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CENTRUM
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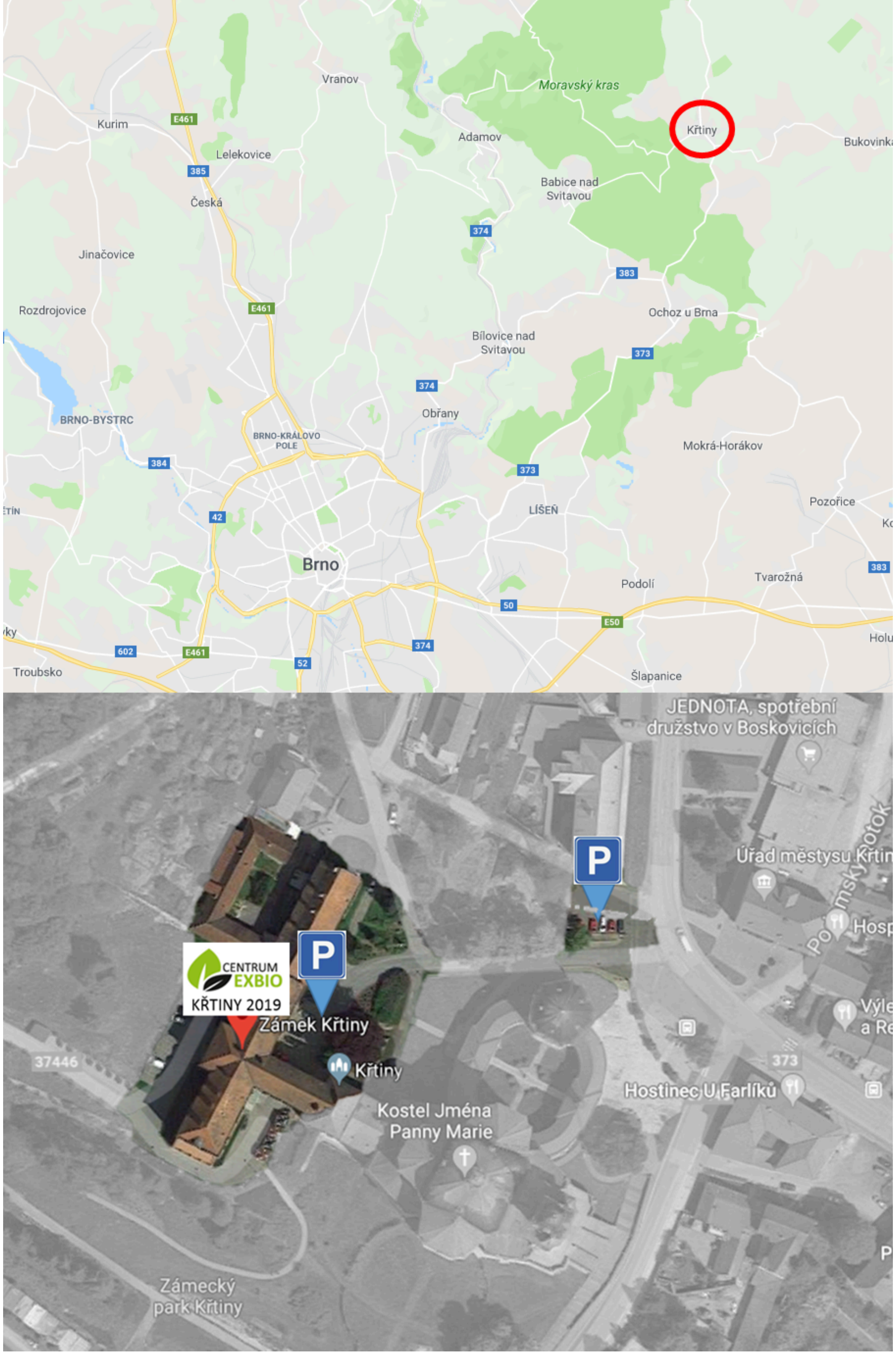
September 5-6, 2019



Château Křtiny

Organizers

Břetislav Brzobohatý, Mendel University in Brno
Martin Černý, Mendel University in Brno
Šárka Koukalová, Mendel University in Brno
Markéta Luklová, Mendel University in Brno
Radomíra Vaňková, Institute of Experimental Botany, CAS

Section chairs

Břetislav Brzobohatý, Mendel University in Brno (WP2)
David Honys, Institute of Experimental Botany, CAS (WP3)
Tamara Pečenková, Institute of Experimental Botany, CAS (WP1)
Helena Štorchová, Institute of Experimental Botany, CAS (WP4)
Radomíra Vaňková, Institute of Experimental Botany, CAS (WP2)

The work was supported from European Regional Development Fund-Project "Centre for Experimental Plant Biology" (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
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MINISTRY OF EDUCATION,
YOUTH AND SPORTS

Programme

Thursday, September 5

11:30	**Opening**
11:35 - 12:00	**Centrum development and key milestones for 2019 (Radomíra Vaňková)**
12:00 - 13:00	**Lunch break**
13:00 - 14:40	**Lectures WP1 (Tamara Pečenková)**
13:00 - 13:20	**Katarzyna Retzer** Dissecting differential control of PIN2 turnover during tropistic responses
13:20 - 13:40	**Markéta Šámalova** Exploring the role of cytokinin-responsive WP1_2 EXPANSINs in the control of cell wall properties and development in Arabidopsis under stress
13:40 - 14:00	**Jan Hejátko**
14:00 - 14:20	**Tamara Pečenková** EXO70B1 and B2 role in plant responses to biotic and abiotic stresses
14:20 - 14:40	**Petr Klíma**
14:40 - 15:00	**Coffee break**

15:00 - 17:00	**Lectures WP2 (Břetislav Brzobohatý)**
15:00 - 15:20	**Radomíra Vaňková** The effect of acclimation and Incyde application on hormonal dynamics during heat stress response
15:20 - 15:40	**Danuše Tarkowská** Gibberellins under a magnifying glass or find a needle in a haystack
15:40 - 16:00	**Peter Hedden** Gibberellin signalling in response to abiotic stress in wheat
16:00 - 16:20	**Lenka Burketová** Actin depolymerization increases plant resistance against pathogens via activation of salicylic acid signalling pathway
16:20 - 16:40	**Petr Maršík** Changes of lipid profile in nanoparticle-stressed plants
16:40 - 17:00	**Martin Černý** Seed proteome and metabolome under changing environmental conditions
17:00 - 17:30	**Coffee break**
17:30 - 19:10	**Lectures WP2 (Radomíra Vaňková)**
17:30 - 17:50	**Jan Martinec** Pattern triggered immunity under heat stress - is there a place for FLOTs?
17:50 - 18:10	**Ivan Petřík** Innovative, effective, robust, fast and cheap purification of phytohormones using dispersive solid phase extraction

18:10 - 18:30	**Tomáš Moravec** Design of infectious viral clones based on the Tobamovirus genus and their use as the markers for detection of viral infection
18:30 - 18:50	**Barbora Jindřichová** Fungal infection modifies oilseed rape attraction to herbivores
18:50 - 19:10	**Jan Rezek** Volatile organic compounds (VOCs) of *A. thaliana* as a response to different stresses
19:10 - …	**Dinner**

Friday, September 6

10:00 – 11:15	**Lectures WP3 (David Honys)**
10:00 - 10:20	**Helene Robert Boisivon** Thermomorphogenesis during seed development in *Arabidopsis* and *Brassica napus*
10:20 - 10:40	**David Honys** Pollen germination during heat; Do HSP90s play a role?
10:40 - 11:00	**Jan Petrášek**
11:00 - 11:15	**Zbyněk Zdráhal** CEITEC Proteomics for CEBR
11:15 - 11:45	**Coffee break** **Jitka Kopková** EXBIO Project management (presentation in Czech)
11:45 - 12:25	**Lecture WP4 (Helena Štorchová)**
11:45 - 12:05	**Helena Štorchová** Three transcriptomic compartments in Silene vulgaris flower buds are affected to various extent by cytoplasmic male sterility
12:05 - 12:25	**Miloslav Juříček** Development of transcriptome analysis pipeline in non model plants
12:25 - 12:30	**Concluding remarks**
12:30	**Lunch**

Abstracts

WP1-01

Dissecting differential control of PIN2 turnover during tropistic responses

Katarzyna Retzer, Kateřina Malínská, Klára Hoyerová, Jan Petrášek

Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany, the Academy of Sciences of the Czech Republic, Prague, Czech Republic

Plant plasma membrane proteins are essential mediators of endogenous and environmental cues in the regulation of various developmental and metabolic processes. Coordinated distribution of auxin throughout the plant body is crucial for proper root growth adaptation. Polar auxin transporter PIN2 is maintaining the asymmetric distribution of auxin during tropistic responses, which affects root growth. Using a “Graviscope”, a novel confocal microscopy setup with vertical sample mounting that allows non-invasive tracking of root growth and PIN2 turnover, we show here that mobility of PIN2 along the plasma membrane and its intracellular trafficking depends on the exposure of roots to light. Roots cultured in darkness had enhanced trafficking of PIN2 in comparison with light-grown roots. Interestingly, only dark-grown roots had enhanced PIN trafficking upon salt stress. We had initiated a reverse phenotyping screen to screen candidates known to interfere with PIN2 trafficking for their reaction to dark-grown salt treatments. From this screen, phospholipase D α1 mutants showed a less pronounced response upon salt stress when their roots were dark-grown, suggesting an auxin-driven molecular interplay between these pathways.

Acknowledgement: This work is supported by the CSF project n.
CZ.02.1.01/0.0/0.0/16_019/0000738 (KR, KH).

WP1-02

Exploring the role of cytokinin-responsive *EXPANSINs* in the control of cell wall properties and development in *Arabidopsis* under stress.

Marketa Samalova[1], Kareem Elsayad[2] and Jan Hejatko[1]

[1] *Central European Institute of Technology (CEITEC MU), Masaryk University, Kamenice 5, Brno, Czech Republic*

[2] *Vienna Biocenter Core Facilities (VBCF), Dr. Gohr-Gasse 9, Vienna, Austria*

The cell wall (CW) is a fundamental constituent of plant cells that has the extraordinary ability to combine extreme tensile strength with extensibility. Studies of growth regulation suggests that turgor-driven cell expansion is the result of a delicate balance between wall relaxation and stiffening linked by a mechanosensing feedback loop. Phytohormones including cytokinins (CKs) are key players in growth regulation responses and are thus important determinants of plant architecture with potential impact on CW properties. CKs were demonstrated to control the equilibrium between cell division and cell differentiation (Dello Ioio *et al*., 2007, Curr Biol 17:1-5), however, the targets of CK-regulated cell differentiation are still elusive.

We hypothesize that CKs control cell differentiation via regulation of biomechanical properties of the CW. Using advanced genome-wide transcriptional profiling and looking for direct targets of CK signalling associated with the control of CW properties we identified *EXPANSINs* as potentially crucial players. Expansins were originally described as pH-dependent CW loosening agents (Cosgrove, 2015, Curr Opin Plant Biol 25:162-172) that become activated during CW acidification triggered by a number of stimuli, including hormones. We have identified a subset of CK-responsive members of the *EXP* subfamily A (*EXPA*) in *Arabidopsis thaliana* and determined their spatiotemporal expression patterns.

We aim to develop a biomechanical sensing model that describes the relationship between the expansins and modulation of biomechanical properties of the CW during cell growth and differentiation and to study its changes under biotic and abiotic stresses.

SoMoPro grant No. 665860 (MS), CZ.02.1.01/0.0/0.0/16_019/0000738 (JH).

WP1-03

[Title]

Jan Hejátko

Central European Institute of Technology (CEITEC MU), Masaryk University,

[Abstract - max 250 words]

WP1-04

EXO70B1 and B2 role in plant responses to biotic and abiotic stresses

Matěj Drs[1], Andrea Potocká[1], Tamara Pečenková[1], Jitka Ortmannnová[1], Peter Sabol[1,2], Viktor Žárský[1,2]

1 Laboratory of Cell Biology, Institute of Experimental Botany, Czech Academy of Sciences, Rozvojová 263, 165 02, Prague 6, Czech Republic

2 Laboratory of Cell Morphogenesis, Department of Experimental Plant Biology, Charles University in Prague, Faculty of Science, 128 44 Viničná 5, Prague 2, Czech Republic

One of our aims is to understand a role of a secretory vesicles tethering complex exocyst in the co-ordination of plant cell responses to simultaneous exposures to biotic and abiotic stresses. Our task is complicated by the fact that the complex consists of eight subunits, most of them being encoded by multigene families in land plants, implying existence of many possible versions of the complex. We focused on the two isoforms of the subunit EXO70, B1 and B2, found to be the most relevant in response to pathogen attack and environmental challenges (Pečenková *et al*., 2011, J Exp Bot 62: 2107-2116; Kulich *et al*., 2013, Traffic 14: 1155-1165). Using Arabidopsis knockout mutants in B1 and B2, we uncovered their roles in resistance toward *Blumeria graminis* and *Pseudomonas syringae* under different conditions. On the cellular level, we developed approaches to decipher previously described role of the two isoforms in stomata guard cells and newly also in root hairs development (Hong *et al*., 2016, New Phytol 209: 624-635; Seo *et al*., 2016, Plant Cell 28: 2952-2973). On the subcellular level, we are verifying if the role of these isoforms is connected to the trafficking of the probable cargo – a protein pathogenesis related 1 (PR1) and we are comparing these data with observations of the intracellular dynamics for different exocyst subunits tagged with GFP. Where possible we extended our analyses on other EXO70/exocyst subunits isoforms using also double and triple mutants in order to understand multifunctional nature of the land plant exocyst complex.

WP1-05

[Title]

Petr Klíma

Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany, the Academy of Sciences of the Czech Republic, Prague, Czech Republic

[Abstract - max 250 words]

[Acknowledgements - if any]

WP2-01

The effect of acclimation and Incyde application on hormonal dynamics during heat stress response

Sylva Přerostová[1], Petre I. Dobrev[1], Barbara Kramná[1], Alena Gaudinová[1], Vojtěch Knirsch[1], Lukáš Spíchal[2], Radomíra Vaňková[1]

[1]*Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany, Czech Academy of Science, Rozvojova 263, 165 00 Prague 6 - Lysolaje, Czech Republic*

[2]*Department of Chemical Biology and Genetics, Centre of the Region Hana for Biotechnological and Agricultural Research, Institute of Experimental Botany CAS and Faculty of Science of Palacky University, Slechtitelu 27, 783 71 Olomouc, Czech Republic*

The impact of heat acclimation (37°C, 1 h) on heat stress response (45°C, 3 h) was evaluated in Arabidopsis thaliana apices, leaves and roots at the levels of phytohormones [cytokinins, auxin, abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and ethylene precursor aminocyclopropane carboxylic acid (ACC)], activity of antioxidant enzymes (NADPH oxidases, superoxide dismutases, guaiacol peroxidases, catalases and ascorbate peroxidases) and electrolyte leakage. Heat acclimation diminished negative effects of heat stress, as indicated by preservation of FeSOD activity and low activity of NADPH oxidase isoenzymes (NOX1 – NOX3) as well as CAT1. The levels of ABA and JA were in apices lower than upon direct heat stress. Active cytokinins did not differ from control conditions and IAA was only slightly elevated. In roots, ABA and ACC contents were significantly elevated in comparison with direct heat stress. This may indicate higher stimulation of defence in the case of acclimated plants.

The effect of timing of cytokinin modulation was evaluated using inhibitor of cytokinin oxidase/dehydrogenase INCYDE-F. In combination with acclimation, INCYDE treatment had a slight positive effect on heat stress tolerance, especially when applied in the end of a short period at optimal temperature (20°C) between acclimation and heat stress. INCYDE treatment resulted after stress in elevation of indole-3-acetic acid in all tested organs and higher trans-zeatin and cis-zeatin content in roots; as well as in increase of the activity of ascorbate peroxidase isoenzymes APX3 and APX4. INCYDE application just before or after heat shock strengthened the negative effects of this stress.

Acknowledgement: The work was supported by the Ministry of Education, Youth and Sports of CR from European Regional Development Fund-Project "Centre for Experimental Plant Biology": No. CZ.02.1.01/0.0/0.0/16_019/0000738 and by MEYS CR program Inter-Excellence LTAUSA17081.

WP2-02

Gibberellins under a magnifying glass or find a needle in a haystack

DanušeTarkowská[1], Marek Szecówka[1] and Peter Hedden[1]

[1] Laboratory of Growth Regulators, Institute of Experimental Botany, Czech Academy of Sciences & Faculty of Science, Palacký University, CZ-783 71 Olomouc, Czech Republic

Many of our current concepts about intercellular communication in plants have been derived from similar studies in animals, in which two main systems evolved: the nervous system and endocrine system. Plants, lacking motility, never developed a nervous system, but they did evolve hormones as chemical messengers. Gibberellins (GAs) are part of a nine-member plant hormones family. They are actively involved in a control of plant development throughout its life cycle, promoting organ enlargement through enhanced cell elongation and/or division, and triggering developmental switches, such as germination or flowering. Like other classes of plant hormones, concentrations of GAs in plant tissues are generally extremely low (generally pg/mg FW). Thus, very sensitive analytical methods are required for their detection. However, levels of GAs may vary substantially even within a plant organ, being concentrated in the elongation zones. Vegetative tissues (stems, roots and leaves) typically contain several pg/mg FW of GAs, while reproductive organs (such as seeds and flowers) may show higher levels. Often there is also a requirement to successfully monitor changes in ultra trace amounts of bioactive plant GAs (GA_1 and GA_4) including their biosynthetic precursors and catabolites in target organs, *i.e.* a description of their distribution and how their levels change during development and in response to environmental stimuli. Our aim is therefore to respond to all these requests and develop sensitive targeted analytical approach for finding very tiny needles (GAs) in a huge haystack (extremely high levels of interfering matrix compounds typically present in plant extracts).

The Czech Science Foundation No. 18-10349S (DT, MS, PH) and the European Regional Developmental Fund Project "Centre for Experimental Plant Biology" No. CZ.02.1.01/0.0/0.0/16_019/0000738 (DT, PH).

WP2-03

Gibberellin signalling in response to abiotic stress in wheat

Klára Ptošková[1], Marek Szecówka[1], Stephen Thomas[2], Andrew Phillips[2], Bethany Wallace[2,3], Matthew Terry[3], Ondřej Novák[1], Iva Pavlović[1], DanušeTarkowská[1] and Peter Hedden[1,2]

[1]Laboratory of Growth Regulators, Czech Academy of Sciences, Institute of Experimental Botany & Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic
[2]Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom
[3]School of Biological Sciences, University of Southampton, Southampton, SO17 1BJ, United Kingdom

Wheat is one of the most important sources of nutrition for humanity globally, but yields can be severely limited by abiotic stress, particularly drought. This work is focused on the role of gibberellin (GA) signalling in the response of wheat seedlings to water limitation. The effect of water restriction on shoot and root growth and on the levels of stress factors and hormones in these organs was determined in soil-grown wheat seedlings. The expression level of genes involved in the GA-biosynthetic and signal transduction pathways was examined in well-watered and stressed leaves and roots by qRT-PCR and RNAseq. GA acts by promoting the degradation of the growth-suppressing DELLA proteins, which accumulate when GA concentrations are low, as can occur under stress conditions. They act by regulating gene expression via interaction with different transcription factors (TFs). Previous experiments indicated that the wheat DELLA protein RHT-1 interacted with PHYTOCHROME INTERACTING FACTOR-LIKE (PIL) TFs, which are involved in light-regulated gene expression. As the rice PIL1 was been shown to regulate stem height in response to drought, mutants of the wheat orthologue TaPIL1 and the closely related TaPIL3 were generated from a TILLING population to determine their response to drought. The interaction between RHT1 and PILs is being further tested by bimolecular fluorescence complementation. Future experiments will include determining the response of wheat mutants with altered GA signalling to water limitation.

We acknowledge funding from The Czech Science Foundation No. 18-10349S (DT, MS, PH) and the European Regional Developmental Fund Project "Centre for Experimental Plant Biology" No. CZ.02.1.01/0.0/0.0/16_019/0000738 (DT, PH)

WP2-04

Actin depolymerization increases plant resistance against pathogens via activation of salicylic acid signalling pathway

Hana Leontovyčová[1,2,4], Tetiana Kalachova[2], Lucie Trdá[2], Romana Pospíchalová[2], Lucie Lamparová[1,2], Petre Dobrev[3], Kateřina Malínská[3], Lenka Burketová[2], Olga Valentová[1], Martin Janda[1,2,5]

[1]Laboratory of Plant Biochemistry, Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague 6, Czech Republic; [2]Laboratory of Pathological Plant Physiology, Institute of Experimental Botany of The Czech Academy of Sciences, Rozvojová 263, 165 02 Prague 6, Czech Republic; [3]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany of The Czech Academy of Sciences, Rozvojová 263, 165 02, Prague 6, Czech Republic; [4]Department of Biochemistry, Faculty of Science, Charles University in Prague, Faculty of Science, 128 44 Hlavova 2030/8, Prague 2, Czech Republic; [5]Ludwig-Maximilians- University of Munich (LMU), Faculty of Biology, Biocenter, Department Genetics, Grosshaderner Str. 2-4, D-82152 Martinsried, Germany

The integrity of the actin cytoskeleton is essential for plant immune signalling. Consequently, it is generally assumed that actin disruption reduces plant resistance to pathogen attack. Here, we demonstrate that actin depolymerization induced a dramatic increase in salicylic acid (SA) levels in *Arabidopsis thaliana*. Transcriptomic analysis showed that the SA pathway was activated due to the action of isochorismate synthase (ICS). The effect was also confirmed in *Brassica napus*. This raises the question of whether actin depolymerization could, under particular conditions, lead to increased resistance to pathogens. Thus, we explored the effect of pretreatment with actin-depolymerizing drugs on the resistance of *Arabidopsis thaliana* to the bacterial pathogen *Pseudomonas syringae*, and on the resistance of an important crop *Brassica napus* to its natural fungal pathogen *Leptosphaeria maculans*. In both pathosystems, actin depolymerization activated the SA pathway, leading to increased plant resistance. To our best knowledge, we herein provide the first direct evidence that disruption of the actin cytoskeleton can actually lead to increased plant resistance to pathogens, and that SA is crucial to this process.

The work was supported from European Regional Development Fund-Project " Centre for Experimental Plant Biology " (No. CZ.02.1.01/0.0/0.0/16_019/0000738), a grant from Czech Science Foundation grant no. 17-05151S, GAUK no. 992416. IEB Imaging Facility is supported by OPPK CZ.2.16/3.1.00/21519 and MEYS LM2015062 from (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

WP2-05

Changes of lipid profile in nanoparticle-stressed plants

Petr Maršík, Kateřina Moťková, Tomáš Vaněk

Laboratory of Plant Biotechnologies, Institute of Experimental Botany, Czech Academy of Science, Rozvojova 263, 165 00 Prague 6 - Lysolaje, Czech Republic

Effect of nanoparticles on organisms is one of the important factors leading to their physiological and biochemical changes associated with stress response. Changes in lipid profile of hydroponically cultivated Arabidopsis thaliana plantlets was evaluated after treatment by silver (Ag) and zinc oxide (ZnO) nanoparticles using triple quadrupole tandem mass spectrometry (MS/MS). Five principal lipid classes including phosphatidic acids (PA), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG) and phosphatidylinositols (PI) were monitored. Lipids were measured by direct infusion using neutral lost (PE) and precursor ion analysis (PA, PC, PG, PI) in positive (PE, PI) as well as negative mode (PA, PC, PG). Particular compared lipids were classified according to their total number of carbons in the fatty acid chain:number of double bounds. The strongest effect on lipid profile was found in the plants treated by ZnO, which significantly induced amount of PC and PE, whereas other lipid groups were reduced or remained unchanged. Treatment with the Ag nanoparticles led only to moderate changes in content of PC, and particularly also in PI in comparison with untreated control.

WP2-06

Seed proteome and metabolome under changing environmental conditions

Hana Habánová[1], Miroslav Berka[1], Vratislav Psota[2], Břetislav Brzobohatý[1], Martin Černý[1]

[1]*Mendel University in Brno, Brno, CZ*

[1]*Research Institute of Brewing and Malting, Brno, CZ*

Plants are sessile organisms, and as such, their persistence and survival in the face of climate change predominantly depend on successful reproduction by seed. Key environmental factors affecting seed production are temperature and drought. Here, we employed proteomics and metabolomics analysis to evaluate seed composition of three barley cultivars collected from 12 different locations all over the Czech Republic. The correlation analysis of proteomics data, metabolomics profile and locality-specific data we collected (e.g. recorded temperatures and rainfalls), four contrasting samples were selected for detailed analysis. Our results confirm a significant impact of the environment on the seed quality, which was higher than cross-cultivar variations.

WP2-07

Pattern triggered immunity under heat stress - is there a place for FLOTs?

Jan Martinec

Institute of Experimental Botany ASCR, Czech Republic

The first part of the presentation will be focused on biotic stress responses triggered by flg22 after exposure to temporary heat stress (HS). In Arabidopsis, flg22 is recognized by the flagellin sensing 2 (FLS2) receptor. Transient early production of reactive oxygen species (ROS) is a well-characterized response to flg22 recognition. We demonstrate the strong reduction of flg22-induced ROS production in Arabidopsis after HS treatment. In addition, a decrease in FLS2 transcription and a decrease of the FLS2 presence at the plasma membrane after HS were shown. Subsequently, a decrease of the resistance of Arabidopsis to Pseudomonas syringae was detected after temporary HS.

In the second part of the presentation, characterisation of SPFH domain proteins Flotillins will be shown. Plant flotillins (FLOTs) are localized in membrane microdomains and involved in endocytosis. Upon activation, FLS2 is endocytosed and this process is an important part of flg22-triggered signalling. FLOT1 was shown to participate in the endocytosis of FLS2. We characterised AtFLOTs in the scope of microdomain formation, their membrane mobility and the linkage with the cell wall.

The work was supported from European Regional Development Fund-Project " Centre for Experimental Plant Biology " (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

WP2-08

Innovative, effective, robust, fast and cheap purification of phytohormones using dispersive solid phase extraction

Ivan Petřík[1,2], Anna Valníčková[1], Karin Ljung[3], Miroslav Strnad[1], Ondřej Novák[1,3]

[1] Laboratory of Growth Regulators, The Czech Academy of Sciences, Institute of Experimental Botany & Palacký University, Faculty of Science, Šlechtitelů 27, CZ-78371 Olomouc, Czech Republic
[2] Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 27, CZ-78371 Olomouc, Czech Republic
[3] Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 901 83 Umeå, Sweden

Cytokinins and auxins are naturally occurring plant growth regulators. They play an important role in controlling growth and developmental processes in plants. Similarly to other phytohormones, their concentrations in plant tissues are usually very low (pmol per gram of fresh weight). Therefore, their identification and quantification are based on highly sensitive analytical approaches, such as an ultra-high performance liquid chromatography coupled with a tandem mass spectrometry (LC-MS/MS). Moreover, sample preparation, especially removal of salts and isolation of analytes from a complex plant matrix, is the most critical procedure to achieve high quality data. For many years, the purification of cytokinins and auxins has been based on a well-established solid phase extraction (SPE). However, a dispersive SPE (dSPE) has been introduced a few years ago as an effective and robust approach for isolation of a wide range of analytes. In the contrast with conventional SPEs, this technique uses free solid phase particles dispersed in a liquid crude sample extract. We tested dSPE as an innovative, fast and cheap approach to purifying selected phytohormonal groups (cytokinins and auxins). Our work has been mainly focused on investigation of the main parameters contributing to the extraction efficiency compared to the conventional SPE technology.

WP2-09

Design of infectious viral clones based on the Tobamovirus genus and their use as the markers for detection of viral infection.

Kateřina Kratochvílová [1,2], Jakub Dušek [1, 3], Helena Plchová [1], Noemi Čeřovská [1], Zuzana Pobořilová [1], Tomáš Moravec [1]

[1] *Laboratory of Virology, Institute of Experimental Botany AS CR, v v.i., Prague, Czech Republic*

[2] *Department of Experimental Plant Biology, Charles University, Praque, Czech Republic*

[3] *Department of Crop protection, Czech University of Life Science, Prague, Czech Republic*

Plant viruses from the genus Tobamovirus infect a wide variety of plant species worldwide and cause substantial yield losses in agricultural crops. Viruses from this genus are non-enveloped rigid rods with helical symmetry and monopartite, linear, ssRNA (+) genome of about 6,5 kbp. Tobamoviruses are one of the best studied group of plant viruses and for their advantageous properties and simple structure are frequently used in biotechnology. To fully exploit the biotechnological potential of these viruses in the future, we need to know more about the course of infection in various plant species. One of the prerequisites is to have access to the simple and robust modular system for cloning of different infectious viral constructs.

The main purpose of this study was to create a simple and robust system for cloning of different infectious viral constructs based on Tobamovirus genus. Next, we want to follow the course of viral infection in different plants species.

Here we used Tobacco mosaic virus and Tobacco mosaic virus Cg8 for design of the constructs and as a cloning method we have chosen the Golden Braid convention. For the non-invasive detection of viral infection we used fluorescent markers cloned into the constructs. As the alternative method of viral detection and quantification we used simple viral RNA detection method that takes advantage of unique properties of thermostable DNA polymerase and reverse transcriptase RTX (reverse transcription xenopolymerase). We discuss strengths and weaknesses of both alternatives.

ACKNOWLEDGEMENT

We acknowledge the MEYS, CZ.02.1.01/0.0/0.0/16_019/0000738, the GAČR, GA15-10768S and the skilled technical assistance of J. Svobodová and L. Kolčabová.

WP2-10

Fungal infection modifies oilseed rape attraction to herbivores

Barbora Jindřichová[1], Nikoleta Rubil[1], Thure Pavlo Hauser[2], Lenka Burketová[1]

[1]Laboratory of Pathological Plant Physiology, Institute of Experimental Botany ASCR, Czech Republic

[2]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

In nature, plants are exposed to a wide range of pathogens and pests. Most studies published to date have been concerned with plant - pathogen or plant - arthropod interactions. Our study focuses on a combined interaction among plant, fungal infection and arthropod infestation.

Choice and non-choice tests were used for studying of preference a chewing insect (diamondback moth - *Plutella xylostella*) and a sucking insect (cabbage aphid - *Brevicoryne brassicae*) to oilseed rape (*Brassica napu*s) plants infected by the fungus *Leptosphaeria maculans*, an infectious agent of phoma stem canker. The mechanisms underlying insect preferences were analysed.

Our preceded research showed that resistance to *L. maculans* in *B. napus* is mediated by salicylic acid signalling in combination with ethylene. We ascribe this unusual hormone cooperation to a hemibiotrophic nature of *L. maculans*. Similar approach has been chosen to study *B. napus* interaction with the insects. Results clearly show that both insect species prefer the first true leaves to cotyledons and activate defence signalling in plants. While genes implicated in jasmonic acid and ethylene signalling were upregulated in plants infested by *P. xylostella*. This was confirmed also by hormone quantification in infested tissue.

Concurrently, the preinoculated oilseed rape plants with L. maculans were used in a choice test to demonstrate a time-dependent attraction of infected leaves to *P. xylostella*, which was the highest on the third day after inoculation. This effect disappeared during later stages of the infection. In case of *B. brassicea*, *L. maculans* infected leaves were less attractive compared to control leaves.

Acknowledgement: The work was supported from European Regional Development Fund-Project " Centre for Experimental Plant Biology " (No. CZ.02.1.01/0.0/0.0/16_019/0000738) and by a grant from Ministry of Education, Youth and Sports of the Czech Republic INTER-COST ELTC17013 and is solved in a framework of COST Action FA1405.

WP2-11

Volatile organic compounds (VOCs) of *A. thaliana* as a response to different stresses

Jan Rezek

Institute of Experimental Botany, Czech Academy of Sciences

[Abstract - max 250 words]

[Acknowledgements - if any]

WP3-01

Thermomorphogenesis during seed development In Arabidopsis and Brassica napus

Juan Francisco Sánchez López[1], Kateřina Mácová[1,2], Veronika Jedličková[1], Lenka Sochová[1], Lenka Pátková[1], Marie Štefková[1], Hélène Robert Boisivon[1#]

[1]Hormonal Crosstalk in Plant Development, CEITEC - Masaryk University, Brno, Czech Republic
[2]Laboratory Functional Genomics and Proteomics (FGP)-National Centre for Biomolecular Research (NCBR), Faculty of Science, Masaryk University, Brno, Czech Republic
[#]helene.robert.boisivon@ceitec.muni.cz

Seed production is an essential step of flowering plant life cycle. This step is the most sensitive to changes in ambient temperatures. In the past decades, consequently to the climate changes, the average annual ambient temperatures have arisen. High ambient temperatures shorten the life cycles of many crops, reducing their grain yield.Elevated temperatures during the growth of flowering plants limit seed production by impairing fertilization, embryo and seed development and grain filling. We determined the heat-induced changes at phenotypical level in flowering *Arabidopsis thaliana* and *Brassica napus*, an oilseed crop widely cultivated in Europe. In our high-temperature growth conditions, common features were observed in the two species, related to growth speed of the seeds and morphogenesis defects of the embryos. Auxin and cytokinin are two hormones involved in the regulation of plant development, including the correct development of seedpod, ovule, seed and embryo. We aim to dissect the temperature-induced responses in seeds mediated by the regulation of hormone homeostasis.

Funding

This work has received funding from Czech Science Foundation (19-05200S) and from European Regional Development Fund-Project "EXBIO" (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

WP3-02

Pollen germination during heat; Do HSP90s play a role?

Christos Michailidis, Zahra Aghcheh Kahrízí, Karel Raabe, David Honys

Laboratory of Pollen Biology, Institute of Experimental Botany CAS, Prague, Czech Republic

HSP90 proteins have been shown to be required for the promotion and maintenance of protein complexes that have critical roles in signal transduction, cellular trafficking, chromatin remodelling, cell growth and differentiation. In *A. thaliana*, seven members of the HSP90 family have been identified. Amongst them, four are found in the cytoplasm and nucleus (AtHsp90-1 to 90-4), and one in mitochondria (AtHsp90-6), ER (AtHsp90-7), and chloroplasts (AtHsp90-5). Among the AtHSP90s, AtHsp90-1 is stress-inducible and shares comparatively low sequence identity with the constitutively expressed AtHsp90-2 to -4. Sequence identities between cytosolic and other subcellular localized AtHSP90s are at an average of 50%. Application of heat stress on flowering *A. thaliana* plants identified severe loss of fertilization ability on buds harboring unicellular to bicellular stage pollen. Analysis of the HSP90 family T-DNA lines identified a 25% pollen defect at the mature pollen stage for the AtHsp90-1 and AtHsp90-5 lines. To dissect the role of the HSP90 members in pollen development, RNAi was utilized to downregulate the cytoplasmic HSP90 members. The RNAi cassette was spatiotemporally regulated to act upon different stages of pollen development. Overexpression lines for the HSP90-1 and HSP90-5 members with a C- terminal fluorophore fusion were also analysed. These lines, driven by the Lat52 promoter, active during mature pollen stage and throughout the progamic phase of pollen development were utilized for analysing the role of these specific HSP90 members in pollen tube germination and growth.

The work was supported from European Regional Development Fund-Project "Centre for Experimental Plant Biology" (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

WP3-03

[Title]

Jan Petrášek

Institute of Experimental Botany, Czech Academy of Sciences

[Abstract - max 250 words]

[Acknowledgements - if any]

WP3-04

CEITEC Proteomics for CEBR

Zbyněk Zdráhal

Central European Institute of Technology (CEITEC MU), Masaryk University

[Abstract - max 250 words]

[Acknowledgements - if any]

WP4-01

Three transcriptomic compartments in *Silene vulgaris* flower buds are affected to various extent by cytoplasmic male sterility

Helena Štorchová

Plant Reproduction Lab

Institute of Experimental Botany AS CR

We analyzed three transcriptomic compartments (mitochondrial, plastid and cytoplasmic) of flower buds of the gynodioecious plant Silene vulgaris. We compared two genders – females and hermaphrodites. Male sterile females are produced owing to the action of the mitochondrial gene, which expression leads to pollen abortion as a result of mitochondrial dysfunction in the course of pollen development (cytoplasmic male sterility – CMS). The comparison of mitochondrial transcriptomes between both genders identified the differentially expressed (DE) candidate CMS genes. The plastid transcriptomes were identical between females and hermaphrodites, showing just marginal differences among haplotypes. In sharp contrast with organellar transcriptomes, the comparison of nuclear-encoded cytoplasmic transcriptomes revealed high numbers of DE genes between the two genders. They were involved in fatty acid metabolism, respiration, anther development, programmed cell death etc. We found distinctions in the sets of DE genes between two haplotypes with different CMS genes, which suggests the existence of different pathways leading to pollen abortion.

Mitochondria play an important role in plant response to abiotic stress. We aim to analyze mitochondrial function from transcriptomic perspective under elevated temperature. We will follow plant phenotype, pollen viability, and all three transcriptomic compartments under control and elevated temperature, using the methods and results obtained under ambient temperature described above. We have already optimized the conditions suitable to perform heat stress experiments in Silene vulgaris. This plant model provides a unique opportunity to study a complex interaction: genotype (mitochondrial –CMS gene) x genotype (nuclear genes – proteins targeted to mitochondria, factors restoring fertility) x environment (temperature).

WP4-02

Development of transcriptome analysis pipeline in non model plants

Miloslav Juříček

Institute of Experimental Botany AS CR

[Abstract - max 250 words]

[Acknowledgements - if any]

List of participants

Arnaud Dominique

Berka Miroslav

Boisivon Helene Robert ***WP3-01***

Brzobohatý Břetislav

Burketová Lenka ***WP2-04***

Černý Martin ***WP2-06***

Doležal Karel

Dušek Jakub

Hedden Peter ***WP2-03***

Hejátko Jan ***WP1-03***

Hluska Tomáš

Hlusková Lucia

Honys David ***WP3-02***

Jindřichová Barbora ***WP2-10***

Juříček Miloslav ***WP4-02***

Kertbundit Sunee

Klíma Petr ***WP1-05***

Knirsch Vojtěch

Kopková Jitka

Kratochvílová Kateřina

Krčková Zuzana

Luklová Markéta

Maršík Petr ***WP2-05***

Martinec Jan ***WP2-07***

Moravec Tomáš ***WP-09***

Müller Karel

Novák Ondřej

Pečenková Tamara ***WP1-04***

Petrášek Jan ***WP3-03***

Petřík Ivan ***WP2-08***

Retzer Katarzyna ***WP1-01***

Rezek Jan ***WP2-11***

Strnad Miroslav

Šámalová Markéta ***WP1-02***

Štorchová Helena ***WP4-01***

Tarkowská Danuše ***WP2-02***

Tyč Dimitrij

Vágner Martin

Vaňková Radomíra ***WP2-01***

Wiese Anna Johanna

Zdráhal Zbyněk ***WP3-04***

The work was supported from European Regional Development Fund-Project "Centre for Experimental Plant Biology" (No. CZ.02.1.01/0.0/0.0/16_019/0000738).

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research,
Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS