of calamine kidney vetch ecotype were placed on agar media containing 0.0; 0.5; 1.0 and 1.5 mM Pb. The photosynthetic apparatus efficiency, as well as the lipid peroxidation level after 4 and 8 weeks of culture were examined.

The highest malondialdehyde (MDA) level, with simultaneously chlorophyll a and total carotenoids content decrease was observed in plant shoots from 1.5 mM Pb.

The carotenoids and chl a content decrease under lead stress, combined with the plastochinone pool increase as well as the efficiency of linear electron transport, indicating the acclimatization of the photosynthetic apparatus. This allows for the most efficient use of light radiation without exposing plants to photooxidation.

P3.62

Programmed cell death is involved in the formation of Zn-related lesions in tobacco leaves

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Recent studies indicate that in the leaves of tobacco plants exposed to high Zn concentration in the medium pre-lesion regions developed from mesophyll cells accumulating high amount of Zn [1]. These regions subsequently turn into lesions with high amount of Zn inside. Accumulation of Zn in the lesion regions likely contributes to protecting the neighbouring cells from Zn toxicity. We proposed that formation of lesions could be a mechanism of tolerance to Zn, rather than only the symptoms of Zn sensitivity. In this study the involvement of programmed cell death (PCD) mechanisms in the formation of Zn-related lesion in tobacco leaves was investigated.

Five-week old hydroponically grown tobacco plants were exposed to 200 μM Zn. All analysis were performed on the second leaf (counting from the bottom).

To determine the involvement of PCD mechanisms in the formation of lesions in tobacco leaves, the TUNEL assay method was used. Fragments of leaf blades were fixed in 2% paraformaldehyde in PBS, embedded in the Steedman's wax and cut on a microtome. The cross-sections were subjected to the TUNEL analysis. They were examined using the Laser-Scanning Confocal Microscopy (CLSM). The co-localization of PCD-positive cells and lesions in the cross-sections of tobacco leaves were expected.

Moreover, the expression of the following PCD marker genes was determined: NtBI-1, NtSIPK, Ntrboh [2,3,4] by Real-Time PCR. The increase in the expression level of the PCD genes in leaves of plants exposed to high Zn was foreseen.

The TUNEL analysis showed the PCD-positive nuclei in the cross-sections of leaves from tobacco plants grown in the presence of high Zn in the medium. They were not present in the control sections. This result suggests that the PCD mechanisms are most probably involved in the formation of lesions in tobacco leaves.

The expression analysis of PCD marker genes showed the increase in their transcript level in the leaves from high Zn-treated plants in comparison to the control plants. The highest differences in the expression between Zn-treated and control plants were noted on the 2nd day of treatment. Results indicate that the initiation of lesions formation takes place on the 2nd day of Zn-treatment, and the PCD mechanisms could be involved in this process.

The presented results showed that Zn-related lesions are most likely a form of programmed cell death, rather than an accidentally cell dying as a result of Zn-toxicity.

[1] Siemianowski, O., et. al, (2013). Plant, Cell and Environmental, 36: 1093-1104

[2] Sanchez, P., et. al, (2000). The Plant Journal, 21: 393-399

[3] Samuel, M.A., et. al, (2005). The Plant Journal, 42: 406-416

[4] Overmyer, K., et. al, (2003). Trends in Plants Science, 8: 335-342

Physiological and anatomical aspects of antioxidant response in heavy metal-tolerant *Daphne jasminea* shoot cultures

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In the course of in vitro selection, two lines of woody shrub *Daphne jasminea* Sibth. & Sm. (Thymelaeaceae) have been obtained that exhibited increased tolerance to lead and cadmium, respectively. Tolerant shoots were able to propagate vigorously and rooted easily in culture media containing 1.0 mM $Pb(NO_3)_2$ or 5.0 μM $CdCl_2$. For over two years shoots had been regularly subcultured onto fresh medium containing heavy metal salts, with no inhibition in growth and multiplication rate.

Regenerated plant material from both tolerant lines was then examined for its antioxidant response to long-term heavy metal treatment. In the root and shoot samples the activity of peroxidase and catalase was analyzed, as well as the content of phenolic compounds. Histological distribution of both enzymes was also studied.

In heavy metal-treated plants the activity of antioxidant enzymes was significantly higher than in non-treated control plants. Differences in enzyme activity occurred also between both tested lines, especially in the case of peroxidase, which activity was the highest in the roots of Cd-tolerant line, cumulating mainly in the cortex parenchyma. In the leaves of both Cd- and Pb-treated plants peroxidase activity was particularly pronounced in epidermal layer. Heavy metal exposure resulted also in enhanced accumulation of catalase in the roots, in comparison with its level in the leaves. Stimulation of antioxidant response in *Daphne* tolerant lines was also reflected in increased content of phenolic compounds. In comparison with control plants, in Pb-tolerant line total phenolic content was 4 times and 15 times higher in the shoots and in the roots, respectively. It can be concluded that heavy metal tolerance in *Daphne jasminea* shoot cultures was induced as a result of enhanced activity of cellular antioxidant machinery.

P3.64

Changes in gene expression profiles during seasonal senescence of *Populus trichocarpa* leaves and fine roots

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Plant ontogenetic development can be divided into few stages: embryonic stage, germination, vegetative development, generative development, and senescence. The last stage, senescence, directly precedes death of a specific group of cells, organs or the entire plant. Although senescence is a destructive process, it is also well organized and precisely regulated. Senescence of plant organs, i.e. leaves and petals, involves the mechanisms responsible for programmed cell death (PCD). During PCD a number of physiological and molecular processes are activated, leading finally to cell death. This is accompanied by multiple changes at cytological, physiological and molecular level, such as degradation of cellular structures, fluctuations in phytohormone level or activation of expression of specific genes. However, there is not much information on the senescence of fine roots, as ephemeral organs as leaves or petals. Fine roots are important for water and nutrient absorption, and at the end of the growing season, when demand for water and minerals decreases, most of them senescence and die.

The aim of our study was to analyze changes in gene expression profiles during leaf and fine root senescence to address the question: if an analogy between fine roots and leaf lifespan can be drawn? *Populus trichocarpa* (Torr. & Gray) plants were grown in system of rhizotron which allows the mapping the natural conditions of development. Gene expression analyses were performed using the Affymetrix GeneChip poplar genome array.

Gene expression analysis confirmed that seasonal senescence of both fine roots and leave is genetically regulated process. We identified 1348 genes in leaves and 1898 genes in fine roots that were differentially expressed during senescence. We mapped these genes to Gene Ontology terms searching for functional groups that were significantly enriched. Among differentially expressed genes, we identified known genes involved in PCD process. In addition, genes that were up-regulated during leaf and fine roots senescence, were assigned to transcription, protein degradation, signaling, autophagy, carbohydrate metabolic processes and reactive oxygen species metabolism. Moreover, despite the functional differences of leaves and fine roots, some similarities in the regulation of senescence in both organs has been revealed and documented, enabling to demonstrate a universal manner of this process.

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Gaseous signaling molecule hydrogen sulfide modulates vacuolar H+-ATPase activity

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Hydrogen sulfide (H_2S) can be generated in mammalian and plant cells. It has been suggested that endogenous H_2S acts as a biologically active molecule that, along with nitric oxide (NO) and carbon monoxide (CO), is a member of the gasotransmitters controlling intracellular processes. In plant cell, current knowledge about H_2S emphasizes its physiological functions in regulation of stomatal movement, senescence, seed germination, organogenesis, and photosynthesis. H_2S has been also proposed to mediate plant responses to stress conditions, including fungal infections, salt, drought, heat, hypoxia, and heavy metals. H_2S production and degradation is tightly regulated. In plant cells, at least three enzymes are responsible for H_2S generation: L-cysteine and D-cysteine desulfhydrases (L-CD; EC 4.4.1.1 and D-CD; EC 4.4.1.15), as well as sulfite reductase (SIR; EC 1.8.7.1). On the other hand, O-acetyl-L-serine(thiol)lyase (OAS-TL; EC 4.2.99.8) consumes H_2S during cysteine biosynthesis and may be responsible for H_2S depletion.

Our results suggest that beside other functions, H_2S is responsible for the regulation of vacuolar H^+-ATPase (V-ATPase) activity under cadmium stress and control conditions. It can modify both ATP hydrolysis and H^+-transport, catalyzed by this enzyme, as well as expression of VHA genes.

P3.66

Abscic acid metabolism in the response to cadmium stress in pea plants

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Abscisic acid (ABA) regulates many aspects of plant growth and development and plays an important role in plant stress tolerance. The protective role of ABA in response to cadmium toxicity may be due to the reduction of Cd uptake, the suppression of Cd translocation to shoot, an involvement of ABA in signal pathways, and an induction of phytochelatins synthesis. The physiological and biochemical processes regulated by ABA are closely correlated with endogenous ABA levels, which result from the balance between to simultaneously occurring processes, biosynthesis and catabolism. Although an increase of ABA content in response to Cd has been reported for many plant species, the site and rate of ABA synthesis and catabolism under heavy metal toxicity are largely not known. Therefore, we studied the spatial and temporal changes in expression of genes engaged in ABA metabolism in pea plants exposed to 50 μM $CdCl_2$. We examined expression of genes encoding enzymes of the ABA biosynthetic (9-cis-epoxycarotenoid dioxygenase – NCED; aldehyde oxidase – AO) as well the catabolic ABA pathway (ABA 8'-hydroxylase – ABA8'OH; ABA uridine diphosphate glucosyltransferase – ABA-GT). In Cd treated plants, ABA accumulation was observed only in leaves, but not in roots, already 5h after stress application and the accumulation was more pronounced thereafter. Among the pea leaves of different age (the youngest, not fully developed leaves, the fully developed leaves, the old leaves), the highest ABA content was observed in the youngest leaves both under control as well stress conditions. However, according to the expression of PsNCED2, PsNCED3 and PsAO3 genes, the fully expanded and the oldest leaves are the main ABA producers. Cd toxicity induced expression of the ABA biosynthetic genes also in roots, where an increase of PsABA-GT1 expression was observed as well. It may suggest that in the first hours of Cd stress, ABA is synthesized also in roots, where it is transformed into its transportable form, ABA-glucosyl ester, and then transported form root to shoot. Cd toxicity did not affect significantly the expression of PsABA8'OH1 and PsABA8'OH2, both in roots and leaves. The obtained results showed that Cd toxicity induces expression of genes engaged in the biosynthesis of ABA, both in roots and leaves; however ABA accumulation takes place only in leaves, most probably for the reduction of transpiration rate and the suppression of Cd transport from root to shoot.

Engineering metal-related traits – contribution of the specific regulation of endogenous metal homeostasis genes

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This study aimed at deciphering mechanisms through which the metal-related phenotype of plants expressing a metal transporter gene is generated. AtHMA4 is a plasma membrane export protein involved primarily in the regulation of Zn root-to-shoot translocation. Therefore, it was expressed in tobacco and tomato to engineer high metal shoot content. However its expression failed to yield one set pattern of accumulation/distribution of Zn (and also Cd) at varying metals concentrations in the medium [1, 2].

Understanding of the molecular background of the regulation of Zn/Cd root/shoot partitioning, that depends on Zn/Cd supply (and differs in wild-type and transgenic plants), is crucial for successful engineering of metal-related traits. To identify genes differentially expressed under a range of combinations of Zn (and Cd) concentrations in the medium, two approaches were adopted. For tomato, comparative expression analysis of transgenic and wild-type plants was performed at the tissue level.

Laser Capture Microdissection was used to isolate two root sectors having different functions in the control of metal root-to-shoot translocation [(i) epidermis+cortex and (ii) stele]; and two leaf sectors [(i) upper epidermis+palisade parenchyma and (ii) lower epidermis+spongy parenchyma]. For tobacco suppression subtractive hybridization (SSH) method was chosen. Subsequently expression analysis was conducted.

In tomato, it was shown that Zn-supply-dependent modification of Zn root/shoot distribution in transformants (different from wild type) involved distinct expression of LeNRAMP1, LeNRAMP2 and LeNRAMP3 primarily in the stele of the root. These were accompanied by respective lower/higher expression of ethylene genes (LeNR, LeACO4, LeACO5) suggesting involvement of the ethylene pathway [3].

In tobacco, seven candidate genes were identified as key players in the regulation of the phenomenon of Zn/Cd supply-dependent of Zn/Cd root/shoot partitioning in wild-type tobacco, and in modifications resulting from expression of AtHMA4. The coordinated responses of NtZIP1, NtZIP2, NtZIP4, NtIRT1-like, NtVTL, NtNAS, NtMTP1A accompanied metal supply-dependent metal distribution in transgenic plants [4].

Research performed on tobacco and tomato expressing AtHMA4 demonstrates that deregulation of a metal balance due to activity of a protein encoded by a transgene changes metal status at a cellular/tissue/organ level, leading to activation of endogenous metal homeostasis mechanisms to combat generated imbalance. These secondary effects induced by expression of a transgene are an integral part of mechanisms underlying development of transgenic's characteristic features (so far unstudied).

[1] Siemianowski, O. et al.,2011, Plant Biotechnol J.6: 64

[2] Kendziorek, M. et al., 2014 J Plant Physiol 171:1413

[3] Barabasz et al., 2016, J Exp Bot 67: 6201

[4] Kendziorek et al., 2016, BMC Genomics. 17

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Role of selective autophagy in plant response to nutrients deficit and other abiotic stresses

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Autophagy, an important cellular degradation system plays an important role in plant growth, development and response to environmental stresses. In stress conditions it protects plant cells from the oxidative stress by degrading oxidized proteins and it recovers valuable substances from the degraded cellular components. On the other hand selective degradation of certain cellular targets (such as transcription factors or other regulatory proteins) by the plant autophagy machinery might be an effective strategy of reprograming the cellular metabolism during shortage of nutrients. The role of selective autophagy and the selective autophagy cargo receptors is quite well characterized in mammals. Plants also possess selective autophagy cargo receptors, of similar to mammalian proteins domain architecture, named, depending on the plant species, NBR1 (*Arabidopsis*) or Joka2 (tobacco, potato). Through binding to ATG8s proteins NBR1/Joka2 deliver cargo to autophagosomes. However, their other partners, such as upstream regulators and degradation targets are not yet sufficiently investigated. Therefore, one of the focus of our work is identification of the protein partners of NBR1/Joka2 in normal and sulfur-deficiency conditions.

Moreover, plants with increased level of selective cargo receptors have been obtained in our laboratory and characterized in various growth conditions. Phenotypic differences between the wild type plants and the lines with the changed level of NBR1/Joka2 were rather subtle in normal growth conditions, however suggested that NBR1/Joka2 might be involved in nutrients deficiency response. Comparison of the transcriptomic profiles in shoots (rosette leaves) and roots of the wild type and the NBR1 overexpressing *Arabidopsis* revealed that NBR1 might be involved in certain processes related to abiotic stress response, such as cold or heat acclimation, regulation of circadian rhythm, flowering and ribosome biogenesis. Interestingly, increased level of NBR1 protein seems to affect the expression of numerous transcription factors. Involvement of NBR1 in processes and stresses concluded from the transcriptomic analysis is currently under investigation.

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P4.1

The role of *Medicago truncatula* ABCG membrane transporter as a modulator of carbon flow in the phenylpropanoid pathway

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Members of the so-called G subfamily of ABC transporters have been shown to be involved in numerous physiological processes associated with plant response to biotic and abiotic stresses. In model legume plant *Medicago truncatula* ABCG10 has been reported as: (i) a plasma membrane protein accumulating in leaves and roots of *Medicago* upon pathogen infection and elicitor treatment, (ii) a protein required for efficient de novo production of medicarpin. Medicarpin is a phytoalexin of *Medicago* and a product of a legume-specific isoflavonoid biosynthesis pathway. What is interesting, the expression pattern of genes encoding pivotal enzymes from medicarpin biosynthesis pathway, namely phenylalanine ammonia -lyase (PAL) and isoflavone synthase (IFS) goes along with that of MtABCG10 upon biotic stress conditions.

Silencing of *MtABCG10* expression results in a lower accumulation of medicarpin and its intermediates, suggesting a disturbance in the early stages of medicarpin biosynthesis. This impairment can be averted by exogenous application of 4-coumarate, an early precursor from the core phenylpropanoid pathway and isoflavonoid formononetin onto *MtABCG10*-silenced hairy roots. Transport experiments of various *Medicago* phenolic compounds performed in tobacco suspension cell cultures (BY2) overexpressing *MtABCG10* and membrane vesicles derived from them revealed that MtABCG10 is responsible for membrane translocation of molecules representing key branching points in the phenylpropanoid pathway, namely 4-coumarate and liquiritigenin. Provided data show a new potential role of plant ABCG transporters as precursors distributors and modulators of carbon flow in medicarpin biosynthesis pathway.

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Post-infection changes of salicylic acid (SA) levels and phenylalanine ammonia-lyase (PAL) activity in *Lupinus* L. in response to *Colletorichum gloeosporioides*

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Anthracnose, caused by the fungal pathogen *Colletotrichum gloeosporioides*, is the most serious disease of lupin. The most obvious symptom in lupins is brown lesions with orange conidial masses and typical twisting stems or petioles. Salicylic acid (SA) is an endogenous plant growth regulator of phenolic nature, which plays an important regulatory role in multiple physiological processes, including its central role in controlling immunity. Studies conducted on many pathosystems have shown that SA as a signaling molecule is an important compound inducing plant defense against a variety of pathogens. The aim of the presented study was to determine the participation of salicylic acid (SA) in defense mechanisms of two lupin species varying in their susceptibility to *Colletotrichum gloeosporioides*, i.e yellow lupin (*Lupinus luteus* L.) and narrow-leaved lupin (*Lupinus angustifolius* L.). Yellow lupin is much more susceptible to anthracnose than narrow-leaved lupin. Additionally, the aim of the study was to determine the activity of phenylalanine ammonia-lyase (PAL), a key enzyme which catalyses non-oxidative deamination of phenylalanine to trans-cinnamate. This is the first step in the phenylpropanoid pathway and is an important regulation point between primary and secondary metabolism. PAL plays an important role in plant defense and it may be involved in SA biosynthesis. At 96 h a significantly elevated level of SA and high activity of PAL were recorded in *L. angustifolius* leaves infected with *C. gloeosporioides*. Moreover, SA level in leaves of *L. angustifolius* was significantly higher than in *L. luteus* leaves. At the same time it was found that *L. angustifolius* plants exhibited lesser development of anthracnose than *L. luteus* plants. Our results highlight the important role of SA in defense mechanisms of *Lupinus angustifolius* during infection caused by the pathogenic fungus *C. gloeosporioides*.

P4.3

The structure, cytological characterizations and calcium localization in *Cycas revoluta* and *Cycas circinalis* roots under symbiotic conditions with blue-green algae of the genus *Nostoc*

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Hetreocystous cyanobacteria found in the genus Nostoc form symbioses with selected Prokaryotic and Eukaryotic organisms. Such symbiont partners are found in all kingdoms. For instance, cyanobacteria appear to associate with lichenized and non-lichenized fungi, with a range of photosynthetic algae with lower (liverwort) and higher plants (*Azolla*, cycads and *Gunnera*), even a few representatives within the animal kingdom (sponges, ascidians and some worms). All hosts that harbor heterocystous cyanobacteria rely on the N2-fixation capacity of the cyanobacteria to cover their need for combined nitrogen. The cyanobacteria-cycad symbioses are the only known symbiosis between a gymnosperm and prokaryotic diazotrophs. All members of this taxon form diazotrophic symbioses and they are the only symbiosis in which *Nostoc* invades the coralloid, apogeotropic and adapted to gas exchange roots of plants. On cyanobacterial invasion of the *Cycas revolut* and *Cycas circinalis* roots the cyanobacterial zone were formed midway between the stele and the epidermis. The filamentous cyanobacteria are found intercellulary within mucilage between elongated cells interconnecting two cortical layers. Ultrastructurally the host cells show the characteristics of transfer cells. The cell wall becomes thickened and presented numerous ingrowths. Plasmodesmata are closed. The cytoplasm is condensed and the number of plastids, mitochondria, ribosomes, Golgi apparatus, endoplasmic reticulum is strongly increased. Nuclei are hypertrophied and acquire irregular shapes. The precipitations of calcium were observed in tracheal elements and also in cortex cells.

Between the phytohormone translocation and nodulation process – unravelling the role of ABC transporters

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Cytokinin (CK) constitutes one of the foremost signalling compound involved in a wide variety of plant growth and development processes as well as responses to environmental stimuli. In legumes, CK plays a crucial role in regulation of the *Rhizobium* beneficial associations. In response to symbiotic bacterial infection and NOD Factor (NF) signalling, the level of CKs increases in the root susceptible zone, both in rhizodemis and root cortex. Interestingly, presumed role of rhizodermal cytokinins as a mobile signal joining outer and inner root tissue responses has been proposed recently [1]. However, elucidation of the molecular mechanism enabling cytokinin translocation to cortical cells, where they are perceived by membrane-localized receptors, as well as within cortical cell layers remains elusive.

We have identified and classified 30 full-size ABC transporters from the G subfamily, in model legume plant *Medicago truncatula*. Phylogenetic analyses of ABCGs from various plant species revealed presence of legume specific protein cluster. Among selected transporters from this cluster expression of two of them is significantly induced upon *Sinorhizobium meliloti*, isolated NOD factor and cytokinin treatment. Moreover, observed upregulation is partially dependent on cytokinin receptor CRE1. Both cytokinin induced transporters are plasma membrane proteins. Interestingly, analyzed ABCGs exhibit different tissue-specific expression patterns. Furthermore, lack of one of them decreased nodule number in comparison with wild-type control. Influence on nodulation efficiency might be correlated with phytohormone translocation, since conducted transport experiment evidenced its participation in efflux of bioactive cytokinins.

[1] Boivin et al. (2016) Front. Plant Sci. 7:1240.

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P4.5

Cell wall modifications of oilseed rape genotypes during *Alternaria brassicicola* infection

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Necrotrophic fungus *Alternaria brassicicola* causes black spot disease to all *Brassica* species in Europe, as well as in both Americas and Asia. The plants of 160 oilseed rape (OSR) (*Brassica napus*) cultivars (both winter and spring ones) have been phenotyped for their resistance/susceptibility to *A. brassicicola* under laboratory conditions in triplicate. Using Win_DIAS3 system necrosis formation on third leaf of each plant at 5 dai was analyzed. Analysis of necroses revealed that 111 of OSR cultivars were susceptible or highly susceptible, whereas 49 cultivars were resistant or highly resistant to *A. brassicicola*. Samples of infected and control plants were harvested and subjected to the spectrometric analysis for total content of phenylopropanoids and lignin as well as a fluorescent microscopy analysis of callose deposition. The above-mentioned experiments allowed selecting four OSR cultivars, two of them highly susceptible to *A. brassicicola* infection: 'MONTY-028DH' and 'Zairai Chousenshu', and two highly resistant: 'Savannah' and 'Askari'. The plants of these four cultivars were subjected to quantitative Real-Time PCR at 24, 72 and 120 hai. The expression profiles of genes encoding enzymes involved in phenylopropanoid pathway: phenylalanine ammonia-lyase (PAL), polyphenol oxidase, cinnamoyl CoA reductase (CCR), as well as callose biosynthesis pathway: glucan synthase-like (GSL) revealed significant differences in regulation of these genes in resistant and susceptible cultivars during infection.

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Studies on HopBF1, a novel type three secretion effector from *Pseudomonas syringae*, that displays kinase activity

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Plants have developed various defense strategies against pathogens. One of the mechanisms is based on activation of a MAPK (mitogen-activated protein kinase) pathway, a process by which consecutive phosphorylation of proteins leads to the necessary responses. Pathogenic bacteria employ several effector proteins to disrupt at various levels plant MAPK signaling and thus promote pathogenesis.

Pseudomonas syringae, a pathogen of many plant species, utilizes a type III secretion system to deliver effector proteins into the plant cell. One such effector is HopBF1, of which little is known. Bioinformatics analyses showed that HopBF1 contains specific features characteristic of kinases. However, it also displays an amino acid sequence variation not usually found in the classic kinases. In order to elucidate its role and mechanism of action we constructed a set of mutants with amino acid substitutions at the sites predicted to be crucial for protein activity. Variants containing single and double mutations within the putative motifs responsible for kinase activity ("kinase-dead") were chosen as being of great interest to our studies. Our preliminary experiments revealed that HopBF1 exhibits enzymatic activity and undergoes autophosphorylation.

Mass spectrometry analysis of wild-type HopBF1 expressed both in *E. coli* and in *Nicotiana benthamiana* show multiple phosphorylation sites which are absent in the kinase-dead variants. Moreover, when purified from *N. benthamiana*, the effector co-purified with plant chaperones. Further experiments verifying these interactions are being conducted.

Protein localization studies in *N. benthamiana* indicate that HopBF1 displays nucleocytoplasmic distribution, which was not influenced by the introduction of mutations.

N. benthamiana plants infected with *Agrobacterium tumefaciens* expressing HopBF1 after a few days start developing chloroses and necroses of leaves and subsequently begin to wilt. These symptoms are absent when infiltrated with bacteria expressing kinase-dead mutants.

Collectively, our studies suggest that HopBF1 may be a founder protein for a novel family of kinases demonstrating an atypical structure. We hope that our results will shed some light on the role and mode of action of HopBF1 in pathogenesis.

P4.7

The role of chloroplasts in the response of cucumber to *Pseudomonas syringae* pv *lachrymans* infection

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Chloroplasts are considered as the most sensitive sensors of environmental changes. Similar to other stresses, pathogenic infection mimics the plant exposure to excess of excitation energy and leads to disorders in the light phase of photosynthesis, leaf gas exchange as well as to perturbations in the redox status of chloroplasts. These changes could be signalled from the chloroplasts and mediate the plant defense responses, both at the local and systemic levels. We studied the changes in chlorophyll fluorescence, leaf gas exchange, activity of superoxide dismutase (SOD) and ascorbate (AA)-glutathione (GSH) cycle enzymes as well as concentration of AA and GSH in chloroplasts of cucumber infected with *Pseudomonas syringae* pv *lachrymans* (Psl), the causal agent of angular leaf spot disease. The third true (inoculated) and the fifth true (non-inoculated) leaves have been analysed for the local and systemic response, respectively. The bacterial infection affected the chlorophyll fluorescence: a decrease in maximum quantum yield of PS II and an increase in non-photochemical quenching in the third true leaves have been observed. The leaf gas exchange (stomatal conductance and transpiration) as well as net-photosynthesis efficiencies have decreased in the late phase of the pathogenesis. The changes in SOD and ascorbate-glutathione cycle activities suggest the maintenance of prooxidative conditions shortly after inoculation and a delayed antioxidant response, both in the third and the fifth true leaves. The antioxidant mechanisms triggered by infection involved mainly MnSOD, ascorbate peroxidase (APX) and dehydroascorbate reductase (DHAR). Our results confirmed that the activation of both local and systemic response to Psl-induced biotic stress can be mediated by chloroplast-dependent redox signalling related to AA and GSH.

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CRISPR/Cas9 gene-editied *Nicotiana tabacum* BY-2 cells as a model for investigation of stress signal transduction pathways in plants

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When stress signal transduction pathways are examined in laboratory, they are often considered as a separate entities. However, in nature plants are not exposed to only one type of stress. In fact, one stress factor can also foreshadow appearance of another (e.g. heat stress may indicate incoming drought).

Therefore the plant that encounters stress combinations must present an integrated response to them. As a result of that, stress signaling pathways in plants often converge and such relation is described as 'cross talk' between them. Such pathways, despite being triggered by different receptors, have the same effector proteins and consequently similar response on cellular level. Therefore, in some cases, it is reasonable to use stress inductor that is not directly related to the pathway tested, which is especially convenient if it is in a form of a cheap chemical compound (e.g. sodium chloride).

We have developed a simple and fast system to verify the involvement of any gene in stress signal transduction cascades, using *Nicotiana tabacum* BY 2 suspension culture as a model cell line. Tobacco plants have been studied for a long time, and have sequenced and well-annotated genome. Form of suspension culture enables fast and uniform distribution of a stress inductor (e.g. salt, heavy metals, low temperature) to every cell, and reduces time and space required for growth of the plant material.

Using CRISPR/Cas9 gene editing technology non-sense mutation is introduced into candidate gene. Protospacer sequence, that determines position of digestion of the Cas9 nuclease, is ligated into vector encoding gRNA backbone which is subsequently transferred into final vector using GATEWAY® system. Resulting plasmid is introduced into *Agrobacterium* and BY-2 cells are transformed. Transformed calli are then screened for the presence of desired mutation (i.e. knock-out in tested gene) and the mutant suspension culture is established.

Wild type and mutant suspension cultures can be tested using simple physiological methods, like determining the growth rate or TTC survivability assay. Differences in these parameters suggests involvement in stress signal transduction pathway. It can be also easily up scaled to perform multilpe gene analysis at once.

The time required for completion of the whole procedure should be about 8 weeks. The most promising candidates can be chosen to verify observations in tobacco plants or extrapolated to different species.

P4.9

Proteins associated with *Prune* dwarf virus replication as element of host plant-virus interaction

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Prune dwarf virus (PDV) is one of the most dangerous pathogens of fruit trees worldwide. One of the most important proteins required for PDV infection is replicase. (P1 protein) which anchored viral RNA and builds replication complex along with RNA depended polymerase (P2 protein). Presence of replication complex proteins alongside PDV coat protein (PDV CP) induce cascade of signal transduction associated with cell ultrastructure and membranous structures alternations. Despite the importance of PDV as a pathogen, our knowledge regarding tissue/cellular localization and structure of PDV P1 protein is still incomplete. The aim of this work was to localize replicase distribution in leaf tissues and cells by immunofluorescent and immunogold labeling of *Nicotiana tobaccum* cv *Samsun* and development of a 3D model of PDV replicase. In this work we demonstrate that PDV replication generates formation of special membranous structures named spherules generated from tonoplast membranes. PDV replication process is similar to that of Alfalfa mosaic virus and is strongly connected with tonoplasts. In addition, PDV replicase and coat protein (CP) were also found to be strongly associated with membranes of endoplasmic reticulum and, indicating the potential involvement of these membrane structures in the processes related to viral infection. Bioinformatic analyzes based on 3D modeling and structure prediction revealed that P1 protein has a potential transmembrane domain which enables protein anchoring to tonoplast during replication complex assembly.

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Investigation of molecular targets of *Pseudomonas syringae* HopQ1 effector

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Hrp outer protein Q, or simply HopQ1, is an effector of Type Three Secretion System (TTSS) of *Pseudomonas syringae* – a potent pathogen of plants such as tomato and bean. It is capable of promoting bacterial virulence by, as of yet, not entirely understood mechanism. Following a delivery via TTSS into the host cell, HopQ1 undergoes phosphorylation of serine residue (S51). This modification stands as a prerequisite for binding of the effector by 14-3-3 proteins – an interaction that promotes stabilization of HopQ1, influences its subcellular distribution and possibly mediates contact with other molecular partners.

Data acquired from Size Exclusion Chromatography coupled to Multi-Angle Light Scattering experiments suggest that, under *in vitro* conditions, pool of HopQ1 includes monomeric, dimeric and trimeric forms. Addition of reducing agents alters this state by abolishing oligomerization and prompting HopQ1 to exist in the monomeric state. This behavior is consistent with exclusive monomerization of the mutated variant displaying dual substitution of serine (HopQ1_C70A_C230A) and point at the involvement of disulfide bridge formation in this process.

On the contrary, calcium depletion promotes reversible formation of dimers. This phenomenon can be successfully replicated by disrupting the non-canonical binding motif of the effector. HopQ1 mutants displaying substitutions (D107A and D108A) are not capable of binding calcium ions and exist primarily in the dimeric state.

Confocal imaging suggests that dynamic equilibrium between monomeric and dimeric forms is the component of the motivating force behind cytoplasmic and nuclear distribution of HopQ1 pool. This is especially pronounced in regard to the predominantly nuclear HopQ1_D107A_D108A and prompts us to believe that HopQ1 possesses a dynamic range of plant protein interactors specific to this compartment. Mass spectrometry analysis of protein samples acquired from *Nicotiana benthamiana* leaves transiently expressing several variants of HopQ1 displaying different subcellular localization seem to support this concept, as multiple, compartment-specific, potential protein interactors have been identified. A study is underway to further narrow down the search list.

P4.11

Heterodera schachtii infection affects arginase activity in *Arabidopsis thaliana*

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The beet cyst nematode *Heterodera schachtii* parasitizes brassicaceous plants including *Arabidopsis thaliana*. The second-stage juveniles penetrate roots and induce formation of a syncytium (a permanent feeding site) being a "sink" for amino acids and sugars on which nematode feeds. Infection is known to induce a defence response in host plants, and we hypothesized that arginase, an important enzyme for nitrogen metabolism, hydrolysing arginine to urea and ornithine, could take part also in the defence against parasitic nematodes since arginase induction was observed after bacterial or fungal infection. To assess whether an infection with *H. schachtii* alters expression and activity of arginase, studies were performed on *Arabidopsis thaliana* roots and shoots at the day of inoculation and at 3, 7 and 15 post-inoculation days (dpi). The infection caused a decrease of arginase activity and arginase I gene expression in roots. Arginine decarboxylase activity competing for substrate with arginase was not enhanced upon infection and similar level of arginine both in control and infected roots, was observed. It suggests that the nematode effectors induce processes leading to inhibition of arginine catabolism in roots to maintain this amino acid level high enough for protein synthesis in developing larva. In shoots of infected *A. thaliana* plants, the gene expression of arginase II increased at 3 and 7 dpi, but the enzyme activity was significantly stimulated only at 3 dpi. To find if oxidative stress is possible upon the nematode infection, the activity and mRNA expression of glutathione reductase (a marker of the redox equilibrium) were measured and found to be significantly stimulated at 7 dpi in shoots of infected plants. It was accompanied by changes in contents of total soluble proteins, protein carbonyl groups and polyphenols. In addition, the level of proline (an osmolyte and scavenger of free radicals) was extremely enhanced. These results indicate that disruption of redox equilibrium, as reflected by increased levels of proline, protein carbonyl groups, polyphenols as well as glutathione reductase activity and its gene expression, accompanies alterations in arginine metabolism in shoots of infected plants, indicating systemic defence responses induced upon nematode infestation.

[1] Labudda M, Różańska E, Cieśla J, Sobczak M, Dzik JM (2016) Arginase activity in *Arabidopsis thaliana* infected with Heterodera schachtii. Plant Pathol 65:1529-1538.

[2] Labudda M, Różańska E, Szewińska J, Sobczak M, Dzik JM (2016) Protease activity and phytocystatin expression in *Arabidopsis thaliana* upon Heterodera schachtii infection. Plant Physiol Biochem 109:416-429.

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N availability determines a possible scenario for mycorrhizal benefits for plants

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Arbuscular mycorrhiza (AM) can have a large variety of effects on N uptake by the plants. However, it is still not clear under which conditions will AM be beneficial for N uptake.

The aim of our study was to investigate the potential impact of AM symbiosis on the plant response to N availability. Maize plants (hybrid Opoka) mycorrhized (AMF1) or not (AMF0) with high quality AMF inoculum were grown in phytotron for 8 weeks. After that the commercial fertilizer was introduced for additional 5 weeks at 4 suboptiomal doses for plant growth and 1 optimal. AMF colonization was high and similar in the range of all suboptimal fertilization doses but the highest dose strongly reduced the mycorrhization rate. Fertilization stimulated leaf N status (measured fluorometrically as NBI Nitrogen Balance Index) in both plants, but AMF1 plants reached the highest difference in NBI to AMF0 plants at the lower dose of fertilizer. Moreover, at the lowest suboptimal dose presence of fungi allowed plants to reduce the decrease in NBI related to leaf position. Gene expression analysis focused on a set of target genes involved in the plant nitrogen metabolism (NR, GS, GDH) and in parallel, root and leaf GS and NR protein expression showed that the AM benefit for plant N nutrition changes with N availability. In roots and leaves, AM symbiosis down-regulated the all of these genes expression GS (ZmGln1-3, ZmGln2) and GDH (ZmGDH) at both, low and high-N supply, showing the positive AM effect at intermediate N nutrition. This regulation was related to leaf position and it was more pronounced in younger leaves of AMF1 than in the oldest ones. Varied expression of GS protein in the older leaves of AMF1 plants, together with GS gene expression may indicate, that the plant's response to the increased dose of nitrogen depends not only on accumulated nitrogen in these organs and directly absorbed by roots, but also on nitrogen provided by micorrhizal fungi. Based on the presented result we discussed a possible beneficial role of AM in N uptake by plants.

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P4.13

Cross-talk between brassinosteroid and sucrose signaling during *Pisum sativum* L. – *Fusarium oxysporum* f.sp. *pisi* interactions

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Plant growth and development as well as plant immunity are regulated by several plant hormones that interact in complex networks. Among them, most recently brassinosteroids (BR), have been proposed to modulate the trade-off between growth and immunity. After the recognition of microbial- or pathogen-associated molecular patterns (MAMPS or PAMPs) by plants using transmembrane pattern recognition receptors (PRRs), there is a shift of activity associated with the growth in the direction of enhancing defense reactions. Effective regulation between growth and defense responses in pathological conditions is crucial for the functioning of plants in the environment. BRs are increasingly implicated in plant responses to pathogen attack. In turn, sugars have important hormone-like functions as primary messengers in signal transduction, regulating plant cell metabolism. The first aim of the study was to determine the role of epibrassinolide and sucrose in the development of infection caused by hemibiotrophic pathogenic fungus *F. oxysporum* f.sp. *pisi* and *Fusarium wilt* in pea embryo axes (*Pisum sativum* L. cv. Cysterski). The second goal of the present study was to examine the effects of crosstalk interactions of epibrassinolide, sucrose and *F. oxysporum* on the expression of phenylpropanoid pathway genes and, specifically, the flavonoid biosynthesis pathway. Also, the accumulation and cytochemical localization of end products, i.e. flavonoids, were analyzed in cells. Analysis of disease symptoms of embryo axes pretreated with epibrassinolide and cultured on the medium with sucrose revealed strong tissue melanization and minor development of *Fusarium wilt* than in other infected embryo axes. Besides, it needs to be stressed that infection of epibrassinolide pre-treated tissues cultured on sucrose-containing medium caused strongly enhanced activity and transcript levels of enzymes involved in flavonoid biosynthesis and accumulation. Thereby new sugar-hormone cross-regulation was evidenced, although the identification and characterization of the molecular mechanisms of convergence of these signaling pathways are still necessary.

Metabolism of ROS and RNS in common bean response to sequential pathogen attack

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The balance of reactive oxygen and nitrogen species and their metabolites concentration play a key role in plant response to every type of stress. These signal molecules are strictly related to direct and indirect reactions of cells to stress factors, including pathogen infection. Metabolism of ROS and RNS is mediated by specific enzymes, which can release or deactivate them. Enzyme particularly associated with NO generation is S-nitrosoglutathione reductase (GSNOR). One of mechanisms using NO involved in modification of proteins activity under various stresses is S-nitrosylation. ROS metabolism is under control of other enzymes maintaining redox state i.a. functioning in the ascorbate-glutathione cycle: glutathione reductase (GR), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR).

The aim of study was to determine the changes in concentration of generated ROS and RNS and activity of enzymes involved in their metabolism in common bean after sequential infection with biotrophic bacteria *Pseudomonas syringae* pv. *phaseolicola* and necrotrophic fungus *Botrytis cinerea*.

The results showed that the infection of common bean with selected pathogens caused significant changes in the generation of ROS and RNS, as well as in the activity of selected enzymes comparing to the control. Plant inoculation with a specific bacterial pathogen significantly affected the final response to the sequential infection and caused the delay of the development of necrotrophic fungus.

P4.15

The role of sugars in the mechanism of *Fusarium oxysporum* f.sp. *lupini* pathogenicity

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A high level of sugars may lead to disturbances in metabolic mechanisms of pathogens, which limits their invasion of plant tissues. A phytopathogenic fungus *Fusarium oxysporum* f.sp. *lupini* is a hemibiotrophic fungus that combines two attack strategies. In the initial biotrophic phase, during which the host's immune system and cell death are actively suppressed, invasive hyphae are able to spread throughout the infected plant tissue. In the next phase the fungus secretes toxins and enzymes that kill host cells and then take up nutrients released from the dead tissue. Fungal plant cell-wall degrading enzymes are among the critical factors contributing to pathogenicity of various plant-pathogenic fungi. The general aim of the study was to determine the correlation between soluble carbohydrate levels and the pathogenicity of the hemibiotrophic fungus *F. oxysporum* f.sp. *lupini*. The first aim of the study was to determine a correlation between endogenous sugar levels in the examined material and the development of infections and *Fusarium wilt*. Therefore, symptomatological analysis was conducted, morphometric measurements were recorded and the level of ergosterol, a fungal growth indicator, was determined in infected embryo axes. The second aim of study was to determine the level of a mycotoxin moniliformin as the most characteristic secondary metabolite of *F. oxysporum* in the infected material in the context of various sugar sources. *F. oxysporum* is responsible for *Fusarium wilt* as well as pre-emergent sprout root and post-emergent seedling rot. The experimental protocol used in this study was based on a model system, i.e. embryo axes from germinating yellow lupine seeds were cultured in vitro on a medium with sugar (60 mM sucrose, 120 mM glucose, 120 mM fructose) or without sugar after inoculation with a spore suspension of *F. oxysporum* f.sp. *lupini*. Additionally, *F. oxysporum* f.sp. *lupini* was cultured in vitro on potato dextrose agar (PDA) either with sugar or without it. Samples were collected for analyses at 0 h, after 72 and 96 h of culture. It needs to be stressed that the level of ergosterol was highest in infected embryo axes with a sugar deficit. At the same time, a significant accumulation of the mycotoxin moniliformin was observed in these tissues. Furthermore, it was found that the presence of sugars in PDA medium inhibited sporulation of *F. oxysporum* in relation to the control, with the strongest effect observed in the case of glucose. Additionally, in 96 h-infected embryo axes with sugar deficit a higher cellulase activity was found in relation to non-infected embryo axes with sugar deficit. In turn, in infected embryo axes with high sugar levels cellulase activity was lower than in non-infected embryo axes with high sugar levels. High sugar levels may lead to reduced virulence of *F. oxysporum* (a decreased activity fusariosis development).

The *Trifolium repens* growth parameters under the *Rhizobium leguminosarum* bv. *trifolii* influence from about 100-yrs old zinc and lead waste heap

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Calamine waste heaps localized in Silesia-Kraków Upland in southern Poland are high heavy metal contaminated areas, mainly with lead, zinc and cadmium as well as deficient with water and nutrients. The root nodulating bacteria (rhizobia) belong to the plant growth promoting rhizobacteria group (PGPR) and improve leguminous plants fitness according to the conversion of atmospheric nitrogen into ammonium which is available to plants, instead of this host-plants supply bacteria in carbohydrates, minerals and create optimal conditions for microsymbionts growth. Heavy metals abundance in soils may adversely affects bacterial microflora resulting in i.e. reduction of their number, biological activity as well as the rate of growth, and as a consequence the host-plant growth indices may be diminished. According to the long-term exposition of rhizobia strains in Bolesław waste-heap area these microorganisms might be well fitted to highly disadvantageous conditions. The aim of study was to determine the influence of symbiotic bacteria *Rhizobium leguminosarum* bv. *trifolii* isolated from root nodules of white clover plants inhabited old (70-100-yrs old) zinc-lead (calamine) waste heap Bolesław area, on chosen growth parameters of the white clover in comparison with bacteria received from nodules of plants growing on control area, with low heavy metal ion concentrations

P4.17

Plant cell wall dynamics in susceptible and resistant potato in response to infection by *Potato virus Y* (PVY^{NTN})

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Plant cell wall not only provide structure to the plant body, but also act as a barrier against biotic and abiotic stress. The frontline of the plant defense system consist of physical and chemical barriers such as antimicrobial enzymes or secondary metabolites (Alexander & Cilia, 2016). Potato virus Y infects more than 170 species belonging to 34 genera and has a worldwide distribution. It is listed as an economically important damaging virus affects economic crop, especially Solanum. The PVY^{NTN} known as a vein necrosis strain induced diverse symptoms with necrotic ringspots decrease the yield and quality of crops (Chen et al., 2016). Virus like many other pathogens triggers a number of inducible basal defense response when recognized by plants, inducing the upregulation of a number of common defensive proteins (enzymes). Enzymes involved in cell wall metabolism play a crucial role in the interaction since they affect the spread of the virus.

Therefore, PVY^{NTN} induced numerous cell wall changes in susceptible (cv. Igor) as well as in resistant (cv. Sapro Mira with HR gene) potato cultivars were analyzed. At ultrastructural level it was abnormal thickening observed due to deposition of callose near the edge of virus induced lesions mainly in phloem tissue, especially in resistant potato. Moreover, modifications to plasmodesmata were very intense with wall protrusions associated. They appear more often in susceptible potato to be due to deposition of new wall material induced by the virus and may lined inside and out with the plasma membrane. Depositions the electron-dense material between the cell wall and membranes highly localized in association with paramular bodies. Furthermore, the localization of two proteins involved in respectively callose and cellulose metabolism in potato – PVY^{NTN} interaction were investigated, it means pathogenesis related protein 2 (PR-2) and cellulose synthase A4 (CESAA4). The immunofluorescence localization of PR-2 in susceptible and resistant potato leaves indicated that PR-2 is induced as an impact of PVY^{NTN} in comparison to non-infected material. However, the data revealed the stronger PR-2 detection in compatible interaction than in hypersensitive response. Moreover, the quantification of immunogold labeling indicated the significant differences between control and infected leaf tissues. The cellulose synthase catalytic subunit (CESA) A4 start to synthesis the secondary wall on primary one. Furthermore, immunofluorescence analyses in susceptible and resistant potato cultivar allowed to state that PVY^{NTN} infection induced Cesa A4 deposition. Analyses of CesaA4 deposition on ultrastructural level revealed the simultaneously visualization in apoplast region and membranous structure in symplast, which intense affirmed the active distribution of Cesa A4 as a step of potato cell wall remodeling in PVY^{NTN} infection.

PIP aquaporins expression in uppermost and ear leaves of mycorrhized maize in response to drought

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Under progressive drought development mature corn plants reduce stomatal gas exchange rate of ear leaves to a much greater extent than uppermost leaves. According to our observations mycorrhizal relationship alleviates this effect and maintains photosynthetic activity at the higher level.

The aim of this work was to verify the hypothesis that mycorrhizal alleviation of ear leaves response is related to alteration of expression patterns of aquaporins from PIP1 and PIP2 subfamilies.

Drought regulation had differential, leaf position specific effect on average mRNA accumulation of both aquaporins subfamilies. Two characteristic AM/NM expression patterns could be distinguished: upper leaves with equal mRNA levels and ear leaves with high AM/ low NM ratio at the deepest drought. Very similar ratios were also specific to well-watered cultures. Both stomatal gas exchange rates and PIPs expression reflected mycorrhiza-related differences in ABA accumulation. We postulate that AM and NM leaf physiology at distant positions are differently regulated by hormonal and leaf aquaporins factors.

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P4.19

Function of tryptophan metabolic network in *Arabidopsis* immunity

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In the model plant *Arabidopsis thaliana* the effective defence towards a broad range of pathogenic microorganisms requires two autonomous but complementary mechanisms that are crucial for plant immunity at different stages of infection time course. Respective molecular mechanisms involve products of the complex network of tryptophan and indole glucosinolate (IG) metabolic pathways. End products of the CYP81F2/PEN2-mediated indole glucosinolate metabolism modulate the pre-invasive resistance that controls entry of filamentous microbial pathogens into epidermal cells. In the case of successful epidermis penetration the post-invasive mechanism based on the activity of indole-type phytoalexin camalexin is triggered to restrict subsequent pathogen development and hyphae spreading to the cells surrounding the initial infection site.

Previous analyses of the *cyp79B2 cyp79B3* mutant revealed that apart from PEN2-dependent products of IG metabolism and camalexin *Arabidopsis* immunity requires other indolic compounds. To investigate whether indole-3-carboxylic acids and/or indole-3-carbonyl nitriles, whose biosynthesis is dependent on CYP71A12 activity, may contribute to post-invasive immunity a set of double and triple mutant combining *pen2, pad3, cyp71A12, cyp71A13* and *cyp82C2* single knockouts was generated. Infection assays with *Plectosphaerella cucumerina* and *Colletotrichum gloeosporioides*, two fungal pathogens representing different lifestyles, revealed no clear phenotype for single *cyp71a12*. However, more severe symptoms were observed on double and triple mutants including *pen2 cyp71a12 cyp71a13* as the most susceptible. This indicates that compounds whose biosynthesis is dependent on CYP71A12 activity act in a highly coordinated manner with products of IG metabolism, contributing to post-invasive resistance against microbial pathogens.

P4.20

Heterooligomeric composition of maize PIP aquaporins and their role in response to mycorrhizae and drought

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Here we present proteomic data regarding mycorrhiza effect on PIP1- and PIP2-type aquaporin monomers interactions within heterocomplexes formed in roots and leaves of mature corn under drought development and recovery. Regulation of heterooligomeric assembly has been recently proposed as a way of achieving a diversification in the transport capacity of these water channels.

13 aquaporin complexes of very reproducible pI positions across samples taken from different drought, organ and symbiotic variants were resolved by IEF-SDS PAGE western blot. After immunoprecipitation and mass spectrometry approach 2D spots were classified according to emPAI protein abundance index values of the most abundant PIP1;1 and PIP2;1 isoforms. The mechanism of root regulation appeared selective accumulation of aquaporins complexes. In result, oligomers comprised almost exclusively of PIP2 monomers, dominated root response observed during rehydration. Immunodetection showed that accumulation of this type oligomers was enhanced by symbiosis.

Immunoblot analysis indicated that leaf regulation during drought followed evenly rising pattern of aquaporins accumulation additionally enhanced by mycorrhiza. In contrast to roots, this was represented mostly by oligomers of comparable proportion of PIP1 to PIP2 monomers.

Considering enhancement of aquaporin complexes conductivity by PIP1-type monomers it seems to be reasonable that mycorrhiza-elevated accumulation of PIP1-comprising oligomers reported here could be responsible for enhanced leaf ability to maintain transpiration and tolerate drought.

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P4.21

The role of antioxidative response in effective protection of *Quercus robur* leaves against *Erysiphe alphitoides*

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Reactive oxygen species (ROS) and reactive nitrogen species (RNS), in particular hydrogen peroxide (H_2O_2) and nitric oxide (NO), can act as second messengers during plant responses to several abiotic and biotic stresses that include salinity, drought, heavy metals and pathogen attack. An important mechanism by which NO regulates plant development and stress responses is through S-nitrosylation. This process is mostly regulated by the activity of specific enzyme S-nitrosoglutathione reductase (GSNOR). Phenolics are widely distributed in oak, some of them occur constitutively, whereas others are synthesized in response to a pathogen attack where their appearance is considered as part of active defense.

The aim of this study was to determine the concentration of nitric oxide, S-nitrosothiols (SNO) and activity of S-nitrosoglutathione reductase, as enzyme regulating S-nitrosylation process, in the *Quercus robur* leaves uninfected and infected with powdery mildew. Moreover visualisation of NO was performed by using confocal microscopy. Additionally, we determined the form of phenolic accumulation in oak leaves and them role in effective protection against pathogen attack.

The study material was constitute one-year old seedlings of pedunculate oak infected with *Erysiphe alphitoides* from the container nursery, Forest District Gidle, Poland. For analysis we used leaves without visible symptoms of disease as control, and leaves with different size of infection area (<5%, 12-15%, 25%).

The results showed the changes in the NO, SNO concentrations and GSNOR activity that may play an important role in defensive system of powdery mildew-infected leaves. HPLC analysis indicated remarkable increases in the concentrations of 23 phenolics belonging to hydroxybenzoic acids, cinnamic acids, catechins, flavonols, flavons and flavanons in the plants infected with powdery mildew. We suggest that the changes in the NO, SNO concentrations, GSNOR activity and accumulation of phenolic acids and flavonoids may strongly contribute to oak protection against *Erysiphe alphitoides* attack.

RNA-seq analysis of resistant and susceptible cucumber plants infected with *Pseudomonas syringae* pv. *lachrymans*

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One of the factors limiting open-field production of cucumber (*Cucumis sativus* L.) is angular leaf spot disease caused by *Pseudomonas syringae* pv. *lachrymans* (Psl). Recently, destructive outbreaks of this disease have been reported in Poland, USA and China, which caused the need of better understanding of this pathosystem. In this study RNA-seq was applied to compare transcriptomic response of two cucumber lines, i.e.: susceptible (B10) and partially-resistant (Gy14) to the highly virulent Psl strain 814/98. Total RNA was isolated from pooled leaves tissue, collected from 2-3 weeks old plants in growth chamber conditions. The leaves were collected three times: before inoculation, and one and three days after inoculation with Psl 814/98 (0, 1, and 3 dpi). Illumina HiSeq2000 platform was used for RNA-seq sequencing. About 4.47 Gb of clean reads were obtained for each sample and 93.18-93.75% of clean reads were mapped on reference genome confirming that samples are comparable. Transcripts representing about 19,000 genes were identified. Transcriptional differences between particular time points, i.e.: 0, 1 and 3 dpi were revealed. Differentially Expressed Genes (DEGs) for susceptible and partially-resistant cucumber lines were identified including signaling-related genes. The higher number of genes up- and down-regulated was identified in partially-resistant line Gy14. This study provides new insights into the transcriptomic response of cucumber to this bacterial pathogen.

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P4.23

Molecular mechanisms and crosstalk in long distance signaling pathways in *Arabidopsis thaliana*

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The plants must respond to a variety of stresses, biotic and abiotic natures and wide range of viruses, bacteria, fungi which often cause a damage to them. Plants respond to these threats by transmiting an information within itself from one organ to another to establish systemic response to various stresses. The Jasmonic Acid plays main role in triggering plants defense mechanisms. To investigate the long distance signaling in plants we are using Jas9:VENUS *Arabidopsis thaliana* biosensor line. The increase in Jasmonic acid level results in Jas9:VENUS degradation and decrease in fluorescence intensity. To study the effects of genetic backgrounds on the long distance signaling and crosstalk between pathways, we are crossing knockout *Arabidopsis thaliana* lines with Jas9:VENUS and mJas9:VENUS lines. To investigate the interactome of long distance signaling pathways gene cloning of crucial for long-distance signaling pathways is being conducted for study of protein-protein interactions.

The comparison of selected antioxidant enzymes activity in *Pinus sylvestris* and *Quercus robur* after infections with fungal pathogens

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The aim of the study was to determine changes in antioxidant enzymes activity in pine needles and common oak leaves after fungal pathogen infection, analysis of peroxidase (POX) and superoxide dismutase (SOD) isoforms and demonstration the comparison of response effectiveness of selected tree species to pathogen infection.

The study material consisted of two-year old seedlings of common pine and common oak, collected from forest plantations in Spała Forest District on the site of a fresh mixed forest, infected in 10-15% with *Lophoderium pinastri* and *Erysiphe alphitoides* respectively. Leaves and needles were collected at two time points: 14 and 28 days after first visible symptoms of infection.

The results showed the increase in POX and SOD activity in infected pine needles and pedunculate oak leaves comparing to the controls. Activation of some elements of defense mechanisms (enhanced POX and SOD activity) as a reaction to infection in both pine needles and oak leaves, confirms the active process of response to pathogen attack. The prolonged increased activity of both enzymes in pine needles indicates the effective defense mechanism of this species against fungal pathogen, which was additionally confirmed by the visualization of the chosen enzymes by means of electrophoretic paterns.

P5.1

Insight into the structure of NuA4 histone acetyltransferase complex in *Arabidopsis*

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Dynamic structure of chromatin plays a key role in genome stability, regulation of developmental processes and interaction with environment in both plants and animals. Chromatin structure may be changed in many ways, including DNA methylation, posttranslational modification of histone proteins, non-coding RNA or histone variants.

Histone acetylation is one of the most intensively studied chromatin modifications, especially in the context of transcription. Enzymes responsible for this modification often take form of multi-subunit protein complexes. Nucleosomal Acetyltransferase of histone H4 (NuA4) is one of such complexes, first discovered in *Saccharomyces cerevisiae* but also present in animals and, as we will show, in plants.

The NuA4 complex can be subdivided into the catalytic and the non-catalytic part. The catalytic part, called Piccolo NuA4, can acetylate nucleosomal histones on its own but depends on the non-catalytic part for proper targeting.

In our work, we focused on protein-protein interactions between identified plant homologs of NuA4 subunits. For this purpose we used affinity purification/ mass spectrometry (AP-MS) of selected NuA4 components, co-immunoprecipitation (CoIP), pull-down and Far-Western blot assays. We discovered direct interactions between putative subunits of Piccolo NuA4 subcomplex. Then, we moved on to see how the plant Piccolo NuA4 is anchored in the holocomplex. Our results provide molecular basis for understanding of NuA4 structure and function in plants.

Construction of innovative platform based on *Arabidopsis*, human cell lines, mice and chemoinformatics modelling for identification of drugs targeting metabolome-related human diseases

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Some human diseases, such as clear cell renal cell carcinoma (ccRCC) are related to metabolome disorders, featured by the TOR (Target of rapamycin) serine-threonine kinase hyperactivation and correlate with aberrant activity of SWI/SNF ATP-dependent chromatin remodeling complex. Given a recent increase in the number of ccRCC cases, there is an urgent need to address and fill the current gaps in our knowledge on the etiology of this disease, and to develop innovative evidence-based treatments.

The TOR kinase is responsible for integrating signals connected to environmental stress, cell energy status and availability of amino acids. Homologues of TOR kinases were among others identified in plants. TOR kinase appears in cytoplasm and nucleus, but its nuclear function remained thus far largely unknown. Given that the TOR kinase pathway regulates transcription, it is highly probable that there is a functional interdependence between the TOR pathway and machineries responsible for chromatin remodeling enabling precise control of gene expression.

Our findings indicate the existence of functional interdependences between TOR and SWI/SNF chromatin remodeling complexes in *Arabidopsis* and human. We showed that functional SWI/SNF is necessary for proper function of TOR pathway as well as for metabolism control. Subsequently, we constructed a unique innovative platform enabling both the study of evolutionary conserved processes behind development of ccRCC and identification of potential drugs targeting the TOR kinase pathways. This *Arabidopsis*, human, mice and chemoinformatics based platform provides an attractive and cost effective alternative to other currently used approaches.

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P5.3

Different DNA methylation patterns associated with BABA-primed defense gene expression in potato immunity to *Phytophthora infestans*

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Systemic acquired resistance (SAR) is a type of plant immunity caused by local infection or chemical treatment. In turn, priming as the sensitization of stress responsiveness improves defensive capacity of plants and induces an alarmed state of defense. Primed plants acquire resistance in local and distal organs to cope with subsequent, much stronger secondary stress [1]. One of the commonly used and effective chemical SAR agents is a non-protein amino acid, i.e. β-aminobutyric acid (BABA). The high efficiency of BABA in induced disease resistance was found earlier in susceptible potato cv. Bintje [2], while in the presented study it was in cv. Sarpo Mira inoculated with virulent *P. infestans*. An important component of epigenetic modifications that regulates and supports gene expression program is DNA methylation acting as a 'memory transcription' for SAR. Our experiments revealed that potato leaves (cv. Sarpo Mira) supplied with BABA showed an enhanced expression of pathogenesis-related genes (NPR1, PR1, PR2 and PR5), correlated with reduced late blight symptoms after challenge inoculation with vr *Phytophthora infestans*. The obtained results provide insight into the interplay between the DNA methylation/demethylation status in potato leaves immunity, at the cell nucleus level, leaving organ global DNA methylation and promoter region of target defense genes methylated in CpG sequence. De novo DNA methylation was sensitive to BABA treatment. Using immunocytochemical method and 5-mC DNA ELISA assay we found that potato DNA was first drastic methylated then hypomethylated and these changes were controlled by DOMAINS REARRANGED METHYLTRANSFERASE (DRM) and CHROMOMETHYLASE. DNA hypometylation was also tuned with deposition of chromatin marks such as H3K27me3 modification which was responsible for the maintenance of short-lasting stress memory.

[1] M. Jaskiewicz, U. Conrath, C. Peterhänsel, (2011), Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response, EMBO Rep., 12, 50–55,

[2] J. Floryszak-Wieczorek, M. Arasimowicz-Jelonek, D. Abramowski, (2015), BABA-primed defense responses to *Phytophthora infestans* in the next vegetative progeny of potato, Front. Plant Sci., 6:844., doi:10.3389

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Towards the identification of HEI10 substrates in *Arabidopsis thaliana* meiocytes

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Arabidopsis HEI10 is an ortholog of the yeast ZIP3 E3-ubiquitin ligase. It had been identified as a key player in recombination and crossover rate regulation. Indeed, HEI10 had been isolated from a quantitative trait loci identified based on recombination differences between two *Arabidopsis thaliana* natural accessions, Col and Ler. Moreover, HEI10 underexpressing lines showed reduced crossover numbers in comparison to the control. Whereas plants overexpressing HEI10 exhibited an increased crossover rate. These data show that HEI10 is a limiting factor for crossover recombination in plants. It is believed that HEI10 acts during meiosis by ubiquitinylating recombination factors targeting them for proteasome-dependent degradation. However, the details of this process are largely unknown.

Identification of specific HEI10 targets would allow us to understand how the crossover decision is made. This work aims to identify HEI10 substrates in *Arabidopsis*.

To do so, we will use Ubiquitin Activated Interaction Trap (UbAIT). This technique relies on the E3-ubiquitin ligase activity. Fusion proteins, composed of a tagged HEI10 fused with a ubiquitin through a poly-GA linker, will be expressed in hei10-/- *Arabidopsis* plants. Substrates that interact with HEI10 will be trapped when ubiquitinylated. HEI10 and its interactors will be purified using the tag and identified by liquid chromatography coupled to mass spectrometry. Interactions spotted by UbAIT will be further confirmed by other assays including pull-down. This project may result in developing new strategies to modulate crossover frequency in plants.

P5.5

Identification of coding sequences of PICKLE (PKL) gene in yellow lupine (*Lupinus luteus*)

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Along with the social development and a growing population, increasing demand for plant protein. Currently, the vast majority used for the acquisition is genetically modified soy. Climatic conditions in Poland are not conducive to the cultivation of this species, which is why it is necessary to import soybean meal. Lack of diversification of sources of plant protein, can have a material impact on the economy and society. Therefore, to reduce the amount of imported soybeans, it becomes necessary to search for more and new, alternative sources of protein. One of them may be a native species of lupine. Particular attention among them deserves yellow lupine (*Lupinus luteus*). The seeds of this species is accumulated to 45% of the storage proteins and their amino acid composition is much more favorable because of the higher incidence of phenylalanine, lysine or arginine.

Seed development is represented in three stages: late embryogenesis, seed filling, and desiccation. A SWI/SNF class chromatin remodeling factor, encoded by PICKLE (PKL) is present during germination. The PICKLE acts to repress main regulators of embryonic identity LEAFY COTYLEDON1 (LEC1) and LEAFY COTYLEDON2 (LEC2). Their role is control of the expression of genes encoding seed storage proteins (SSP) and additional factors associated with the accumulation of SSP.

The aim of the research was to identify the coding sequence of PKL gene at yellow lupine and to determine similarity to genes identified in the related species and to designate evolutionary conserved domain. The study was carried out using the RNA-seq technique of transcriptomes obtained from vegetative and generative organs of yellow lupine, Taper variety.

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Identification of interdependences between the ATP – dependent SWI/SNF-type chromatin remodeling complex and BRCA1 in *Arabidopsis thaliana*

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Within the nucleus of all eukaryotes, DNA is tightly packaged into a nucleoprotein complex called chromatin. This compaction allows the storage of large amount of DNA, but on the other hand nucleosomes inhibit transcription, DNA repair, and other chromosome transactions. One of the best studied complexes, responsible for chromatin structure changes is evolutionary conserved, multiprotein SWI/SNF complex, able to relieve this inhibition by sliding or disassembling nucleosomes, substituting histones with histone variants, or interactions with transcription factors. It is know that hSWI/SNF subunits (BRG1, hBRM), with the ATPase activity, directly interact with BRCA1 protein. The BRCA1 protein is a product of the suppressor gene, that is involved in genome stability maintenance by e.g. participating in DNA double strand breaks repair (DSB), cell cycle control, regulation of genes involved in DSB response as well as control of higher chromatin structure. The aim of presented study was to check, whether interactions between BRCA1 and SWI/SNF complex are evolutionarily conserved in *Arabidopsis thaliana* model plant and assess how the aberration of BRCA1 influence the SWI/SNF complex function. In this order we identified *Arabidopsis* mutant lines carrying T-DNA insertions in the BRCA1 gene and line with overexpression of BRCA1, respectively. Subsequently we constructed double *Arabidopsis* lines carrying mutations in genes coding for SWI/SNF subunits and BRCA1 protein. These lines were subjected for detailed phenotypic analyses including assessment of their response to the treatment with various hormones and abiotic stresses.

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P5.7

Identification of functional interdependence between SWI/SNF dependent chromatin remodeling and pre-mRNA splicing in *Arabidopsis*

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SWI/SNF type chromatin remodeling complexes (CRCs) are evolutionarily conserved multiprotein machineries controlling DNA accessibility by regulating chromatin structure. The core of *Arabidopsis* SWI/SNF complex contains one of the four ATPases (BRM, SYD, CHR12, CHR23), two of the four SWI3 type proteins (SWI3A, SWI3B, SWI3C, SWI3D) and one SNF5 type protein (BSH). SWI/SNF CRCs play an important role in the regulation of transcription, cell cycle and DNA replication. Moreover, recent data indicate that the SWI/SNF complex is likely involved in the regulation of pre-mRNA splicing in humans, *Drosophila* and yeast. In eukaryotes, intron sequences from pre-mRNAs transcribed by RNA polymerase II are removed by the spliceosome to produce mature mRNAs. The spliceosome is composed of small nuclear snRNAs and associated proteins. Catalytic activation of the spliceosome is critically dependent on its association with the evolutionary conserved NineTeen Complex (NTC), which among others carries the core PRP19A/B, CDC5 and SPF27 subunits. The aim of our study is to identify a regulatory link between chromatin remodeling and pre-mRNA splicing in *Arabidopsis*. To investigate the protein-protein interactions of several subunits of SWI/SNF and NTC complexes in *Arabidopsis*, we performed yeast two-hybrid (YTH) interaction studies. Subsequently, we verified the existence of identified protein interactions using bimolecular fluorescence complementation and pull-down assays. In addition, using combinations of existing T-DNA insertion mutations, we identified epistatic genetic interactions between SWI/SNF and NTC, which provide evidence for functional interdependence between chromatin remodeling and pre-mRNA splicing mechanisms in *Arabidopsis*.

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Can MS-HRM technique be used as an alternative tool in DNA methylation study in plant?

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Activity of many genes is highly influenced by changes in the status of DNA methylation in the promoter regions. This phenomenon is known as the epialleles. The level of DNA methylation in plants is higher than in animals mostly limited to cytosine methylation in a CG sequence context. Plants additionally harbor methylated cytosine in CHG and CHH islands where H = A, G or T. DNA methylation is critical for various biological processes including responses to environmental stresses both abiotic and biotic.

Variation in DNA methylation patterns could be responsible for heritable phenotypic differences including acquired resistance traits in plants [1].

A variety of molecular methods exist to assay for global DNA methylation based on immunodetection or restriction enzymes for DNA modifications. These methods are time-consuming and sometimes difficult to perform. A simple and fast method for searching specific regions of gene methylation is the Methylation-Sensitive High Resolution Melting PCR analysis (MS-HRM PCR) first devised by [2]. MS-HRM monitors the change in fluorescence as a PCR amplicon melts in the presence of an intercalating DNA dye. PCR products generated from bisulfite-treated DNA templates have different melting curves if they are differentially methylated Bisulfite reaction leads to transformation non-methylated cytosines to uracils.

Uracil possesses lower melting temperature than cytosine. These differences in melting temperature between methylated and non-methylated sequence is visible on melting curves created in HRM analysis [2]. We have adopted MS-HRM technique for DNA methylation, so far mainly used in human clinical research, in plant disease study with some modifications of this methodology.

In the present study we found changes in DNA methylation patterns in the promoter regions of many genes involved in systemic acquired resistance and compared results with RNA transcript accumulation of these genes under biotic stress conditions caused by β-aminobutyric acid (BABA) treatment and challenge inoculation potato leaves cv. Sarpo Mira with *Phytophthora infestans*.

[1] R. H. Dowen i in., „Widespread dynamic DNA methylation in response to biotic stress", Proc. Natl. Acad. Sci., t. 109, nr 32, s. E2183–E2191, lip. 2012.

[2] T. K. Wojdacz i A. Dobrovic, „Methylation-sensitive high resolution melting (MS-HRM): a new approach for sensitive and high-throughput assessment of methylation", Nucleic Acids Res., t. 35, nr 6, s. e41–e41, mar. 2007.

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P5.9

The role of SWI/SNF-type chromatin remodeling complex in maintaining the energy homeostasis in *Arabidopsis thaliana* plants

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Chromatin is subjected to dynamic modifications that allow an access to DNA for transcription factors and other regulatory proteins. Part of these modifications is executed by evolutionary conserved ATP-dependent SWI/SNF chromatin remodeling complex (CRCs) which are involved in such important processes as regulation of transcription, DNA replication, DNA repair and cell cycle, maintenance of proper chromatin structure and histone variants exchange. However the function of SWI/SNF CRCs is deeply studied many aspects of function of these complexes are still unknown. Our study showed that inactivation of *Arabidopsis* SWI/SNF complexes affects expression of genes involved in plant development, hormonal signaling, control of metabolic processes and stress responses. *Arabidopsis* plants carrying mutations in genes coding for core subunits of SWI/SNF CRCs exhibit alterations in glycolysis and TCA cycle. Moreover, we showed that core subunits of SWI/SNF CRCs directly interact with key enzymes involved in the control of metabolic processes. Here we show, that SWI/SNF complexes may directly control expression of some genes responding to energy deprivation.

Furthermore, our findings indicate that the occupancy of SWI/SNF complexes on these target loci alters upon this type of stress. Consistently, the use of *Arabidopsis* mutant lines indicated that loss of SWI/SNF subunits alters *Arabidopsis* response to energy deprivation. Collectively, our study indicate the chromatin remodeling executed by SWI/SNF CRCs, as one of the possible mechanisms regulating expression of genes responsive to energy deprivation and provide evidences that SWI/SNF complexes may be involved in the maintenance of energy homeostasis in *Arabidopsis*.

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Priming by β-aminobutyric acid modified transient chromatin and improved resistance to *Phytophthora infestans* in potato

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Potato leaves (cv. Sarpo Mira) pretreatment with β-aminobutyric acid (BABA) revealed stress imprint activation, facilitating acquisition of a competence to react faster and stronger after challenge inoculation, in the form of a potentiated rise in PR1, PR2 and PR5 levels of defense genes. An enhanced resistance to virulent pathogens and disease limitation correlated with increased levels of H3 and H4 expression and histone modifications. To examine the dynamics of post-translational histone lysine acetylation antagonistic gene expressions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) were found. BABA (5μM) was also effective factor in the upregulation of histone methylation mediated by the SET domain of histone lysine methyltransferases (Trithorax (TrxG) and SUVH1 belonging to the Polycomb group). The NPR1 protein as a positive co-regulator of systemic acquired resistance (SAR) plays an extremely provocative role in establishing immune response, having systemic character in plants. Presented chromatin immunoprecipitation (ChIP) data revealed that BABA pretreatment caused an early and temporary enrichment of H3K4me2 at the gene regions of primed NPR1 and PR2. In turn, low H3K9 methylation levels of these genes after induction systematically increased only after challenge inoculation. Our study showed that a BABA mimicked pathogen attack primed epigenetic adjustment of potato to repeated exposure to stresses. TrxG by establishing H3K4me2 marks was probably required for the initial NPR1-dependent immune activation, while SUVH1 was responsible for the establishment and maintenance of the H3K9me2 methylation pattern needed for a successful BABA-primed augmented PRs defense systemic responses upon *P. infestans* challenge inoculation.

[1] Jaskiewicz et al.: Chromatin modification acts as a memory of systemic acquired resistance in the plant stress response. EMBO reports. 2011. Vol 12 no 1.

[2] Ding B. Wang G.-L.: Chromatin versus pathogens: the function of epigenetics in plant immunity. Front. Plant. Sci. 2015. 6:675.

[3] Luna et al.: Role of NPR1 and KYP in long-lasting induced resistance by β-aminobutyric acid. Front. Plant. Sci. 2014. 5:184

[4] Ferreira Neto et al.: Epigenetic signals on plant adaptation: a biotic stress perspective. Curr. Protein Pept. Sci. 2017. 18:1-16

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P5.11

The involvement of histone acetylation in somatic embryogenesis induced in *Arabidopsis* explants cultured in vitro

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Epigenetics refers to various changes in chromatin structure controlling gene expression and they involve DNA methylation and posttranslational histones modifications including histone acetylation. Histone acetylation is believed to play a key role in regulation of plant development however, experimental evidences on the involvement of this process in in vitro induced morphogenic processes are limited.

Thus, we used a model, in vitro culture system of *Arabidopsis* to study a role of histone acetylation in somatic embryogenesis (SE), a developmental process that exemplifies a unique developmental plasticity of plant somatic cells. Our previous observation that *Arabidopsis* explants treated with TSA (trichostatin A), an inhibitor of HDACs activity, acquire the capacity for somatic embryo formation has motivated us to profile the expression of HATs and HDACs genes encoding the enzymes of histone acetylation and deacetylation activity, respectively. The level of the gene transcripts was evaluated with use of RT-qPCR in explants cultured on auxin and auxin-free medium that result in SE and seedling development, respectively. We observed a high accumulation of HDACs and HATs transcripts in embryogenic culture in particular, during early phase of SE induction. Further evidence on a role of histone acetylation in SE provided the impaired embryogenic response observed in the mutants with defected activity of the HDACs and HATs genes. In addition, we performed the immunohistochemical analysis in SE-induced explants and to this end, the antibody against H3K18Ac was used. We observed the high accumulation of H3K18Ac associated signals in explant tissue undergoing SE. Altogether, the results of various analytical approaches indicated the involvement of histone acetylation in embryogenic reprogramming of *Arabidopsis* somatic tissue.

Nuclear function of *Arabidopsis* RCF family leucine-rich repeat receptor like kinases (LLR-RLKs)

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The RCF (Receptors for GA Signaled Flowering) family of leucine-rich repeat receptor-like kinases (LLR-RLKs) consists of RCF1, RCF2 and RCF3 proteins. In *Arabidopsis*, RCF proteins play important role in the regulation of important processes like control of development including meristem size and inflorescence architecture, hormonal signaling, etc. In this work we show that inactivation of *Arabidopsis* RCF proteins causes severe impairment of gibberellin (GA) response, down-regulation of gibberellin receptor GID1 (GIBBERELLIN INSENSITIVE DWARF 1) genes and broad transcription changes affecting other important regulatory processes. We found that upon endocytosis, RCFs accumulate in the nucleus, where they interact with the SWI3B core subunit of the SWI/SNF chromatin-remodeling complex (CRCs). Occupancy of promoter regions of GID1 genes by RCF1, RCF2 and SWI3B proteins, together with genetic and comparative transcriptomic studies, suggests that RCFs and SWI/SNF CRC are involved in transcriptional control of GA receptors.

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P5.13

The impact of GA and ABA on *LlFLD-like* expression in vegetative organs of *Lupinus luteus*

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Flowering induction is a main event in the life cycle of higher plants. The optimal time of flowering is one of the factors providing reproductive success and satisfactory the yield of cultivated plants. A molecular characteristic of *Arabidopsis thaliana* mutants made it possible to identify a number of genes that are directly or indirectly involved in the regulation of this process. There are many mechanisms underlying the regulation of induction of generative development. One of the more interesting is the control of chromatin structure by histone and DNA modifying proteins. One such protein is described in *A. thaliana* lysine demethylase FLD. The homolog of the gene coding for this protein was identified in *Lupinus luteus* transcripts. Using the RT-qPCR technique, the expression pattern was examined. The effect of gibberellins and abscisic acid on the changes in the amount of LlFLD mRNA was also determined.

Significant differences in the level of transcripts of the test gene were found, depending on the body in which it was tested. The changes in LlFLD expression depend on the hormones administered. The results show that the response to the hormone is conditioned by the type of organ and the stage of plant development. The highest expression levels were found in upper leaves and growth tips. The lowest transcriptional activity was described and in the upper leaf petioles. With age, the amount of mRNA of the test gene decreased. Based on the results obtained, it can be stated that the studied phytohormones affect changes in LlFLD level of one of the important factors conditioning chromatin condensation.

Identification of genes contributing to variation in *Arabidopsis* crossover frequency

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During meiotic division homologous chromosomes pair and reciprocally exchange their fragments in the process called crossover (CO); as a result, new allelic combinations are generated, which are important for natural variation within the population and play an important role in the evolution. Crossover numbers are strictly controlled and kept at a very low level of 1-3 per chromosome per meiosis. However, the factors responsible for this control are largely unknown. Therefore, identification of genes which act as trans-modifiers of crossover frequency is of major interest.

Interestingly, crossover frequency is extensively variable within and between species. Previously, using natural *Arabidopsis thaliana* accessions (Col×Ler F2 populations), we identified two major recombination quantitative trait loci (rQTLs), which were responsible for the crossover variation between Col and Ler accessions, and were located on chromosome 1 and 4. Fine mapping revealed that the first of them corresponds to HEI10 E3 ubiquitin ligase, an evolutionary conserved protein which orchestrates proteolysis of recombination complexes. The aim of the study is to identify a trans modifier gene in the interval located on chromosome 4 (rQTL4). In this work, F4 plants from Col×Ler F3 individuals containing crossover sites within the credible interval are used for mapping to narrow down the interval. Fluorescent seed reporter system (420 reporter line) is applied to measure crossover frequency of F4 individuals. Candidate genes will be further confirmed by analysis of crossover rate in the mutant background and additional genetic approaches. In the future we plan to extensively characterize the pathway involving rQTL4 at the molecular level.

P6.1

Nitric oxide affects rhizogenesis in fibre flax (*Linum usitatissimum* L.) cultures *in vitro*

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Plant in vitro culture is a convenient material for investigating the mechanisms of nitrogen oxide (NO) in the regulation of plant growth and development. However, there are only few studies about effects of NO on plants regeneration in in vitro cultures. Positive effect on shoots and roots organogenesis were obtained e.g. in the cultures of flax (Kalra, Babbar 2010) or vanilla (Tan et al. 2013). These experiments refer to improvement of organogenesis after exposure to NO donors, including sodium nitroprusside (SNP) and S-nitrozo-N-acetyl-DL-penicillamine (SNAP).

The aim of our study was to determine the effect of SNP on the regenerative efficiency in fiber flax cultures in vitro and the answer to the following questions: i) whether the vapours from the SNP (containing NO) generates oxidative stress in tissues; ii) to what extent the observed changes in root organogenesis depend on the activity of the antioxidant system?

Cultures of fiber flax (*Linum usitatissimum* L., cv. Selena) in which differences in rhizogenesis were observed due to the action of vapors from 5 mM SNP. Hypocotyl explants were cultured on Gamborg (B5) medium with hormones: α-naphthylacetic acid (NAA, 1 mg l-1) and 6-benzyladenine (0.025 mg l-1) or medium without hormones, and then exposed to the SNP. Determination of tissue antioxidant capacity on the reduction of stable 1,1-diphenyl-2-picrylhydrazine free radical (DPPH), TBARS and H_2O_2 content and antioxidant enzymes activity: catalase (CAT), superoxide dismutase (SOD) and its isoforms were measured.

The vapours from the SNP solution were involved to improvement of tissue regeneration, both in NAA-enriched root cultures and in cultures grown without hormones. Experiments with c-PTIO have confirmed NO's contribution to regulation of organogenesis in fiber flax. Cultures treated with NO showed better antioxidant capacity than control in reaction with DPPH. At the early stage of organogenesis these cultures were also characterized by reduced H_2O_2 and TBARS content. Increased synthesis of H_2O_2 was observed at the later stage of growth in hormone-free cultures. SNP fumigation did not affect SOD and CAT activity in most cases, but in the later stage of cultures growth, the increased activity of MnSOD was observed. The stimulation of rhizogenesis efficiency, as the result of NO action, probably occurred due to interaction of the NO and phytohormones in the nutrient medium, or via hormones synthesis (e.g. cytokinins) and/or signaling molecules (e.g. H_2O_2) stimulation.

[1] Kalra C., Babbar S.B. 2010. Plant Cell Tiss. Organ Cult., 103: 353-359.

[2] Tan B.C., Chin C.F., Alderson P. 2013. In Vitro Cell. Dev. Biol.-Plant, 49: 626-630.

Alteration in ROS metabolism during digestion in pitcher fluid of *Nepenthes ventrata*

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Carnivorous plants attract animals, trap them, kill and absorb nutrients from digested bodies. The specific and bizarre type of nourishing was possible to achieve because typical photosyntheticaly active leaves of the plants were converted into special traps. This adaptation was a strategy allowing survival in the low-nutrient environment (Król et al. 2012). The genus *Nepenthes* (pitcher plants) contains about 120 species that belong to almost 700 species of carnivorous plants. Pitcher plants are characterized by the presence of passive traps of pitcher shape, filled with a digestion fluid, of still not well described chemical composition (Król et al. 2012). Digestion of prey is accompanied by increased activity of different enzymes. Participation of reactive oxygen species (ROS) in digestion process has been proposed (Chia et al. 2004). Nitric oxide (NO) is involved in digestion process in animals, also in human stomach (Gago et al. 2007). The aim of our work was to demonstrate the presence of ROS and alteration of ROS content in pitcher fluid of *Nepenthes ventrata* during digestion in response to stimulation by addition of sodium nitrite (NaNO) into the trap. Our results indicate enhancement of generation of superoxide anion (O -.) and stimulation of total antioxidant activity during digestion in pitcher fluid. ROS action during digestion was confirmed by measurement of the level of carbonylated proteins from pitcher fluid.

[1] Gago et al. 2007. Free Radical Biology & Medicine 43:1233-1242 Król et al. 2012. Annals of Botany, 109:47-64

[2] Chia et al. 2004. Redox Report 9:255-261

P6.3

Non-protein amino acids impact polyamines metabolism in roots of tomato seedlings

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Polyamines (PAs: putrescine – Put, spermidine – Spd, spermine – Spm) belong to the group of plant growth regulators. They take part in regulation of root development and plant reaction to stresses. PAs biosynthesis depends on activity of arginase (ARG) catalyzing reaction of ornithine formation from arginine. Put is synthesized by decarboxylation of ornithine. Biosynthesis of Spd and Spm requires activity of: S-adenosyl-methionine decarboxylase (SAMdc) necessary for formation of aminopropyl donor, and a transferase (Spd synthase – SPDs or Spm synthase – SPMs) catalyzing transfer of the animopropyl group to Put or Spd, respectively. Control of the cellular level of PAs, and their degradation (mainly Spd and Spm) depends on activity of polyamine oxidases (PAO).

In the studies we have used two non-protein amino acids (NPAA): meta-Tyrosine (m-Tyr), and L-cananvanine (CAN), inhibiting growth of tomato plants. m-Tyr is a component of root exudates of fescues species and is antimetabolite of phenylalanine. CAN is an antimetabolite of arginine and is present in sprouts of alfalfa. Both NPAAs induce nitro-oxidative stress in plants. Tomato seedlings (*Solanum lycopersicum* L. var. Malinowy Ożarowski) were treated with m-Tyr or CAN. Concentration of PAs (High Pressure Liquid Chromatography) and expression of genes (qPCR), encoding proteins of PAs metabolism (SAMdc, SPDs, SPMs, PAO, ARG) were investigated.

Our data indicate that both NPAAs modify transcript of the genes in concentrate dependent manner. After prolonged culture transcription of almost all genes were down regulated. Alterations in free PAs level were observed.

It may be assumed that toxicity of m-Tyr and CAN tomato seedlings is due to modification of PAs metabolism, resulting in disturbances in roots growth.

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The effect of reactive nitrogen species on *Phytophthora infestans*

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The metabolic fate of nitric oxide (NO) gives rise to a further series of compounds, collectively known as the reactive nitrogen species (RNS), which possess their own unique characteristics. Apart from NO, reactive nitrogen species include its derivatives, e.g. peroxynitrite (ONOO-). It is known that pathophysiological states are accompanied by an excess of RNS inducing nitrosative stress and cell damage. However, under physiological conditions the RNS formation is tightly regulated by various balancing systems.

In order to verify whether and to what extent reactive nitrogen species affect the oomycete pathogen *P. infestans* (Mont.) de Bary, analyses using two RNS donors were undertaken. Briefly, the experiments were conducted on two pathogen isolates, i.e. avirulent (avr) and virulent (vr) in reference to the potato genotype `Bzura`, which were treated with SIN-1 (3-morpholino-sydnonimine), simultaneously generating NO and superoxide, as well as SNP (sodium nitroprusside), an NO donor.

Bio-imaging with APF (3′-p-aminophenyl fluorescein) and DAF-2DA (4,5-diaminofluorescein diacetate) fluorochromes revealed RNS generation in pathogen structures. The application of different concentrations of RNS donors to synthetic medium modified growth of avr and vr *P. infestans*. However, exposure to RNS doses in the range of millimoles caused only a slightly reduced growth rate of pathogen hyphae. To gain insight into metabolic changes associated with RNS over-accumulation in pathogen structures, the studies included measurements of both lipid peroxidation and peroxiredoxin activity. The results indicated that the oomycete pathogen exposed to exogenous RNS sources is able to tightly regulate the balance between NO and other RNS via the peroxiredoxin system. Therefore, the observed effect may have been a consequence of an adaptation mechanism to the presence of huge amounts of RNS derived from internal and external sources.

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P6.5

Protein nitration and S-nitrosylation: what's new in *Phytophthora infestans*?

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Plant–pathogen systems are dynamic and in the course of evolutionary changes pathogens have developed numerous invasion strategies, to which the host organisms have responded with an extensive range of defense traits known as „fight for their lives". Recent advances in our understanding of plant-pathogen interactions have shown that also pathogenic microorganisms are capable of synthesizing nitric oxide (NO), although the role of NO in this systematically heterogeneous group of organisms has not been clarified.

It is known that the functional role of NO in living organisms may be realized by directly altering protein activity by post-translational modifications (PTMs) via S-nitrosylation and tyrosine nitration.

Our previous studies revealed that the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, a cause of late blight disease, is able to generate NO in hyphae and zoospores growing on the medium and in planta. Therefore, in this study by combining immunological and proteomic approaches we identified proteins potentially targeted by NO via nitration and S-nitrosylation in *P. infestans* structures. An experimental approach involved two *P. infestans* isolates, i.e. avirulent (avr) and virulent (vr) in reference to the potato genotype 'Bzura', thus creating a useful background for a comparative study.

Using the biotin switch assay, a pattern of 15 immunopositive protein bands was found in both pathogen isolates growing on a synthetic medium; however, an enhanced expression of S-nitrosylated proteins was observed mainly in vr *P. infestans*. Similarly, the resulting 2D-nitroproteome profiles showed quantitative and qualitative differences between avr and vr *P. infestans* isolates. A common pool of 13 immunoreactive protein spots was found in the structures of both pathogen isolates. Importantly, as many as 21 spots were specific to the virulent *P. infestans*.

The differences observed between avr and vr *P. infestans* isolates in relation to the protein pools modified via NO might involve factors engaged in pathogen virulence. Therefore, the characterization as well as physiological relevance of protein S-nitrosylation and tyrosine nitration phenomenon are essential in providing insight into the function of NO in the oomycete pathogen.

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AtAnn1 in ROS metabolism

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Annexins are evolutionary conserved family of calcium and phospholipid-binding proteins. Genomes of almost all plant species encode several annexins and expression levels of certain of them are rather high. Annexins from different species (i.e. *Brassica juncea*, *Nelumbo nucifera*, *Gossypium hirutum* and *Solanum tuberosum*) that exhibit high amino acid identity to annexin 1 from *Arabidopsis thaliana* (AtAnn1) improved stress tolerance of tobacco, cotton and *Arabidopsis*. AtAnn1 was identified in a genome-wide search of *Arabidopsis* sequences that were capable of rescuing *Escherichia coli* oxyR growth on high H_2O_2 concentrations. In *Arabidopsis* its expression is induced by exogenous H_2O_2 and in response to factors that induce accumulation of reactive oxygen species (ROS). In potato plants overexpressing AnxD36 (homologues to AtAnn1) accumulation of xenophyles: zeaxanthin and violaxanthin were enhanced and steady-state level of abscisid acid (ABA) was significantly reduced.

Violaxanthin is a precursor in ABA biosynthesis but under high light stress it can also be converted into zeaxanthin by zeaxanthin epoxidase (ZEP). The latter reduction requires reduced ascorbate as an electron donor, whereas stress-induced oxidation of ascorbate pool promotes ABA biosynthesis. In our current project we analyze the possible mechanism of AtAnn1-mediated ability to ameliorate ROS accumulation during stress. We assume that it could be due to enhanced accumulation of reduced ascorbate, which would also improve ascorbate-glutathione cycle capacity. We analyzed the concentration of ascorbate and glutathione and the redox status of *Arabidopsis* plants with various levels of AtAnn1. We also analyzed the expression of genes coding for enzymes involved in redox homeostasis. Our results indicate significant correlation between annexin 1 expression level and ascorbate-glutathione cycle capacity.

P6.8

Methionine sulfoxide reductases – the new differentiating component of antioxidant system in orthodox and recalcitrant developing seeds of *Acer* species

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Norway maple (*Acer platanoides* L.) and sycamore (*Acer pseudoplatanus* L.) produce seeds that are resistant and sensitive to desiccation, respectively, what is reflected in seed categorization. During 11-24 weeks after flowering they continually lose water and accumulate dry matter in a similar manner but differ in physiology. Norway maple and sycamore seeds present dissimilar production and accumulation of reaction oxygen species during their development as well as different antioxidant capacity. Antioxidant protection is always ameliorated in embryonic axes and lower in the cotyledons. Orthodox seeds of the Norway maple shows largely higher antioxidant capacity than recalcitrant sycamore seeds what is established in soluble thiol-protein extracts as well as in extracts containing polyphenols, carotenoids, ascorbic acid and tocopherol. Methionine sulfoxide reductases (Msr) are enzymes involved in the regeneration of oxidatively modified methionine residue in proteins sustaining proper structure and function of proteins. Among all B-type isoforms the two, MsrB1 and MsrB2, were detected in analyzed *Acer* seeds. Both *Acer* species are genetically related, however the expression of MrsB isoforms is different. Interestingly, MsrB1 is expressed uniquely in sycamore seeds, both in embryonic axes and cotyledons and increased MsrB1 production is detected during seed maturation. MsrB2 is detectable in both Norway maple and sycamore species, however, this protein is expressed uniquely in embryonic axes of sycamore whereas in Norway maple this protein is present both in embryonic axes and cotyledons and accumulates as the maturation is progressing. Msr activities strongly contribute to the reduction potential established in *Acer* developing seeds, making them a new component of antioxidant system that differentiate *Acer* seeds from orthodox and recalcitrant category.

Arbuscular mycorrhizal symbiosis affect tyrosine nitration in Al-treated plants

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Background and Aims Protein tyrosine nitration under physiological and different (a)biotic-stress conditions being insufficiently studied. Moreover, almost no information about this post-translational modification exists for arbuscular mycorrhiza (AM). Aluminum (Al) toxicity is one of the major factors that limit plant growth and development in acid soils, but heavy metals availability to plants can be modulated by soil microorganisms, including arbuscular mycorrhizal fungi. Using *Medicago truncatula – Rhizophagus irregularis* symbiosis exposed to short Al stress the immunolocalization and the profile of protein tyrosine nitration in roots were analysed.

Results Immunolocalization with 3-nitrotyrosine antibodie revealed lower protein nitration in mycorrhizal roots under Al stress. In non-treated, non-mycorrhizal roots, the nitrated proteins was localized in vascular tissues, epidermis and cortex cells. Al stress increase accumulation of nitrated proteins in this locations. In non-treated, mycorrhizal roots tyrosine nitrated proteins appeared in cortex cells, vascular tissues, and on the surface of arbuscules and intercellular hyphae, and Al-treatment decrease fluorescence intensity. Additionally, immunoblot results indicated the presence of four immunoreactive bands with molecular masses of 60, 50, 37 and 17 kDa in non-mycorrhizal roots and Al stress induced a higher intensification of 37 and 50kDa bands. A new immunoreactive bands of 70 and 95 kDa, which were not present in non-mycorrhizal plants, was detected in mycorrhizal roots and the most abundant being nitrated protein having 50kDa and 37kDa subunit sizes. AM symbiosis induces intensification of specific bands compared with non-mycorrhizal, non-treated roots. Moreover, AM symbiosis decrease intensification of specific bands under Al stress.

Conclusions These results provide evidence of nitrated proteins localization at the surface of fungal structures demonstrated they participation in infection process and development of AM symbiosis.

Moreover, AM symbiosis results in lower protein tyrosine nitration (which is equivalent to lower nitrosative stress) in Al-treated plants, which indicate the protective role of AM symbiosis for Al-treated plants.

Non-protein amino acids induce alterations in protein nitration in roots of tomato seedlings

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Canavanine (CAN) and *meta*-Tyrosine (*m*-Tyr) belong to non-protein amino acids (NPAAs). CAN is found in seeds of some *Fabaceae* and sprouts of alfalfa and acts as defense against predators. m-Tyr is a component of exudates of fescues species, and is considered as a strong phytotoxin. Both NPAAs disturb RNS and ROS metabolism (Krasuska et al. 2016a,b). As alterations in cellular RNS/RNS level influence posttranslational modification of proteins, the main object of our work was to investigate protein nitration in roots of tomato (*Lycopersicum esculentum* L.) seedlings exposed to *m*-Tyr (50 and 250 μM) or CAN (10 and 50 μM). The immunoprecipitation of nitrated proteins was done using antibody against 3-nitro-Tyr. Inhibition of elongation growth of tomato roots was accompanied by a decline of NO emission after CAN treatment, while *m*-Tyr slightly enhanced RNS formation. CAN declined level of nitrated proteins, mainly at the beginning of the culture, m-Tyr stimulated protein nitration in tomato roots particularly after prolong exposition to NPAA. Alterations in modified proteins were also observed after 24 h and 72 h of the culture.

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Index of Authors

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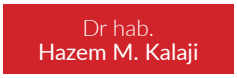

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