



Content

Introduction	3
Institute Representatives	4
Supervisory Board	4
Board of IEB	4
Organizational Structure of the Institute	5
Buildings of IEB	6
Centre of Plant Structural and Functional Genomics	8
Imaging Facility	12
Isotope Laboratory	14
Laboratory of Biologically Active Compounds	16
Laboratory of Cell Biology	20
Laboratory of Growth Regulators	24
Laboratory of Hormonal Regulations in Plants	30
Laboratory of Mass Spectrometry	36
Laboratory of Pathological Plant Physiology	38
Laboratory of Plant Biotechnologies	42
Laboratory of Plant Reproduction	48
Laboratory of Pollen Biology	52
Laboratory of Signal Transduction	58
Laboratory of Virology	60
Station of Apple Breeding for Disease Resistance	62
Research Projects 2015–2017	68
Publications 2015–2017	72
Patents 2015–2017	92
Apple Varieties 2015–2017	94
Science Outreach	96
Journals	98



Discovering
the World
of Plants

Introduction

The 2015–2017 Research Report of the Institute of Experimental Botany (IEB) provides the overview of our mission, activities, and outcomes achieved in three past years. I am proud to say that IEB employees made these years at the IEB very prolific and successful.

Based on the excellent results of our institute's international evaluation, the years 2015–2017 brought a positive change to the financing of the IEB. Following five years of palpable funding cuts, which resulted in a progressive decrease in founder subsidy, 2017 saw the pattern reverse with our non-competitive subsidy, in absolute numbers, almost matching that of 2009. Out of the total funding of the IEB, more than two thirds of our budget depends on grant systems, competitive resources, and our income. Our scientists' time-consuming work of preparing grant proposals has paid off in the past three years: recent and ongoing grants and projects are all listed in Research Report.

The most important measure of scientific work on the plane of fundamental research are publications in high-impact scientific journals. In this regard, it is gratifying that researchers of IEB authored and co-authored more than 460 papers in journals with a impact factor during the years 2015–2017. The number of papers published is unprecedented for the IEB, and the following pages contain short descriptions of most of these manuscripts. The increase in quantity of published papers was also matched with a qualitative improvement: about a third of our papers appeared in journals belonging to the first AIS quartile.

Even though fundamental research is our priority, an important part of this research brings applied

results. Between 2015 and 2017, researchers got thirteen new patents in Czech Republic, Canada, Korea and E.U. The Station of Apple Breeding for Disease Resistance cultivates scab-resistant apple trees. The Station registered the breeding rights to newly bred apple varieties resistant to scab twenty-four times. The IEB sells licenses to grow these varieties all over the world, and revenue from these licenses is an important component of our budget. Based on this licensing, almost five million trees have been sold during 2015–2017.

For more than half a century, the IEB has also published its own academic journals. I am proud that *Biologia Plantarum* and *Photosynthetica* are rated among the top scientific journals in the Czech Republic. Both are indexed in the WOS database.

Few institutes of the Czech Academy of Sciences have fulfilled the Academy's motto – “quality research for public good” – as impeccably as has the IEB. The team of Prof. Jaroslav Doležel coordinates the program Foods for the Future, which is part of the larger project Strategy 21 of the Czech Academy of Sciences.

The IEB also widely collaborates with universities, both through our participation in common projects and the education of students. A number of our researchers actively teach university students: the Joint Laboratory of Growth Regulators is excellent example of close cooperation between the IEB and Palacký University in Olomouc.

After completing two building projects in 2012, 2015–2017 saw the IEB build a storage hall at the Centre of Functional Genomics in Olomouc and reconstruct older building and build a new glasshouse and

hall for apple storage with controlled atmosphere in Střížovice (Station of Apple Breeding).

I wish to thank all of my colleagues at the IEB, who are responsible for the excellent work enumerated above and detailed in the pages of this Research Report. The success of this institute stems from their knowledge, hard work, collegiality, and enthusiasm.

Martin Vágner, director of IEB



Photo: Dylan Lowe



Institute Representatives

DIRECTOR:

RNDr. Martin Vágner, CSc.

DEPUTY DIRECTOR:

RNDr. Jan Martinec, CSc.

Supervisory Board

Chairman:

prof. RNDr. Jan Zima, DrSc. – IVB CAS, Brno

Deputy Chairman:

Ing. Jiří Malbeck, CSc. – IEB CAS

Members:

Ing. Pavel Kriegsman – KM Ltd., Prague (till April 30, 2017)

JUDr. Miloš Kvasnička – Prague (till April 30, 2017)

prof. RNDr. Jana Albrechtová, Ph.D. – FS CU, Prague (since May 1, 2017)

Ing. Petr Hejl – mayor of the municipal district Prague-Suchdol (since May 1, 2017)

Ing. Jan Škoda – IBT CAS, Prague

Secretary:

Ing. Alena Trávníčková – IEB CAS

Abbreviations

Institutions of the Czech Academy of Sciences (CAS):

IBT – Institute of Biotechnology

IEB – Institute of Experimental Botany

IVB – Institute of Vertebrate Biology

Others:

CRI, Prague – Crop Research Institute, Prague

FS CU, Prague – Faculty of Science, Charles University, Prague

MENDELU, Brno – Mendel University, Brno

RIFC Ltd., Troubsko – Research Institute for Fodder Crops Ltd., Troubsko

UCT, Prague – University of Chemistry and Technology, Prague

Board of IEB

Chairman:

RNDr. Radomíra Vaňková, CSc. – IEB CAS

Deputy Chairman:

prof. Ing. Miroslav Strnad, DrSc. – IEB CAS

Members (till January 19, 2017):

prof. RNDr. Břetislav Brzobohatý, CSc. – MENDELU, Brno

doc. Ing. Lenka Burketová, CSc. – IEB CAS

doc. RNDr. David Honys, Ph.D. – IEB CAS

Mgr. Jan Lipavský, CSc. – CRI, Prague

RNDr. Jan Nedělník, CSc. – RIFC Ltd., Troubsko

Mgr. Lukáš Spíchal, Ph.D. – IEB CAS

RNDr. Martin Vágner, CSc. – IEB CAS

prof. RNDr. Olga Valentová, CSc. – UCT, Prague

prof. Ing. Zdeněk Wimmer, DrSc. – IEB CAS

Members (since January 20, 2017):

prof. RNDr. Břetislav Brzobohatý, CSc. – MENDELU, Brno

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Mgr. Jan Lipavský, CSc. – CRI, Prague

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Ing. Václav Motyka, CSc. – IEB CAS

Mgr. Tomáš Moravec, Ph.D. – IEB CAS

RNDr. Jan Nedělník, CSc. – RIFC Ltd., Troubsko

RNDr. Martin Vágner, CSc. – IEB CAS

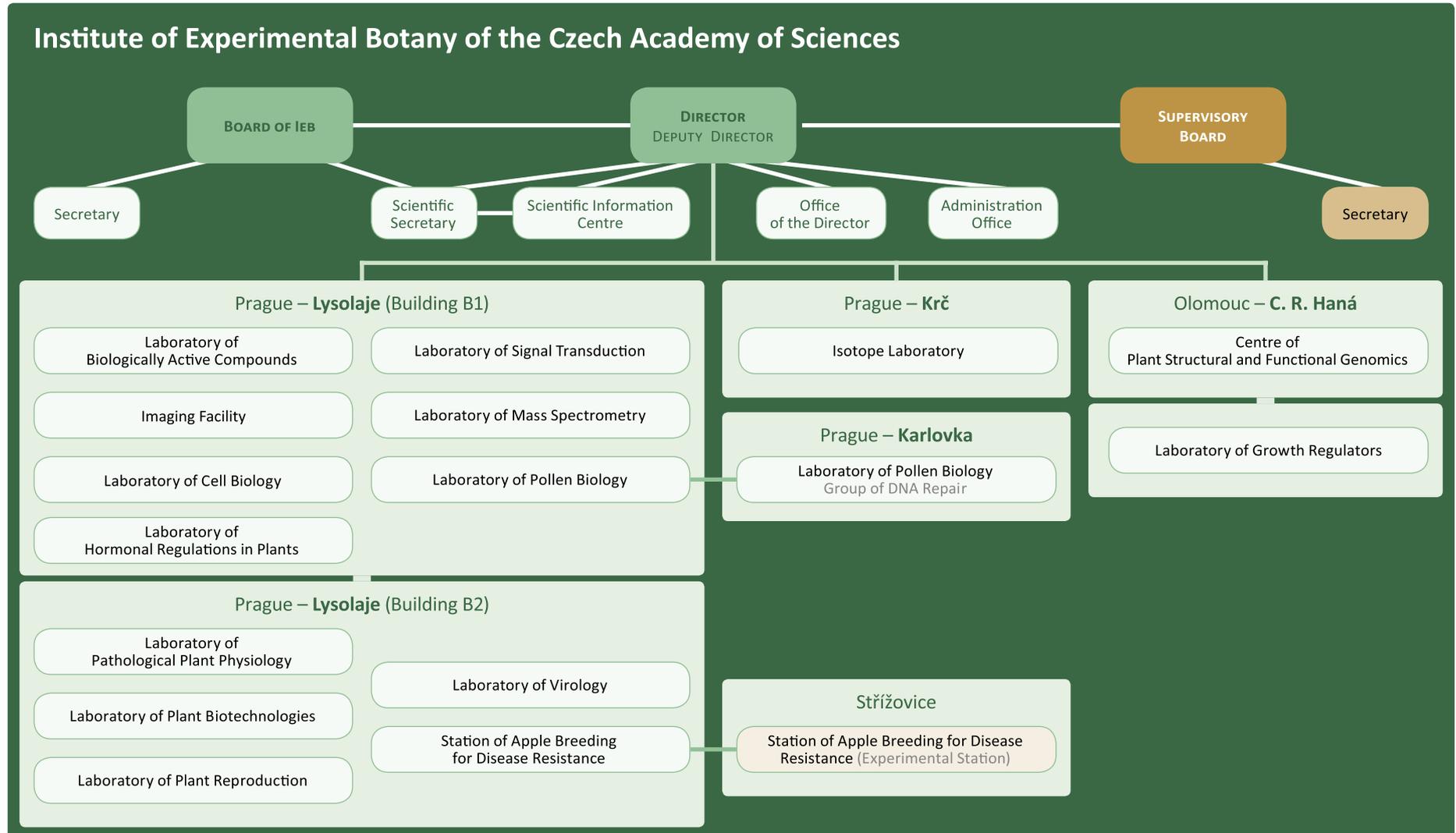
prof. RNDr. Olga Valentová, CSc. – UCT, Prague

Secretary:

Dr.rer.nat. Ing. Helena Plchová – IEB CAS



Organizational Structure of the Institute





Buildings of IEB



Building B1: Rozvojeová 263, Prague 6 – Lysolaje (7 laboratories)



Building B2: Rozvojeová 313, Prague 6 – Lysolaje (5 laboratories)



C. R. Haná: Šlechtitelů 31, Olomouc (Centre of Plant Structural and Functional Genomics)



C. R. Haná: Šlechtitelů 31, Olomouc (Centre of Plant Structural and Functional Genomics)



C. R. Haná: Šlechtitelů 27, Olomouc (Laboratory of Growth Regulators)



Střížovice, Pěnčín u Liberce (Station of Apple Breeding for Disease Resistance)

Others:

Vídeňská 1083, Prague 4 – Krč
(Isotope Laboratory)

Na Karlovce 1a, Prague 6 – Dejvice
(Laboratory of Pollen Biology, Group of DNA Repair)



Centre of Plant Structural and Functional Genomics

Head of laboratory:

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Our research focuses on the molecular organization, evolution and function of plant genomes. The emphasis has been on crop plant species with polyploid genomes and, in particular, those originating from interspecific hybrids. We are also active in characterizing genetic diversity at the DNA level to support its conservation and use. We further aim to produce novel data and biological resources to support the use of molecular and genomic tools in plant breeding. Most of our research involves three groups of plants: (a) cereals of the tribe Triticeae (wheat, barley, rye and their relatives); (b) forage grasses (fescue, ryegrass and their hybrids); and (c) banana. Our team has extensive experience in a broad range of cytogenetic, flow cytometric, molecular biology and genomic methods that permit multidisciplinary and original experimental approaches. For these reasons, our team has been a popular partner in international projects and has also been working on species outside the above three groups of plants.



In the picture (from the left):

Front row: Anna Nowicka, Ph.D. / postdoctoral fellow, Fen Yang / Ph.D. student, István Molnár, Ph.D. / researcher, Mgr. Iva Ilíková, Ph.D. / postdoctoral fellow, Mgr. Miroslav Valárik, Ph.D. / researcher, Mahmoud Said, Ph.D. / postdoctoral fellow, Pranav Sahu, Ph.D. / postdoctoral fellow, Bc. Jitka Weiserová / technician, Helena Tvardíková / technician.

Second row: Ing. Radoslava Kvasničková / project manager, Mgr. Veronika Kapustová / Ph.D. student, Mgr. Kateřina Perničková / Ph.D. student, Mgr. Helena Toegelová, Ph.D. / postdoctoral fellow, Mgr. Jana Čížková, Ph.D. / postdoctoral fellow, Dr.habil. Aleš Pečinka / researcher, Mgr. Jana Zwyrtková / Ph.D. student, Mgr. Miroslava Karafiátová, Ph.D. / postdoctoral fellow, Mgr. Veronika Burešová, Ph.D. / postdoctoral fellow, Mgr. Martina Bednářová / Ph.D. student, Zdeňka Dubská / technician, Bc. Romana Šperková / technician, Radomíra Tušková / technician, Prof. Ing. Jaroslav Doležel, DrSc. / head of the centre.

Third row: Ing. Beáta Petrovská, Ph.D. / postdoctoral fellow, Mgr. Denisa Šimoníková / Ph.D. student, Mgr. Eva Janáková / Ph.D. student, Mgr. Eva Hříbová, Ph.D. / researcher, Mgr. Kateřina Holušová, Ph.D. / postdoctoral fellow, Mgr. Hana Jeřábková, Ph.D. / postdoctoral fellow, Mgr. Jan Bartoš, Ph.D. / researcher, Ing. Hana Šimková, CSc. / researcher, Ludmila Švubová / secretary, Ing. Petr Navrátil / technical assistant, Mgr. Jan Vrána, Ph.D. / researcher, Nicolas Blavet, Ph.D. / postdoctoral fellow, Eva Jahnová / technician, Ing. Marie Seifertová / technician.

Top row: RNDr. David Kopecký, Ph.D. / researcher, Mgr. Marek Glombík / Ph.D. student, Mgr. Radim Svačina / Ph.D. student, Mgr. Tomáš Beseda / Ph.D. student, Ing. Zbyněk Milec, Ph.D. / postdoctoral fellow, Mgr. Petr Cápál, Ph.D. / postdoctoral fellow, RNDr. Jan Šafař, Ph.D. / researcher.

Not in the picture:

RNDr. Roman Hobza, Ph.D. / researcher, Mgr. Pavla Christelová, Ph.D. / postdoctoral fellow, Kashif Nawaz, Mgr. Alžběta Němečková, Mgr. Zuzana Tulpová / Ph.D. students.

Genome analysis and gene cloning in cereals

The analysis of many plant genomes is complicated by their enormous size and sequence redundancy owing to high DNA repeat content and the presence of homoeologous genomes in polyploids. To overcome these difficulties, we have pioneered chromosome-centric approaches which rely on dissection of nuclear genomes to chromosomes by flow cytometric sorting. In line with the strategy of the International Wheat Genome Sequencing Consortium, chromo-

somal DNA served as the main resource to sequence the bread wheat genome using the clone-by-clone approach. As a part of this effort, several chromosomal physical maps have been constructed [2, 11, 63, 344] and new methods were developed for their anchoring [26], validation [275, 307] and sequencing [291]. In barley, DNA prepared from individual chromosome arms was used to assign 15,622 gene-bearing BAC clones to particular chromosomes [86]. The chromosomal approach was applied successfully to

explore the secondary and tertiary gene pools of bread wheat. Particular chromosomes were sequenced in wild wheat relatives and ancestors such as *Aegilops* spp. [1, 84, 135, 234], *Triticum dicoccoides* [3] and *Haynaldia villosa* [457]. The results revealed syntenic relationships between these species, shed light on the evolution of bread wheat and provided opportunities to develop genomic resources from wild germplasm to facilitate wheat improvement [204].

Flow sorting of a chromosome bearing a gene of interest enables focused analysis, reduces cost and provides advantages in positional cloning projects in wheat and barley, targeting genes underlying resistance to the Russian wheat aphid [125], stem rust [482], and pre-harvest sprouting [266, 424]. While these projects employed the traditional clone-by-clone approach, cloning powdery mildew resistance gene [262] relied on a new approach we have developed in collaboration with Prof. Beat Keller (University of Zürich) and Dr. Brande Wulff (John Innes Centre, Norwich). The approach called MutChromSeq permits identification of induced causal mutations without the need for positional fine mapping. This dramatically reduces the time needed for gene cloning and enables isolation of genes located in non-recombining genomic regions. The second approach that facilitates gene cloning in species with complex genomes called TACCA (targeted chromosome-based cloning *via* long-range assembly), combines chromosome flow sorting with Dovetail Genomics Chicago long-range linkage and was developed in collaboration with Dr. Simon Krattinger (University of Zürich). Its application to wheat resulted in high quality de-novo assembly of chromosome 2D and permitted cloning broad-spectrum leaf-rust resistance gene Lr22 in only four months [438].

When a chromosome of interest cannot be discriminated and sorted from a standard karyotype, we have

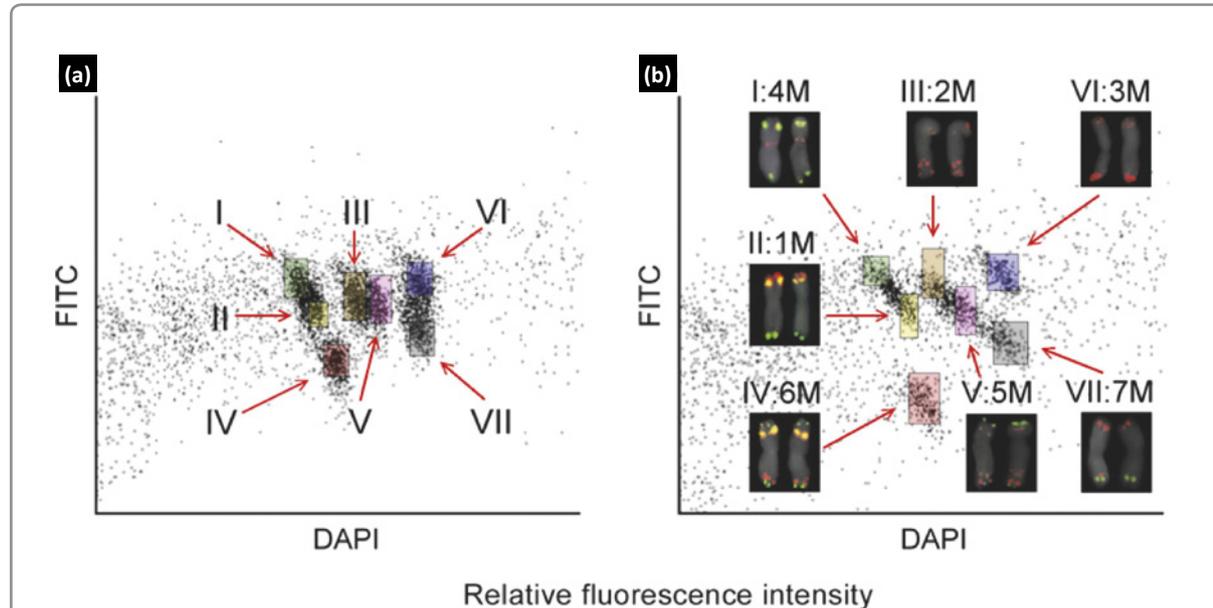


Figure 1: Flow cytometric chromosome analysis and sorting in *Aegilops*. Bivariate flow karyotypes obtained after the analysis of chromosomes isolated from (a) *Ae. speltoides* (S genome) and (b) *Ae. markgrafii* (C genome). Prior to the analysis, GAA and ACG microsatellites were labeled by fluorescence *in situ* hybridization (FISH) in suspension with FITC-labelled probes and chromosomal DNA was stained by DAPI. This approach resolved all S-genome and C-genome chromosomes, which could be flow-sorted at purities of 84–99 % and 80–97 %, respectively. Chromosomes were assigned to the colored regions by FISH using probes for 18S rDNA (yellow), Afa family repeat (red) and pSc119.2 repeat (green). Chromosomes were counterstained by DAPI (grey). Modified from [234].

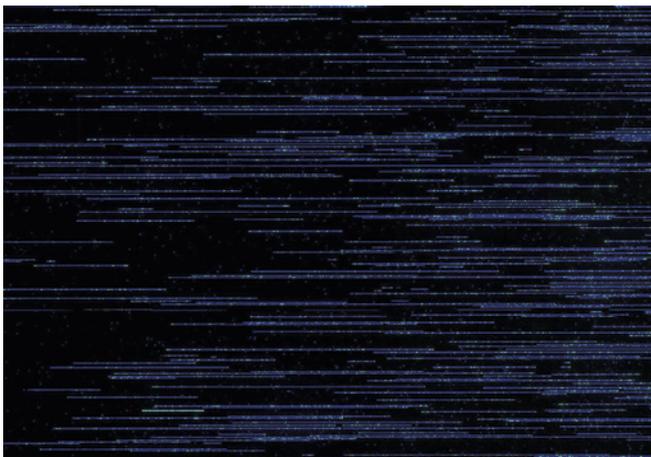


Figure 2: Optical mapping of the short arm of bread wheat chromosome 7D (7DS). Over one million copies of 7DS chromosome arm were isolated by flow cytometry and used to prepare high molecular weight DNA. Molecules of hundreds kilobases in length were then fluorescently labelled at sites cut by Nt.BspQI nicking endonuclease (green colour), counterstained with DAPI (blue colour) and analysed on Irys instrument (BioNano Genomics). Single-molecule data were aligned to construct *de novo* consensus optical map of the chromosome arm. The figure shows a subset of linearized DNA molecules captured in nanochannels of Irys chip during the analysis. Modified from [275].

developed a novel solution [22]. Single chromosomes are flow sorted one-by-one and the minute amounts of their DNA are amplified *via* multiple-displacement amplification. This results in microgram quantities of chromosome-specific DNA, which is suitable for various applications, including next-generation se-

quencing. In a pilot study [22], chromosome-derived sequences allowed identification of missing genic sequences in pseudomolecule of wheat chromosome 3B. This approach was then used to identify chromosomes with a transgene insertion in three different transgenic lines of wheat, providing a stepping stone for analysing transgene function [172]. The portfolio of applications of flow sorted chromosomes was further expanded by demonstrating their suitability for optical mapping [275]. Chromosomal optical maps are a powerful tool to validate and improve chromosomal sequence assemblies and study tandemly organized DNA repeats located on particular chromosomes.

Genetic diversity of cereal fungal pathogens

Apart from understanding crop genomes, detailed knowledge of pathogen genomes is a prerequisite to understanding plant-pathogen interactions. Such knowledge may open doors to fast and efficient development of resistant crops for sustainable agriculture. The fungus *Blumeria graminis* is a causal agent of powdery mildew disease in cereals with an interesting life cycle. In one season it multiplies asexually in great numbers and although its spores are short living, it can cause pandemics with significant yield losses. In winter, the fungus survives as sexually derived structures which ensure recombination and high genetic variability in the next season. We have developed a panel of molecular markers for molecular characterization of *B. graminis* f. sp. *hordei* (*Bgh*) and characterized the complex *Bgh* population of Central Europe and compared it with an Australian population. The isolates were also placed into a global context using isolates collected around the world. The analysis revealed a high level of diversity of Central European populations comparable to the rest of the world. This contrasts with the limited diversity of the Australian population [214].

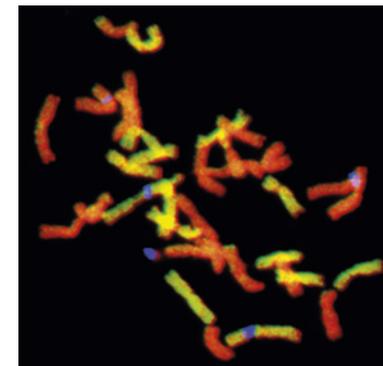


Figure 3: Genomic constitution of a pentaploid hybrid of forage grasses *Festuca apennina* ($2n=4x=28$) and *F. arundinacea* ($2n=6x=42$). Genomic *in situ* hybridization (GISH) was done with fluorescently labelled probes for genomic DNA of *F. pratensis* (green colour) and 45S rDNA (blue pseudocolour), and unlabelled genomic DNA of *F. glaucescens*. Chromosomes were counterstained with DAPI (red pseudocolour). Genomics probe from *F. pratensis* highlighted 14 chromosomes representing two sets of *F. pratensis* chromosomes (one originating from *F. apennina* and the other from *F. arundinacea*). The remaining 21 chromosomes remained unlabelled (one set from the second subgenome of *F. apennina* and two sets representing subgenomes of *F. glaucescens* present in *F. arundinacea*). Modified from [480].

Genome analysis in forage and amenity grasses

Intergeneric hybrids between species from fescue (*Festuca*) and ryegrass (*Lolium*) genera of grasses, referred to as Festuloliums, combine beneficial agronomic traits from both genera and are widely used for agricultural and amenity purposes. With the aim to identify SNP (single nucleotide polymorphism) markers suitable for high-throughput characterization of genomic constitution of Festulolium hybrids, we sequenced transcriptomes of meadow fescue and Italian ryegrass. This work resulted in identification of over 25,000 SNPs



which distinguish *F. pratensis* from *L. multiflorum*, and over 15,000 SNPs which discriminate *F. pratensis* from both Italian and perennial ryegrass. The new markers will enable characterization of genomic composition and gene expression analysis in prospective *Festuca* × *Lolium* hybrids [278]. We have also focused on identification of wild relatives of cultivated grasses which could be used for introgression of agriculturally important traits to elite cultivars. We have established a collection of three tetraploid fescues (*F. mairei*, *F. apennina* and *F. glaucescens*) and genotyped them using DArT (Diversity Arrays Technology) markers and GISH (genomic *in situ* hybridization). Surprisingly, many *F. apennina* accessions were found to be triploid and using GISH we revealed they originate from hybridization between tetraploid *F. apennina* and diploid *F. pratensis*. They display high fitness and predominate in many locations, presumably due to rhizome formation. For this reason, they can be highly valuable for plant breeding [480]. Moreover, all three tetraploid fescues exhibit diploid-like chromosome pairing. This behaviour prevents formation of multivalents and homoeologous recombination in meiosis and the discovery of its nature could help in stabilizing genomes of new Festuloliums.

Banana (*Musa* spp.) genetic diversity and genome organization

Bananas are a staple food for millions in the humid tropics. Their production is threatened by rapid spread of various diseases and pests, and by adverse environmental conditions. One way to breed improved cultivars is to utilize natural genetic diversity. Our team serves as a global *Musa* Genotyping Centre and as part of this mandate we have been genotyping the world's largest banana germplasm collection maintained at the Bioversity International Transit Centre (ITC) in Leuven

(Belgium). Within the framework of this project we examined nuclear DNA content and genomic distribution of ribosomal DNA in diploid accessions added recently to ITC [29]. The accessions were also genotyped using a set of microsatellite (SSR) markers and the results have expanded the number of wild *Musa* species where nuclear genome size and the genomic distribution of rDNA loci are known. SSR genotyping identified *Musa* species closely related to previously characterized ITC accessions and provided data to aid in their classification [350]. In a similar work, DArT markers were used to characterize *Musa* diversity and elucidate centres of banana domestication [264]. The joint effort of Bioversity International and our team has resulted in new prospects for the utilization of genome-wide association study for banana improvement [264]. In order to facilitate the use of molecular and genomics tools in banana improvement programs and to contribute to understanding *Musa* evolution, we have contributed to the production of an improved version of banana reference genome sequence [231] and evaluated genomic prediction models in a multiploidy training population [392].

Nuclear proteome

Nuclear proteins are a vital component of eukaryotic cell nuclei and have a profound effect on the way the genetic information is stored, expressed, replicated, repaired and transmitted to daughter cells and progeny. While plant genomes are becoming well understood at the DNA level, there remains a dearth of information on plant nuclear proteins with the possible exception of histones and a few other proteins. The lack of knowledge hampers efforts to understand how the plant genome is organized in the nucleus and how it functions. Characterization of nuclear proteins requires extraction methods which minimize their alteration

and reduce the extent of contamination with non-nuclear proteins. Conventional multi-step fractionation procedures are laborious and prone to contamination. To overcome these difficulties, we have established a novel protocol combining flow cytometric sorting of cell nuclei with protein/peptide separation and mass spectrometric analysis [104, 491]. The method allows separate analysis of G1, S and G2 phase nuclei and minimizes the risk of contamination by non-nuclear proteins. Its application has facilitated identification of the highest number of nuclear proteins so far in plants. The proteins are deposited in a dedicated database UNcleProt [314], which we have created and which will provide an important resource for studying the functional organization of plant genomes in interphase nuclei.

Research projects: 1, 5, 13, 16, 20, 24, 38, 42, 47, 48, 53, 55, 56, 62, 63, 65, 67, 69, 83, 96, 100, 115



Imaging Facility

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The imaging facility of the institute provides high-end instrumentation and expertise for demanding tasks in the field of confocal microscopy, structured illumination and transmission electron microscopy. In 2015–2017, the facility served in a number of projects performed in laboratories of the IEB CAS, including studies of membrane trafficking, cytoskeleton dynamics, hormonal regulations, involvement of membrane lipids in signalling pathways, the reaction of plants to pathogen attack and the dynamics of cell wall biosynthesis. In addition, it also offered microscopy services for external users from the Czech Republic and abroad.

The imaging facility was officially classed as an independent unit within the organization structure of IEB CAS at the end of 2015. It offers the instrumentation and expertise for advanced fluorescence microscopy and routine transmission electron microscopy.

The confocal laser scanning microscopy (CLSM) equipment consists of two CLSM inverted microscopes Zeiss LSM 880 (**Fig. 1a**) and Zeiss LSM 5 Duo. Together, these instruments offer a whole spectrum of techniques for fast and sensitive spectral detection of fluorescence signals, including photo-manipulation techniques FRAP (**Fig. 2b**) and FRET and correlation



In the picture (from the left):

Ing. Kateřina Malínská, Ph.D. / researcher,
RNDr. Jan Petrášek, Ph.D. / head of the facility.

Not in the picture:

Mgr. Tomáš Moravec, Ph.D. / researcher.

spectroscopy techniques FCS and RICS. The recent introduction of the Airy Scan detector for LSM 880 further improved the spatial resolution of CLSM imaging. Methods of non-invasive *in vivo* fluorescence microscopy in high time and spatial resolution (**Fig. 2a**) are performed on the inverted spinning disk confocal microscope Nikon Eclipse Ti-E with Yokogawa CSU-X1 (**Fig. 1b**), equipped with Andor EM CCD iXon3 897 ultrasensitive camera and sCMOS camera Zyla 4.0 for imaging in higher resolution. Finally, the upright fluorescence microscope Zeiss AxioImager Z2 with Apotome 2 structured illumination unit serves for more routine analyses and allows, among others, for automated imaging of large objects. The light imaging facility is further equipped with an automated station for *in situ* hybridization and immunohistochemistry on whole mounts and sections Intavis InSituPro VS_i, system of microscopic perfusion chambers with peristaltic pump and heating insert for microscopy stages. In contrast to light microscopy, where the imaging unit is on the frontiers of current technical development, our transmission electron microscope FEI Morgagni serves primarily for routine applications.

Between 2015–2017, the microscopes of the Imaging Facility were used every year by more than 50 independent users (researchers, masters and PhD students), from both IEB CAS laboratories and external locations, including foreign research institutions. The imaging facility is a partner of the project in the framework of the “National Infrastructure for Biological and Medical Imaging” (Czech-Biolmaging, CzBi; www.czech-bioimaging.cz), which has been approved for funding by the Ministry of Education, Youth and Sports for the period 2016–2019 [project 99]. Through this project, the imaging facility of IEB CAS is integrated within Prague’s node into the large pan-European research infrastructure Euro-Biolmaging (www.eurobioimaging.eu). On the European level, the Czech-Bioimaging has been evaluated positively and is thus validated for future applications within the ESFRI (European Strategy Forum on Research Infrastructures) network. From 2017, the unit has also taken part in the project “Modernization and support of research activities of the National Infrastructure for Biological and Medical Imaging”.

A plethora of original contributions were published by local and external users between 2015–2017, including among others, reports on cytoskeletal dynamics, the role of integral and peripheral plasma membrane proteins, plasma membrane associated-tethering complexes, lipid dynamics, endomembrane dynamics, cell wall biogenesis and reaction to pathogens [55, 66, 168, 197, 225, 259, 270, 276, 318, 347, 400–402, 408, 415, 417, 420, 423, 428, 432, 450; Havelková et al. 2018, *Plant Sci.* 241: 96–108; Schnablová et al. 2017, *Ann. Bot.* 120: 833–843].

Research projects: 7, 9, 99

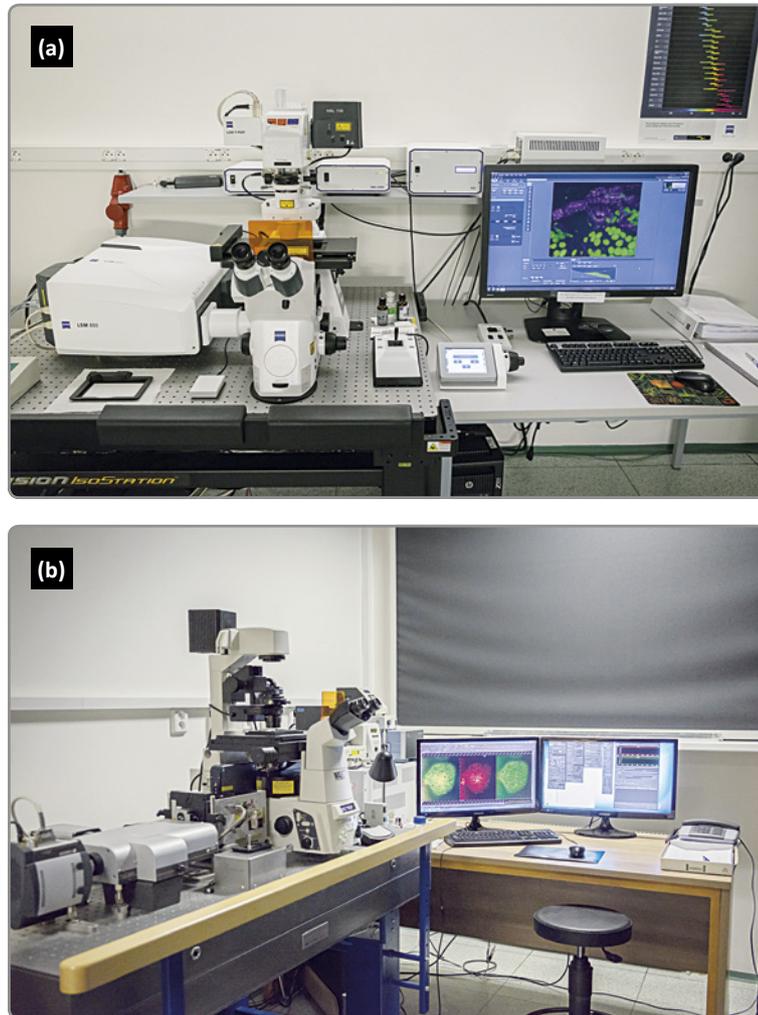


Figure 1: Microscopes for advanced fluorescence microscopy in the Imaging Facility of IEB CAS. **(a)** CLSM inverted microscope Zeiss LSM 880 with Airy Scan and spectral GaAsP detectors. **(b)** Spinning disk confocal microscope Nikon Eclipse Ti-E with Yokogawa CSU-X1, equipped with Andor EM CCD iXon3 897 ultrasensitive camera and sCMOS camera Zyla 4.0 for imaging in higher resolution.

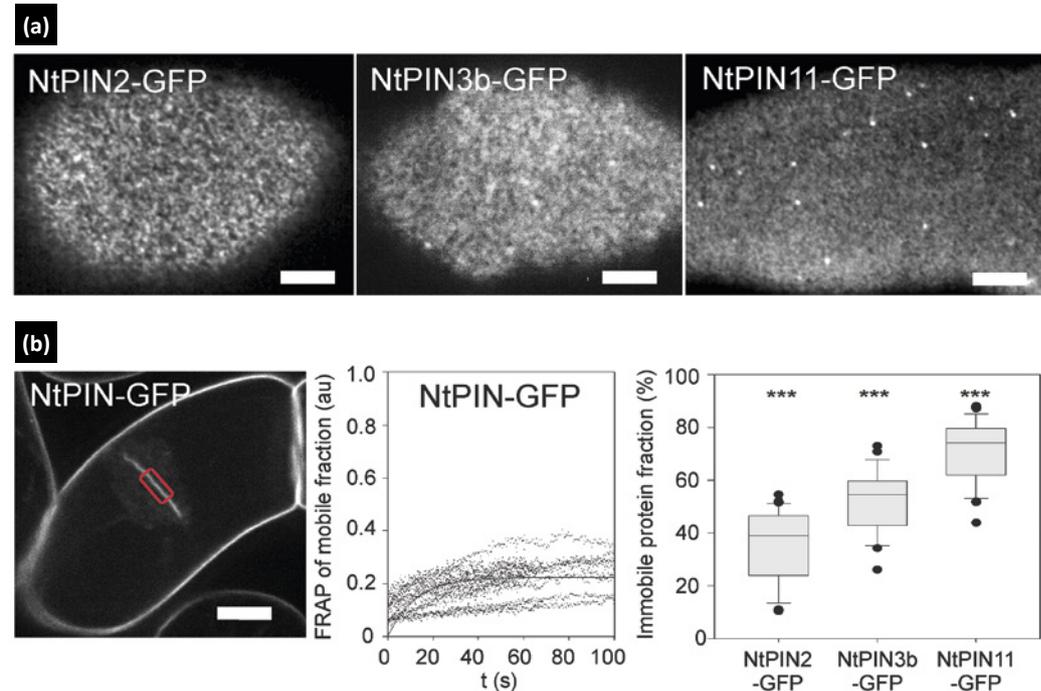


Figure 2: *In vivo* confocal fluorescence imaging of tobacco auxin efflux carriers. **(a)** Heterogeneity of the distribution of three members of NtPIN family within the plasma membrane studied with Nikon Eclipse Ti-E with Yokogawa CSU X1 spinning disk unit. Fluorescence signal of NtPIN2-, NtPIN3- and NtPIN11 GFP is distributed within the plasma membrane with clear patterns that differ for three studied proteins. Images were captured using 100x oil immersion objective (NA 1.45), excitation 488 nm and emission 525/30 nm. **(b)** Fluorescence recovery after photobleaching (FRAP) analysis of NtPINs performed in the central part of developing cell plates (red rectangle, left panel). The amount of recovered fluorescence during 100 s corresponds to mobile fraction of the protein (middle panel for NtPIN11-GFP). Mobility of NtPIN2-GFP, NtPIN3-GFP and NtPIN11-GFP, expressed as the percentage of immobile fraction after 120s is summarized in right panel. Only 40 % of NtPIN2 molecules stayed immobile compared to 80 % immobile molecules of NtPIN11 indicating differential regulation of mobility for these two auxin carriers. Inverted Zeiss LSM880 laser scanning microscope with C-Apochromat 40x/1.2 W, excitation 488 nm and emission 500-550 nm. Rectangular regions (red rectangle, left panel) were bleached within 6-10s. Post bleach images were acquired with 300 ms frame rate, for 90-120s. Values of immobile fraction were calculated within FRAP module implemented in ZEISS Zen software and according to [225] with single exponential fit. For both (a) and (b) error bars represent 5 μ m. pER8 driven expression of NtPIN2-GFP, NtPIN3-GFP and NtPIN11-GFP genes in BY-2 cells was induced with β -estradiol (3 μ M) for 48 hours.



Isotope Laboratory

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Research in the Isotope Laboratory is focused in three basic directions:

(1) Investigation of medicinally important plant products with special attention to their derivation, resulting in semisynthetic compounds with improved physico-chemical characteristics and mostly greater cytotoxicity. Cancer cell apoptosis was observed in compounds displaying high cytotoxicity. High antimicrobial activity has been achieved with several new compounds. Multifarious activity (i.e. pleiotropic effect) was found in several highly active compounds. The ability to form supramolecular aggregates in aqueous media and/or in organic solvents was found and investigated.

(2) Development of heterocyclic derivatives of synthetic origin (purine-based compounds), including radiolabeling whenever required.

(3) Preparation of cytokinin derivatives and other required compounds with radiolabeling.

The research is oriented to biomolecules, their structure and analysis, activity, molecular and cellular mechanisms of action as well as applications in different fields.

A comprehensive screen of land plants is presented suggesting that cytokinins (CKs) of *cis*-zeatin (*cZ*)-type occur generally in the plant kingdom. A survey of the employed bioassays illustrates the ability of high doses of *cZ*-type CKs to induce various physiological responses and CK signaling. These data argue against the image of *cZ*-type CKs as the non-active or weakly active natural adjuncts to the *trans*-isomers suggesting their conceivable function as delicate regulators of CK responses under growth-limiting conditions. This work was enabled due to the development of new *cZ* synthesis [441, 458, 464].



In the picture (from the left):

RNDr. Bohuslav Černý, CSc. / former researcher, Dr. Josef Holík / researcher, Ing. Zülal Özdemir / Ph.D. student, doc. Ing. Libor Havlíček, CSc. / researcher, doc. Ing. Milan Pavlík, CSc. / researcher, Bc. Pavla Štangelová / former diploma student, PharmDr. Lenka Zahajská, Ph.D. / research assistant, Bc. Tereza Jíšová / former diploma student, Mgr. Ivona Blažková / Ph.D. student, Ing. Jana Šusteková / former Ph.D. student, Mgr. Uladimir Bildziukevich / Ph.D. student, Martina Wimmerová / technician, Ondřej Čáslavský / technician, prof. Ing. Zdeněk Wimmer, DrSc. / head of the laboratory.

Not in the picture:

RNDr. Sándor T. Forczek, Ph.D., RNDr. Martin Vlk / researchers, RNDr. Jaroslav Nisler, Ph.D. / researcher in biology and Ph.D. student in organic chemistry, Jana Maňáková / technician.

Natural phytochemicals as potential drug candidates

We have developed drug candidates with an unknown molecular mechanism of action. This is due to our expertise in cellular and molecular testing of new drug candidates.

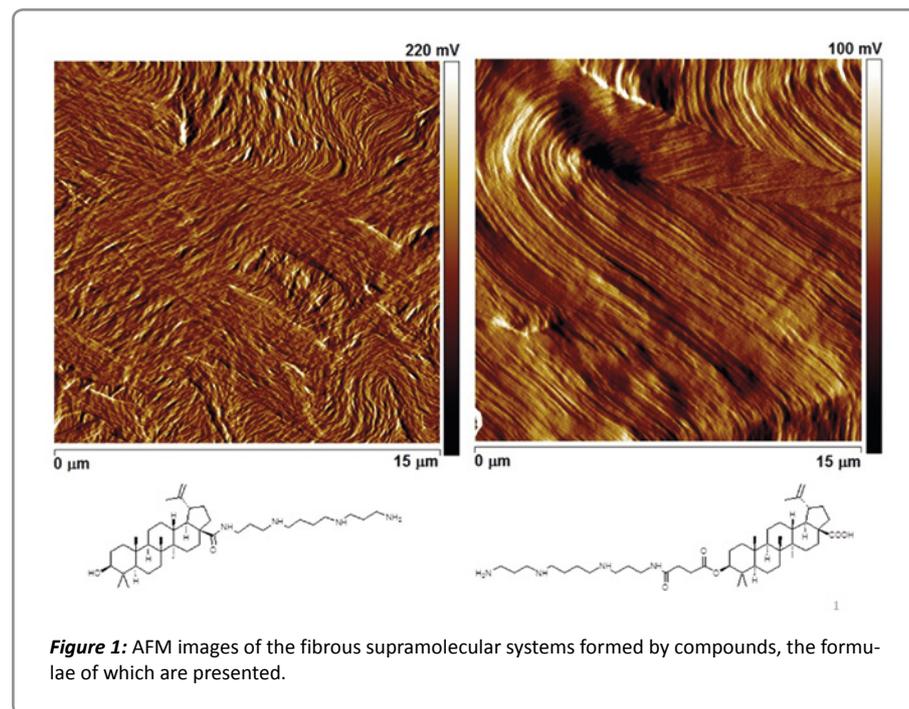
Phytosterols and cholesterol were subjects of structural modification with the objective of obtaining novel compounds displaying either cytotoxicity and antimicrobial activity or the ability to self-assemble into chiral supramolecular systems.

The current interest of the IL team is investigation of novel amides of triterpenoid acids with potential cytotoxicity. The synthetic protocol was designed in as simple a way as possible, and divided into several general methodologies applicable to the compounds synthesized. The cytotoxicity was tested on cells derived from human T-lymphoblastic leukemia, breast adenocarcinoma and cervical cancer, and compared with tests on normal human fibroblasts. ADME parameters as well as selected physico-chemical parameters were either measured or calculated to support experimentally obtained



results [16]. The derivation of steroids resulted in synthesis of porphyrine derivatives and steroidal ribbons [229, 274] for potential application in pharmacology.

We studied the physico-chemical characteristics of the prepared compounds. This is important for investigation of the supramolecular behavior of potential biological systems, and contributes to the newly emerging area of supramolecular chemical biology. A number of the prepared compounds, especially those derived from natural triterpenoid acids, suffer from low bioavailability due to their low solubility in aqueous media. Even triterpenoid acids display low solubility in these media. This aside, several compounds (betulinic acid, oleanolic acid and ursolic acid) are known for their positive effects in treating different types of cancer. A series of cationic polyamine-based amides of plant sterols and triterpenoids was prepared. These compounds are able to bind to negatively charged DNA and are also able to enter the lipid bilayer to participate in the formation of ion channels [312, 395, 435, 454].



Other fields of investigation

The distribution of halogens in nature, and investigation of their natural origin, have been intensively studied by analyzing natural sources [38, 188]. The role of environmental stress has been studied as an emerging topic in agricultural research from different aspects [171, 299, 397, 398, 461, 462, 492].

The synthesis and complex biological investigation of pyrimidine derivatives has also been realized during the evaluated period [296, 301].

Finally, as part of continuing to publish articles on different tastes of natural compounds, natural taste-sour substances have been reviewed [74]. In addition, a complex of all articles on natural sources was published in 2016 as a book [470], prepared in close collaboration with the University of Chemistry and Technology in Prague, and this has been accepted as a text book for students of the Faculty of Food and Biochemical Technology of the UCT Prague.

Future perspectives of the Isotope Laboratory in 2018–2022:

It is expected that all three basic areas of investigation will be continuously developed.

Investigation of plant products will be ongoing. For the near future, we will focus on designing highly bioavailable conjugates of plant triterpenoids by application of short-chained oligopeptides as derivation agents of the target molecules. Another possibility is afforded by the formation of conjugates of the target molecules with hydrophilic polymers of natural origin (mostly those classified as hemicelluloses, e.g., hyaluronic acid, agar-agar etc.).

Earlier prepared compounds will be further modified, and other compounds, e.g. porphyrine derivatives, different adaptogens, polyamines and aromatic amines, sugars etc. will be investigated.

In the area of supramolecular chemical biology, we plan conjugation of the developed supramolecular hydrogels using a broad spectrum of plant products with observed remedial effects on serious diseases. Biological screening tests will be realized through interinstitutional collaboration, as well as through international collaboration with biological and medical teams. Radiolabeling of perspective compounds will be carried out on recently purchased devices for ^3H - and ^{14}C -labeling, for better application in the area of medical biochemistry.

Research projects: 11, 17, 51, 78, 92, 117



Laboratory of Biologically Active Compounds

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The main research interest of our laboratory is the metabolism and physiological functions of growth regulators, polyamines and phenolic compounds in plants. We follow the role of these biologically active compounds in plant development and in the response of plants to abiotic stress. We use both whole plants as well as cell cultures as model systems in our investigations.

The process of somatic embryogenesis of conifers represents our main interest; we aim to study the regulation of somatic embryo development, the role of phytohormones in somatic embryogenesis and the effect of stress factors on embryo development. We have investigated the regulation of seed dormancy and embryo development in beechnuts during stratification (in cooperation with the Forestry and Game Management Research Institute, research station Kunovice).



In the picture (from the left):

Front row: RNDr. Milena Cvikrová / researcher, RNDr. Lucie Fischerová, Ph.D. / researcher, Mgr. Lenka Gemperlová, Ph.D. / researcher, ing. Jana Pavlíčková / graduated technical assistant.

Second row: RNDr. Martin Vágner, CSc. / head of the laboratory and director of the institute, RNDr. Zuzana Vondráková, CSc. / researcher, Mgr. Kateřina Eliášová, Ph.D. / researcher.

Not in the picture:

ing. Kateřina Raková / graduated technical assistant, Jana Kališová, Jaroslava Špačková / technicians.

Seed dormancy

The investigation of dormancy and stratification of beechnuts was carried out within the project Q102A256 that was supported by the Ministry of Agriculture of the Czech Republic.

We were interested in the changes occurring in beech embryos during moist stratification and dor-

mancy breaking. Our experiments were conducted in three steps: characterization of the process of beechnut breaking dormancy in terms of the changes of endogenous ABA level in embryos during stratification; determination of the changes in fumarase activity in embryonic axes during stratification and description of beechnuts dormancy breaking at the histological

level – we have described the changes in distribution of storage proteins, starch grains and calcium oxalate crystals.

We concluded that three tested parameters provide three different directions of dormancy breaking characterization. Both decrease in endogenous ABA and increase in fumarase activity in embryos can be used as indicators of the depth of dormancy in beechnuts. The main storage compounds (starch, proteins and calcium oxalate) were found in beech embryos during stratification. We found no differences in size or location of starch grains and/or CaOx crystals linked with stratification. The storage proteins localized in vacuoles were exhausted during the process of stratification; the first histological changes were evident after imbibition. The results have been summarized in [35].

The role of polyamines and phenolic compounds in somatic embryogenesis of conifers

The process of somatic embryogenesis in conifers is strictly regulated by phytohormones and the changes in endogenous phytohormone as well as polyamine levels characterize all the steps of somatic embryo development. The investigation of the role of polyamines in somatic embryo development and during stress reactions of embryogenic cultures and mature embryos was supported by COST projects LD13051 and [77].

We specified the effects of putrescine treatment during maturation and/or proliferation of spruce embryogenic culture and described the effect of elevated putrescine levels on somatic embryo development. We determined the endogenous polyamine levels in the embryogenic cultures treated with exogenous putrescine and correlated the changes of polyamines level with the anatomical structure of embryogenic cultures and with the yields of mature embryos. We concluded that exogenous putrescine induced intensive changes in

embryo structures but without any positive effect on the yields of mature embryos [143].

Polyamines are primarily known as the key substances in plant response to stress factors. They stabilize the molecular composition of membranes and suppress lipid peroxidation in this way preventing membrane injury. They retain plasma membrane permeability and reduce leakage under stress conditions. Alterations in polyamine levels occur in response to a wide variety of stress situations. We used somatic embryogenesis as a model system for the investigation of plant stress response to UV-B exposure and the changes of polyamines as the marker of a stress reaction. We assessed the polyamine content and the activities of their biosynthetic enzymes in irradiated embryogenic tissues and desiccated embryos. The extent of lipid peroxidation was indicated by the changes in MDA levels. Determination of the viability of irradiated embryogenic cultures and characterization of emblings developed from embryos exposed to UV-B radiation was the criterion of the rate of damage of embryogenic culture and/or desiccated embryos induced by UV-B stress. The effect of UV-B irradiation on fully developed embryos was marginal compared with that on embryogenic cultures. The increase in MDA levels in irradiated ESMs correlated with the decrease in their PA content. Neither significant increase in MDA levels nor significant changes in PA contents were observed in the fully developed embryos after irradiation, which may indicate that the plants' defence mechanisms are particularly active in these tissues [175].

The next investigation was focused (besides polyamines) on phenolic compounds that are often associated with plant strategy of protection against UV-B radiation. Somatic embryos exposed to UV-B during desiccation were characterized first by accumulation of higher levels of spermidine and spermine (by about

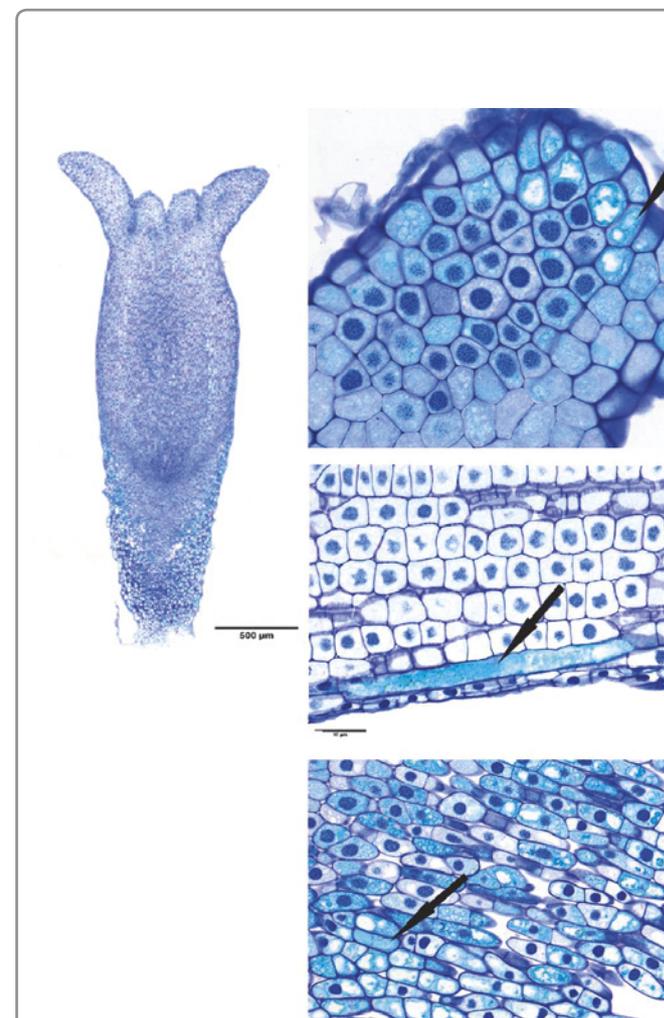


Figure 1: Localisation of phenolic compounds in spruce somatic embryos irradiated by UV-B. Arrows point to the cells accumulating phenolics. Metachromatic staining with Toluidine blue.



20 %) and then by increase in total content of phenolics (about 25 %). UV-B radiation elicited their accumulation in the junction zone between root cap and hypocotyl and in the epidermal and subepidermal cells of hypocotyl and cotyledons. UV-B irradiation evoked striking polyphenolic accumulation in specialized idioblastic cells localized beneath the epidermis of the somatic embryo hypocotyl and cotyledons. The fluorescence due to flavonoids, detected under confocal laser scanning microscope, increased dramatically after UV-B irradiation in the epidermis of the hypocotyl and cotyledons and on the surface of the root cap of spruce somatic embryos. These results indicate the roles of polyamines and phenylpropanoids in the prevention of oxidative damage provoked by UV-B treatment [330].



Figure 2: The longitudinal section of embryonal axis of the beechnut (seed of *Fagus sylvatica*) with a part of cotyledons. Paraffin section was stained with Nuclear Fast Red and Alcian blue.

Physiology and the role of plant growth regulators in somatic embryogenesis

We published a review on the role of phytohormones in somatic embryogenesis in *Vegetative Propagation of Forest Trees* (Park YS, Bonga J, Moon HK eds.) – a book containing authoritative reviews on the development of somatic embryogenesis and other vegetative propagation technologies and their applications in industrial production, first distributed at the Fourth International Conference of the IUFRO unit 2.09.02: Somatic Embryogenesis and Other Vegetative Propagation Technologies in Argentina.

Somatic embryogenesis is characterized as the developmental process by which somatic cells, under suitable induction conditions, undergo restructuring through the embryogenic pathway to generate embryogenic cells and consequently the whole plant. Within this process, a single cell or a group of cells with similar morphology and genetic background respond to external stimuli produced by the surrounding tissue, in the case of natural settings, or present in the tissue culture medium. These stimuli launch a genetic program that leads to the establishment of cell lineages with an altered gene transcription pattern, and a different morphology and developmental fate. The key substances controlling the whole process of somatic embryogenesis are phytohormones.

In our review, we focused on seven main groups of regulators that have a fundamental influence on different developmental stages of somatic embryogenesis – auxins, cytokinins, abscisic acid, ethylene, jasmonic acid, polyamines and phenolic compounds. We provided an overview of current knowledge of phytohormonal regulation of embryo development including the effect of crosstalks between phytohormones and/or plant growth regulators in terms of highly coordinated interactions within phytohormones signalling pathways. We considered the main mechanism of regulation in plant/embryo development as revealed by studies on zygotic as well as somatic embryos using the current approaches of molecular biology and advanced microscopic techniques. Where possible, examples from SE of conifers have been provided [505].

Research projects: 77, 103, 105, 107





Laboratory of Cell Biology

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The Laboratory of Cell Biology explores selected molecular regulatory modules of plant cell polarity and morphogenesis operating mostly at the plasma membrane, at the interface of secretory pathway, membrane lipids and cytoskeleton. Plant morphogenesis is based essentially on two processes – oriented cell division and differential cell growth. As model plants we use angiosperm *Arabidopsis* and tobacco along with moss *Physcomitrella patens*. We focus on intracellular molecular mechanisms driving cellular morphogenesis such as exocytosis. Proteins participating in these mechanisms are, despite major differences in cell structure and behaviour, often very similar to those found in the fungal and animal kingdoms. The laboratory is centered around the detailed characterization and regulation of the plant vesicle tethering complex exocyst in various cell types across plant species, including plant-pathogen interactions. A significant aspect of the research is understanding of minor membrane lipids in the maintenance and establishment of cell polarity.



In the picture (from the left):

Bottom row: Mgr. Klára Aldorfová / Ph.D. student, Ing. Andrea Potocká, Ph.D. / Research assistant.

Middle row: Mgr. Eva Kollárová / Ph.D. student, Mgr. Edita Janková Drdová, Ph.D. / researcher, Patricia Scholtz / internship student, Mgr. Lucie Břejšková, Ph.D. / research assistant, Mgr. Hana Soukupová, Ph.D. / research assistant, Mgr. Vedrana Marković / Ph.D. student, Doc. RNDr. Jiří Luštinec, CSc. / emeritus scientist, Mgr. Peter Sabol / Ph.D. student.

Top row: Mgr. Jitka Ortmannová / Ph.D. student, Mgr. Samuel Haluška / Ph.D. student, Bc. Matěj Drs / MSc. student, Tamara Pečenková, Ph.D. / researcher, Ing. Martin Potocký, Ph.D. / researcher, RNDr. Viktor Žárský, CSc. / head of the laboratory, Mgr. Jáchym Metlička / Ph.D. student, Ing. Přemysl Pejchar, Ph.D. / researcher.

Not in the picture:

RNDr. Michal Hála, Ph.D. / researcher, Mgr. Denisa Oulehlová, Ph.D. / researcher – maternity leave, Ing. Roman Pleskot, Ph.D. / postdoctoral researcher until 2017, Mgr. Anamika Ashok Rawat, Ph.D. / Ph.D. student until 2017, Mgr. Juraj Sekereš / Ph.D. student, Mgr. Lukáš Synek, Ph.D. / researcher – currently abroad, Bc. Jana Štovičková / technician, Mgr. Nemanja Vukašinić, Ph.D. / Ph.D. student until 2016.

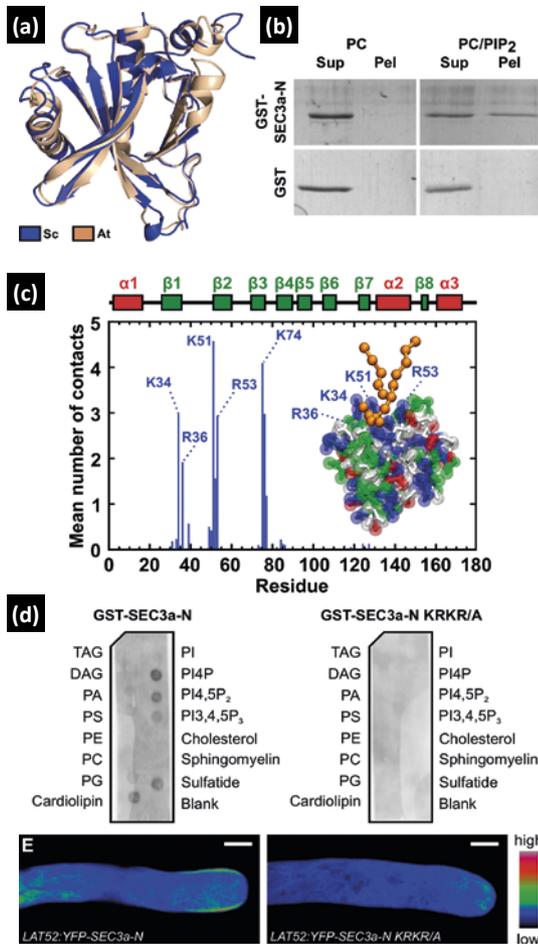


Figure 1: SEC3a N-terminus binds phosphoinositides via four positively charged residues that mediate its association with the plasma membrane. (a) Comparison of the SEC3a-N homology model (At) with the yeast template structure (Sc). (b) Lipid-binding assay of GST-SEC3a-N tested using 200-nm vesicles containing 5% PIP2 : 95% PC or PC alone. After incubation of GST-SEC3a-N with the vesicles, they were recovered by ultracentrifugation, and protein bound (Pel) was analyzed by SDS- PAGE. As a negative control, GST alone was used. Sup, Supernatant. (c) Analysis of MD simulations displaying polar contacts of SEC3a-N with a PIP2 molecule. The inset image represents a coarse-grained model of SEC3a-N coordinating the PIP2 molecule. (d) Lipid-binding properties of wild-type and mutated GST-SEC3a-N KRKR/A recombinant proteins, as determined using a protein-lipid overlay assay. Reproduced from [168].

Research highlights 2015–2017

In the years 2015-2017, we significantly expanded our understanding of the exocyst role in pollination and polar expansion of pollen tubes. In collaboration with the laboratory of Prof. Shaul Yalovsky from Tel-Aviv University in Israel, we performed detailed characterization of the molecular function of the SEC3 exocyst subunit during pollen tube tip growth [168]. Along the same lines, we performed a comparative functional study of multiplied exocyst subunit EXO70 in *Arabidopsis* and tobacco pollen [423, 432]. Since the exocyst subunit EXO70 evolved from three conserved clades with assumed distinct roles, we also studied the function of moss *Physcomitrella patens* exocyst subunit PpEXO70.3d from the as yet poorly characterized EXO70.3 clade [415]. Another role for the exocyst was found in tracheary elements, where we showed that microtubule-dependent targeting of the exocyst complex is necessary for xylem development in *Arabidopsis* [450]. Outside the exocyst framework, we continued studying the relationship between cell polarity, membrane traffic and plant-pathogen interactions [242, 400, 420].

The role of the exocyst complex in pollen tube growth

We have shown that the pollen expressed SEC3 exocyst subunit is an essential gene in plants, i.e. loss of its function results in zero male function/transmission [168]. Surprisingly, loss of N'-term membrane binding ability of the PH domain does not affect any apparent exocyst function. Furthermore, the localization of SEC3 to the cytoplasmic domain is very dynamic and clearly correlates with the direction of pollen tube growth/exocytosis (Fig. 1). We also studied the functional diversity of the EXO70 exocyst subunits which is required for targeting of the complex and is represented by

many isoforms in angiosperm plant cells. This diversity could be partly responsible for the establishment and maintenance of membrane domains with different composition. To address this hypothesis, we performed large-scale expression, localization, and functional analysis of tobacco (*Nicotiana tabacum*) and *Arabidopsis* EXO70 isoforms in pollen [423, 432]. In tobacco, various isoforms localized to different regions of the pollen tube plasma membrane, apical vesicle-rich inverted cone region, nucleus, and cytoplasm. NtEXO70A1a and NtEXO70B1 occupied two distinct and mutually exclusive plasma membrane domains [423]. In contrast, members of the EXO70 C class which are specifically expressed in tip-growing cells, exhibited exocytosis-related functional effects in pollen tubes despite the absence of apparent plasma membrane localization. This was further corroborated by genetic study in *Arabidopsis* where the loss-of-function EXO70C2 allele resulted in a significant male-specific transmission defect due to aberrant pollen tube growth. Mutant *Arabidopsis* pollen tubes grown in vitro exhibited enhanced growth rate and a decreased thickness of the tip cell wall, causing tip bursts (Fig. 2). However, *exo70C2* pollen tubes frequently recovered and resumed their speedy elongation, resulting in a repetitive stop-and-go growth dynamics [432]. Taken together, our data support the existence of multiple membrane-trafficking domains regulated by different EXO70-containing exocyst complexes within a single cell and suggest that members of the EXO70C subfamily are negative exocyst regulators of tip growth in pollen tubes.

Exocyst regulates various morphogenesis-related processes in distinct cell types

We investigated the subcellular localization of exocyst subunits in the xylem of *Arabidopsis thaliana* and analyzed the functional significance of exocyst-mediated

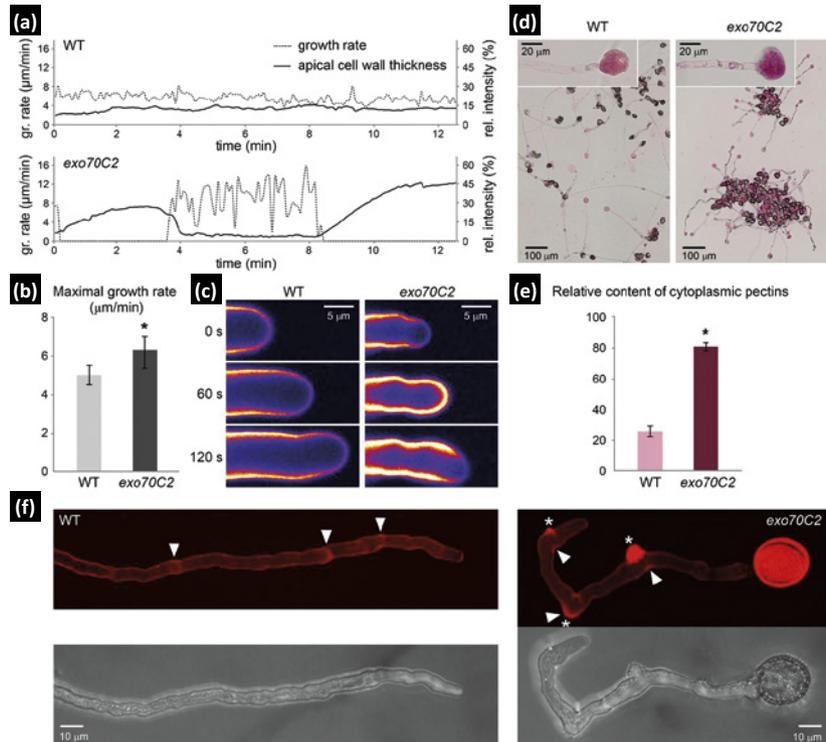


Figure 2: Growth rate and cell wall characteristics of *exo70C2* and wild-type (WT) pollen tubes. (a) Growth rate of typical wild-type and *exo70C2* pollen tubes correlated with cell wall thickness (Calcofluor White fluorescence) at the tube apex. (b) The averaged maximal growth rate of *exo70C2* is significantly higher than that of the wild type. (c) Calcofluor White fluorescence represented as an intensity color scale (purple to white) shows differential cell wall deposition at the tube apex during the highly fluctuating growth rate of the *exo70C2* pollen tube compared with the wild type characterized by low oscillations. (d) Pollen tubes of the wild type and *exo70C2* germinated and stained on the same slide with Ruthenium Red diluted in distilled water to cause the extrusion of cytoplasm. Details of burst tips that were used for quantification are shown in the insets. (e) Quantification of the Ruthenium Red staining in extruded cytoplasm as relative intensity in the red channel subtracted from the background. (f) Propidium iodide staining of growing pollen tubes. Maximum intensity projections over a confocal Z-stack are shown. Asterisks mark sites of collapse; arrowheads point to sites of stopped growth. Reproduced from [432].

trafficking in tracheary elements (TE) development. Exocyst subunits were localized to the sites of secondary cell wall (SCW) deposition in an MT-dependent manner. We demonstrated the importance of a functional exocyst for normal TE development and showed that the deposition of SCW constituents is compromised as a result of the mislocalization of secondary cellulose synthase in the exocyst mutant [450].

We have also contributed to the work of colleagues from the Department of Experimental Plant Biology at the Faculty of Science, Charles University in Prague which resulted in the discovery of callose deposition in trichomes of *Arabidopsis* leaves and its dependence on the exocyst complex function [66]. Unexpectedly this deposition is also linked to the heavy metal accumulation in trichomes opening a new perspective on molecular mechanisms of heavy metal accumulation in plants.

Since several studies including our previous work have implicated the exocyst complex in the reaction to plant pathogens, we further analyzed the involvement of exocyst-mediated polarized secretion during the reaction to microbial pathogens. Together with colleagues from Department of Experimental Plant Biology at the Faculty of Science, Charles University we found an interaction between the *Arabidopsis* EXO70B1 exocyst subunit, and RIN4 protein, the best studied member of the NOI protein family and a known regulator of plant defense pathways. We discovered that RIN4 recruits the exocyst subunit EXO70B1 to the plasma membrane [420]. We have also described that similarly to growth-promoting microorganisms, plant-pathogenic bacteria also stimulate *Arabidopsis* root-hair growth and found that this stimulation requires functional ethylene signalling and an efficient exocyst-dependent secretory machinery [400].

Biochemical and computational analysis of exocyst binding to the membrane

Based on available data on the yeast exocyst complex structure and localization we have proposed a structural and molecular dynamics model of exocyst cooperative docking at the cytoplasmic membrane regulated by exocyst-lipid and exocyst-RHO GTPases interaction [109]. This model not only indicates how exocyst is able to accommodate both membrane lipids and GTPase interactions, it uncovers new regions of membrane interaction along EXO70 subunit surface. In collaboration with colleagues from the lab of prof. Natasha Raikhel (University of California, Riverside) we contributed to the characterization of the first drug – Endosidin2/ES2 – that specifically interferes with the exocyst complex function by compromising EXO70 subunit interaction with the membrane [300]. This has far reaching implication as this drug was also shown to be active in animal cells.

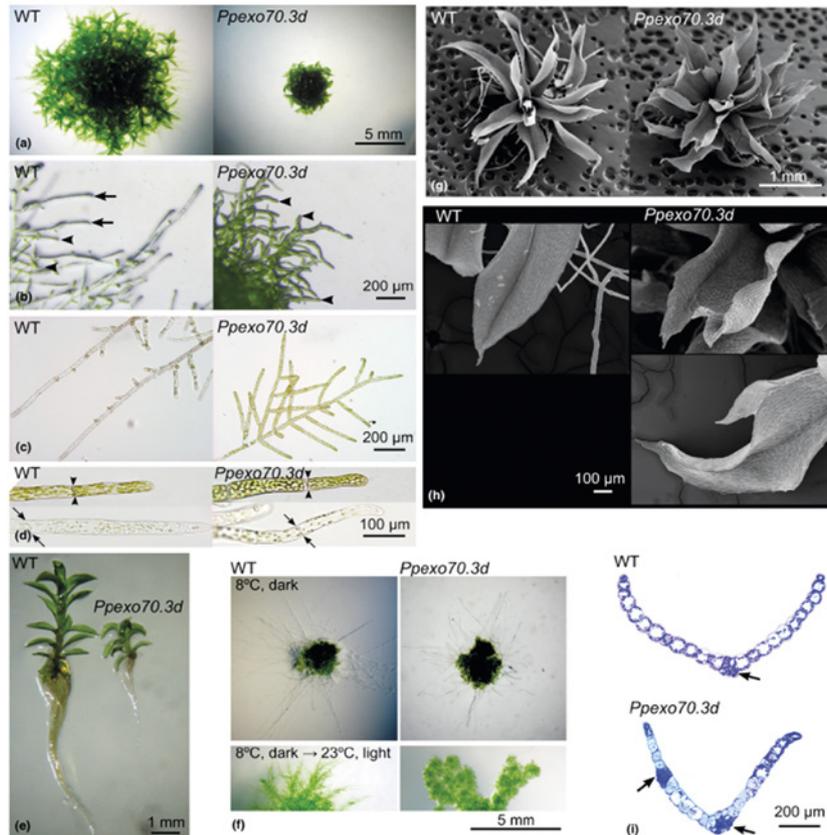


Figure 3: The phenotypic analysis of mutation in exocyst subunit Ppexo70.3d in moss *Physcomitrella patens*. (a) *Physcomitrella patens* wild-type (WT) and Ppexo70.3d#6, 1 month after inoculation. (b) Close-up of the edge of 10-d-old colony showing typical caulonema (arrow) and chloronema (arrowhead) growth in WT, while only filaments of chloronemal appearance (arrowhead) protrude beyond the colony edge in Ppexo70.3d#6. (c) Branching pattern of filaments originating from the edge of 14-d-old plants showing Ppexo70.3d#6 forming more side branches than WT. (d) Types of filaments present in 14-d-old WT and Ppexo70.3d#6 plants. Arrows show oblique cell walls typical for caulonemata, and arrowheads indicate transverse cell walls in chloronemata. (e) Comparison of 4-wk-old gametophores of WT and Ppexo70.3d#6. (f) WT and Ppexo70.3d#6 after growth in the dark at 8°C for 3 wk and then 10 d of transfer to normal growth conditions. (g) Scanning electron micrographs showing structure of gametophores of WT and Ppexo70.3d#6. (h) Scanning electron micrographs of phyllid apices of WT and Ppexo70.3d#6. (i) Transverse sections of WT and Ppexo70.3d#6 phyllids showing ectopic midrib formation in the mutant. Arrows indicate midribs. Reproduced from [415].

The first functional analysis of exocyst complex in moss plants

We have adopted the moss *Physcomitrella patens* as an ideal model to study exocyst functions in cell polarity and morphogenesis in an evolutionary-developmental context. We initiated the study of the 13 EXO70 moss paralogs and described the function of PpEXO70.3d, member of the evolutionarily well-conserved but experimentally poorly characterized EXO70.3 clade. Disruption of PpEXO70.3d caused pleiotropic cell elongation and differentiation defects in protonemata, altered response to exogenous auxin and increased endogenous auxin concentrations. We found that PpEXO70.3d was necessary for female gametogenesis and therefore for completion of the *P. patens* life cycle (**Fig. 3**). The mutants also exhibited altered cell wall and cuticle deposition, as well as compromised cytokinesis, consistent with the protein localization to the cell plate [415].

As cell morphogenesis is also regulated by the cytoskeleton we are contributing to the efforts of our colleagues from the Department of Experimental Plant Biology at the Faculty of Science, Charles University to characterize the function of major F-actin nucleators in plants – formins. Using cotyledons epidermal pavement cells we could show, that *Arabidopsis* Formin1 affects cell morphogenesis/pavement cells lobing not only directly via F-actin, but equally indirectly by affecting the dynamics of cortical microtubular cytoskeleton [259]. We put forward a model of the relationship between F-actin and microtubuli mediated by formins in [27].

In these three years, we were also active in summarizing/discussing our research in a broader context and we wrote or contributed to preparing mostly invited review articles on membrane-lipid interactions and domains in plants [117], formin functions at the interface between membranes and cytoskeleton [27] and vesicles tethering complexes in *Arabidopsis*. Based on our work on the exocyst, autophagy and defense we summarized current knowledge on exocyst and autophagy-related membrane trafficking in plants [401] and proposed a hypothesis on the involvement of autophagy in R protein regulation [242].

Research projects: 18, 29, 34, 59



Laboratory of Growth Regulators

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Research at the Laboratory of Growth Regulators (LGR) is directed to biomolecules: their structure, analysis, activity, molecular and cellular mechanisms of action as well as applications in various fields. LGR pursues research mainly on cytokinins but recently also on other groups of hormones and growth regulators.

One globally renowned contribution of the LGR in this field, is discovery of a number of cytokinins, especially the aromatic cytokinins and their olomoucine-derived derivatives. These compounds are used in biotechnology and medicine. Olomoucine was the first anti-tumor agent derived from cytokinins. The development of other more effective inhibitors of cyclin-dependent kinases, key enzymes in the cell division cycle, followed such as bohemine, roscovitine, olomoucine II and others. Roscovitine is completing phase II clinical trials for cancer treatment in Europe and in the USA. The development of anti-cancer drugs is not the only field that the LGR conducts advanced research in.

LGR is also successful in the agricultural research area. For example, we discovered how to increase endogenous cytokinins using an inhibitor of cytokinin oxidase/dehydrogenase called INCIDE which



In the picture (from the left):

Front row: prof. Ing. Miroslav Strnad, CSc., DSc. / head of the laboratory, Mgr. Ota Blahoušek / research assistant, Jitka Hansgutová, DiS / secretary, Mgr. Lucie Mikulíková / research assistant, Mgr. Vladimír Skalický / Ph.D. student, Mgr. Hana Svobodová / research assistant, Mgr. Jana Oklešťková, Ph.D. / researcher, Mgr. Lenka Plačková / Ph.D. student, prof. RNDr. Martin Fellner, Ph.D. / associate professor.

Second row: doc. Mgr. Ondřej Novák, Ph.D. / researcher, Ing. Jana Kočířová / technician, Ing. Věra Doleželová / research assistant, doc. RNDr. Vladimír Kryštof, Ph.D. / associate professor, Mgr. Veronika Malínková / researcher, Olga Hustáková / technician, Mgr. Helena Rezková / research assistant, Asta Zukauskaitė Dr., Ph.D. / researcher, Mgr. Daniela Konrádová / Ph.D. student, Mgr. Martin Höning / Ph.D. student, Mgr. Barbora Pařízková / Ph.D. student, Mgr. Ivan Petřík / research assistant, Mgr. Jakub Hrdlička / Ph.D. student, RNDr. Miroslav Kvasnica, Ph.D. / researcher, Pharm.Dr. Jitka Šířová, Ph.D. / researcher.

Third row: Mgr. Jan Walla / researcher, Mgr. Karel Doležal, Dr. DSc. / researcher, Mgr. Danuše Tarkowská, Ph.D. / researcher, RNDr. Jiří Pospíšil, Ph.D. / researcher, Ing. Jakub Vylčíl / research assistant, Mgr. Eva Řezníčková, Ph.D. / researcher, Ing. Ludmila Ohnoutková, Ph.D. / researcher, Mgr. Aleš Pěnčík, Ph.D. / researcher, Mgr. Jan F. Humplík, Ph.D. / researcher, Mgr. Tomáš Vlčko / Ph.D. student.

Not in the picture:

doc. RNDr. Jitka Frébortová, Ph.D., prof. Peter Hedden, doc. RNDr. Jan Krekule, DrSc. / associate professors, Ing. Zdeňka Babíková, Ing. Jarmila Greplová, Mgr. Jiří Grúz, Ph.D., Mgr. Radek Jorda, Ph.D., Mgr. Alena Kadlecová, Mgr. Martina Kopečná, Ph.D., Mgr. Barbara Nardelliiová, Mgr. Jaroslav Nisler, Ph.D., Mgr. Andrea Novotná, Mgr. Lucie Plíhalová, Ph.D., Mgr. Lucie Rárová, Ph.D., Mgr. Jiří Skořepa, Mgr. Lukáš Spíchal, Ph.D., Marek Szecowka, Ph.D., RNDr. Veronika Turečková Ph.D., Mgr. Terezie Urbanová, Ph.D., Mgr. Marie Vitásková, Mgr. Jiří Voller, Ph.D. / researchers, Mgr. Magdaléna Bryksová, Mgr. Petra Kořínková, Mgr. Pavla Pokorná, Mgr. Zuzana Skrášková, Mgr. Jan Šimura / Ph.D. students, Ing. Jaromír Mikulík, Ph.D., Mgr. Michaela Mrvková, Mgr. Kateřina Šmídová / research assistants, Kateřina Faková, Lenka Gajdošíková, Eva Hirnerová, Eva Hrdličková, Bc. Dita Jordová, Hana Martínková, Pavel Sedláček, Ing. Michaela Šubrtová / technicians.

supports important plant growth and developmental processes. Owing to this discovery, we were able to increase the yield of a number of agricultural crops and plant stress resistance. Patents covering this know-how were licensed to Syngenta. Recently, we developed a product which restores the

skin to its youthful state and aids in the treatment of skin diseases. Cytokinins which also retard ageing in plants, were used in this development. The product with the trade name Pyratine® not only treats skin roughness, wrinkles, and pigmentation, it is also effective for treating various forms of acne.



LGR engages in scientific research especially in the preparation of new, phytohormone-based growth regulators with potent biological activities, the development of relevant analytical methods, study of the functions and effects on growth and developmental processes in normal and tumor cells, including the development of anti-tumor agents derived from plant hormones. Research on tumor suppressor genes, mechanisms that regulate their expression and the design of mutant organisms with controlled gene expression, are included in our scientific profile.

New phytohormone probes and biomolecules

New phytohormone standards, probes and labelled derivatives

LGR has long standing experience in organic synthesis, labelling of phytohormones and production of heavy and radioactively labelled phytohormones [6–9, 12, 18, 58, 107, 112, 146, 147, 149, 158, 160, 223, 247, 276, 294, 329, 361, 363, 374, 377, 407, 419, 428, 448, 455, 456, 458, 463]. For example, to screen for putative OPDA metabolites, the vegetative tissues of flowering *Arabidopsis thaliana* were extracted with 25% aqueous methanol (v/v), purified by single-step reversed-phase polymer-based solid-phase extraction, and analyzed by high throughput mass spectrometry. This enabled the detection and quantitation of a low abundant OPDA analog of the biologically active (+)-7-iso-JA-L-Ile in plant tissue samples [187]. The identified OPDA-Ile suggests that OPDA specific responses might be mediated upon formation of OPDA-Ile. We tested OPDA-Ile-induced gene expression in wild type and JA-deficient, JA-insensitive and JA-Ile-deficient mutant background. Tests on putative conversion of OPDA-Ile during treatments revealed only negligible conversion. Expression of two OPDA-inducible genes, GRX480 and

ZAT10, by OPDA-Ile could be detected in a JA-independent manner in *Arabidopsis* seedlings but less so in flowering plants. The data suggest a bioactivity in planta of OPDA-Ile [161].

We also published data showing that not only exogenous 2,4-D but also its amide-linked metabolite 2,4-D-Glu display an inhibitory effect on plant growth via the TIR1/AFB auxin-mediated signaling pathway. To further investigate 2,4-D metabolite conversion, identity and activity, we have developed a novel purification procedure based on the combination of ion exchange and immuno-specific sorbents combined with a sensitive liquid chromatography-mass spectrometry method. In 2,4-D treated samples, 2,4-D-Glu and 2,4-D-Asp were detected at 100-fold lower concentrations compared to 2,4-D levels, showing that 2,4-D can be metabolized in the plant. Moreover, 2,4-D-Asp and 2,4-D-Glu were identified as reversible forms of 2,4-D homeostasis that can be converted to free 2,4-D. This work paves the way for new studies of auxin action in plant development [185].

A novel enzymatic activity dependent on NADP⁺ converting *trans*-zeatin (tZ) to 6-(3-methylpyrrol-1-yl) purine (MPP) was detected. MPP shows weak anti-cytokinin properties and inhibition of CK dehydrogenases due to the ability to bind to an active site in the opposite orientation than substrates. Although the physiological significance of tZ side-chain cyclization is not anticipated as MPP occurrence in maize tissue is very low, the properties of the novel CK metabolite indicate its potential for utilization in plant *in vitro* tissue culture. Furthermore, feeding experiments with different isoprenoid CKs revealed distinct preferences in glycosylation of tZ and *cis*-zeatin (cZ). While tZ is preferentially glucosylated at the N9 position, cZ forms mainly O-glucosides. Since O-glucosides, in contrast to N9-glucosides, are resistant to irreversible cleavage

catalyzed by CK dehydrogenases, the observed preference of maize CK glycosyltransferases for O-glycosylated zeatin in the *cis*-position might explain why cZ derivatives are over-accumulated in different maize tissues and organs [201].

The phenylpropanoid 3,4-(methylenedioxy)cinnamic acid (MDCA) is a plant-derived compound first extracted from the roots of *Asparagus officinalis* and further characterized as an allelochemical. Our data provide a novel molecular explanation for the phytotoxic properties of MDCA [276, 428]. The synthesis and antiproteasomal activity of novel O-benzyl salicylamide-based inhibitors built from leucine and phenylalanine and microwave-assisted synthesis of phenylpropanoids and coumarins together with total synthesis of osthol have been also described [353, 361].

A series of 2-chloro-N⁶-(halogenobenzylamino)purine ribosides was also developed. Most derivatives did not trigger cytokinin signaling via the AHK3 and AHK4 receptors from *Arabidopsis thaliana* in the bacterial assay but some specifically activated the ZmHK1 receptor from *Zea mays* and were also more active than the aromatic cytokinin BAP in an ARR5::GUS cytokinin bioassay using transgenic *Arabidopsis* plants (Vylíčilová H et al. 2016, *Phytochemistry* 122: 22-33). The results of new development in different plant hormone fields is also summarised in several reviews [247, 508; Zwanenburg B et al. 2016, *Planta* 243: 1311-1326].

Modulation of phytohormone perception, biosynthesis and degradation

Recently, we have contributed to studies of transgenic plants with auxin/cytokinin receptor loss-of-function mutations, plants with mutations in auxin/cytokinin-synthesizing genes, and auxin/cytokinin-deficient plants with genetically enhanced degradation [147, 194, 198, 233, 249, 250, 255, 283, 295, 302, 309, 322,



334, 416]. These studies showed that such changes lead to distinct alterations in shoot growth parameters, retarded leaf senescence, increased seed size, accelerated germination and enhanced root systems. Chemical inhibitors of cytokinin perception, biosynthesis and degradation are thus very promising as powerful tools for studying further the mechanism of cytokinin action as an alternative to genetic approaches. Further, they are expected to influence plant growth and development and might thus find interesting applications as growth regulators for modifying traits in crop plants. Several compounds regulating both the perception and metabolism of brassinosteroids and cytokinins have been developed over the last five years [235, 248, 363, 458, 463; for patents see www.espacenet.com and patents 1, 2, 5, 7] and their beneficial activity for plant growth and development have been described. Many new regulators have been also isolated in plant tissue cultures and can find potential applications in plant biotechnology [5–9, 12, 18, 19, 67, 107, 112, 158, 160, 305, 372, 386].

Recently we used *in silico* modeling to design, synthesize and characterize twenty new thiazuron (TDZ) derivatives with improved inhibitory properties. Two compounds, namely 1-[1,2,3]thiadiazol-5-yl-3-(3-trifluoromethoxy-phenyl)urea (3FMTDZ) and 1-[2-(2-hydroxyethyl)phenyl]-3-(1,2,3-thiadiazol-5-yl)urea (HETDZ), displayed up to 15-fold lower IC₅₀ values compared with TDZ for AtCKX2 from *Arabidopsis thaliana* and ZmCKX1 and ZmCKX4a from *Zea mays*. Crystal structure complexes, solved at 2.0 Å resolution, revealed that HETDZ and 3FMTDZ bound differently in the active site of ZmCKX4a: the thiadiazolyl ring of 3FMTDZ was positioned over the isoalloxazine ring of FAD, whereas that of HETDZ had the opposite orientation, pointing toward the entrance of the active site. We suggest that the combination of simple synthesis,

lowered cytokinin activity, and enhanced inhibitory effects on CKX isoforms, make 3FMTDZ and HETDZ suitable candidates for *in vivo* studies [235].

We also reported that two cytokinin (CK) analogues, 2-chloro-6-(3-methoxyphenyl)aminopurine (INCYDE) and CK receptor antagonist 6-(2-hydroxy-3-methylbenzylamino)purine (PI-55) were used as a tool to elucidate the auxin-CK crosstalk under *in vitro* conditions in the medicinally important plant, *Eucomis autumnalis* subspecies *autumnalis*. INCYDE generally favoured shoot regeneration while the effect of PI-55 was more evident in root proliferation. Overall, INCYDE promoted the accumulation of higher concentrations and varieties of endogenous CK relative to the PI-55 treatments. In contrast, higher concentration of indole-3-acetic acid and 2-oxindole-3-acetic acid were generally observed in PI-55-supplemented cultures compared to plantlets derived from INCYDE. These results provided insight on how to alleviate root inhibition, a problem which causes considerable loss of several elite species during micropropagation [9].

From X-ray structure, NMR and stability-in-solution study of 6-(furfurylamino)-9-(tetrahydropyran-2-yl) purine (Pyratine), a new very active cytokinin derivative was determined [206]. We further prepared a series of eight N9-substituted kinetin derivatives, and characterized them with available physicochemical, biochemical and biological assays [487]. We hypothesized that a potentially suitable phenotypic marker is root curling induced by CK, as observed in the auxin biosynthesis mutant CK-induced root curling 1/tryptophan aminotransferase of *Arabidopsis* 1 (*ckrc1/taa1*). Phenotypic observations, genetic analyses and biochemical complementation tests of *Arabidopsis* seedlings displaying the trait in large-scale genetic screens showed that it can facilitate isolation of mutants with perturbations in auxin biosynthesis, transport and signaling. Mutants al-

lelic to several known auxin biosynthesis mutants were re-isolated, but several new classes of auxin-deficient mutants were also isolated. The findings showed that CK-induced root curling provides an effective marker for discovering genes involved in auxin biosynthesis or homeostasis [147].

Development of new drug candidates

Over the last two years, we have continued development of increasingly effective anticancer drug candidates. This research also led to the discovery of several other potent compounds with various structural motifs [43, 56, 71, 72, 82, 83, 85, 88, 89, 106, 113, 114, 122, 142, 145, 150, 156, 162, 164, 166, 167, 206, 213, 220, 222, 239, 254, 267–269, 286, 298, 301, 316, 324, 329, 348, 353, 354, 364, 377, 384, 388, 395, 419, 448, 456]. The potential of drugs in different therapeutic areas has also been reviewed several times [78, 93, 98–100, 502, 504]. New generations were prepared following well-established methods, including our previously described syntheses of purines, pyrazolo[4,3-d]pyrimidines, 8-azapurines and arylazopyrazoles [see patents 6, 7 and www.espacenet.com]. Selected examples are enclosed: A series of pyrazolo[4, 3-d]pyrimidine [114, 301], purines [113, 142, 150, 156], 7-azaindole [162] and arylazopyrazoles [56, 206] were synthesized and their kinase inhibitory activity and cytotoxicity against several cancer cell lines has been evaluated. A new potent CDK2 inhibitor with pyrazolo[4,3-d]pyrimidine scaffold has been synthesized, characterized, and evaluated in cellular and biochemical assays [114]. Anaplastic thyroid carcinoma (ATC) is an extremely aggressive human malignancy characterized by a marked degree of invasiveness, absence of features of thyroid differentiation and resistance to current medical treatment. Therefore, in the present study, the effect of a novel purine cyclin-dependent kinase

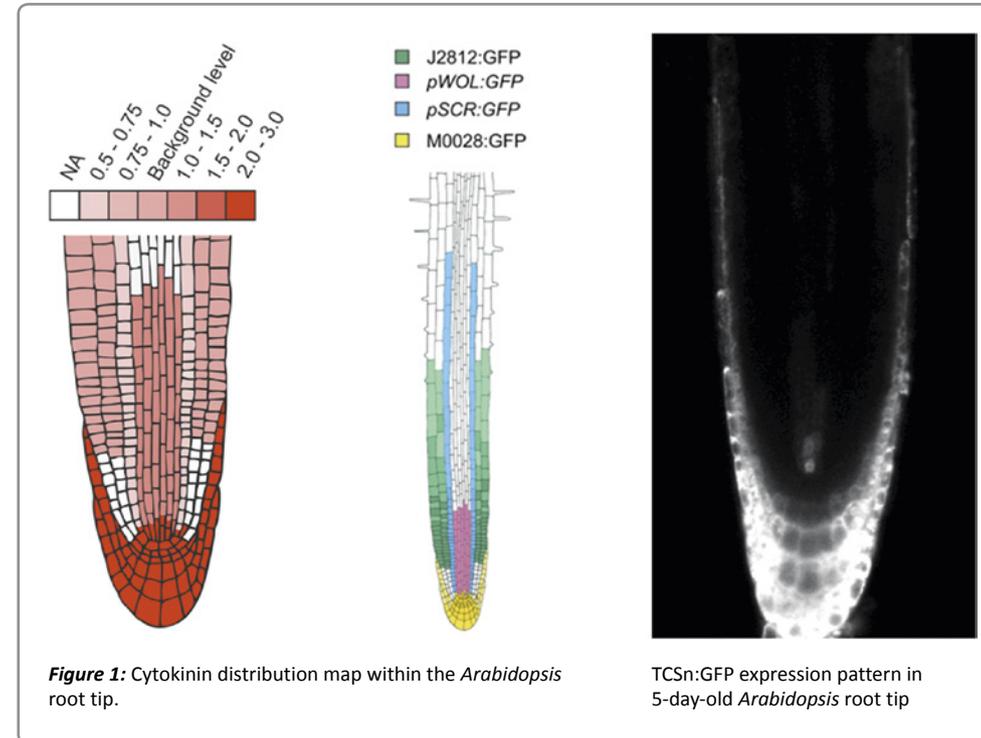
inhibitor, BP-14, was investigated in three human ATC cell lines. Our data indicated that BP-14 is a potential new compound effective against ATC [150]. Cyclin-dependent kinase 5 (CDK5) has recently emerged as an attractive target in several tumour entities. Inhibition of CDK5 has been shown to have anti-angiogenic effects *in vitro* and *in vivo*. We have recently developed a new series of 5-substituted 3-isopropyl-7-[4-(2-pyridyl)benzyl]amino-1(2)H-pyrazolo[4,3-d]pyrimidines that show a preference for inhibiting CDK5 and tested them *in vitro* and *in vivo* in a murine model of hepatocellular carcinoma [301]. Recently, we synthesized and screened a library of 2,6,9-trisubstituted purines that were screened for interaction with Cdc2-related protein kinase 3 (CRK3) and subsequently for activity against parasitic Leishmania species. Its mode of binding to CRK3 was investigated by molecular docking using a homology model [113]. We prepared and studied a series of 3,5,7-trisubstituted pyrazolo[4,3-d]pyrimidines, a new CDK inhibitor scaffold, to assess their CDK2 inhibitory and antiproliferative activities. The dual mode of action involving apoptosis induction in cancer cells and the blocking of angiogenesis-like activity in endothelial cells has therapeutic potential [114].

Recent studies have also indicated that brassinosteroids (BRs) also have antiproliferative, anticancer, antiangiogenic, antiviral and antibacterial properties in animal cell systems, and thus have potential medical application. Among others, BRs can inhibit replication of viruses in confluent human cell cultures, sometimes with high selectivity indexes, inducing cytotoxic effects in various types of cancer cells but not normal human cells. Thus, they provide promising leads for developing potent new anticancer drugs [93, 213, 222, 254]. We have also been working on cytotoxic derivatives of diifferent groups of neutral compounds like triterpenoids (lupanes, saponins) [71, 105, 122, 220, 267–269, 502] alkaloids [88, 89], and salicylamides [324].

New phytohormone bioanalytical methods and their applications

In order to understand better the network regulation of hormone action, we need to measure multiple hormone concentrations simultaneously, i.e. characterize the ‘hormone-metabolome’. Most plant hormones are found in plant tissues in extremely low concentrations which makes qualitative and quantitative analysis difficult and therefore very sensitive analytical tools are required. The analysis of plant hormones is challenging not only because these compounds are present in trace amounts but also because many other substances in plant extracts interfere with the analysis, such as pigments, lipids, phenolics and proteins.

Currently, the most suitable and most used analytical technology for phytohormone analysis is based on liquid chromatography-tandem mass spectrometry ((U



HPLC-MS/MS) as evidenced in our reviews [285, 391, 396, 487, 488, 501]. Since 2008, this methodology has gradually been developing in the LGR for plant hormone analyses (cytokinins, auxins, JAs, ABAs, gibberellins, brassinosteroids, phenolics, etc.) [5, 61, 199, 246, 284, 393, 404]. Further, UHPLC-MS/MS quantitative profiling of tryptophan-related neuroactive substances in human serum and cerebrospinal fluid was developed [199].

An on-line HPLC/EC/HR ESI-MS method had been also used to investigate the oxidation of selected cytokinin compounds. Electrochemical oxidation of isopentenyladenine resulted in five products, including hydroxylated and dehydrogenated products which correlates very well with its *in vivo* metabolism. Electrochemical conversion of *trans*-zeatin revealed six products, with two dehydrogenation products corresponding to its *in vivo* occurring metabolites. *cis*-Zeatin oxidation in the

electrochemical cell gave rise to eight products, resembling similarity to *trans*-zeatin oxidation. All three compounds underwent a complete turnover mainly through two oxidation reactions occurring in the electrochemical cell—dehydrogenation and a less typical aliphatic hydroxylation. The resulting products correlate with their known *in vivo* metabolism [61].

Here, we also applied fluorescence-activated cell sorting of green fluorescent protein (GFP)-marked cell types, combined with solid-phase microextraction and an ultra-high-sensitivity mass spectrometry (MS) method for analysis of CK biosynthesis and homeostasis at cellular resolution. This method was validated by a series of control experiments, establishing that protoplast isolation and cell sorting procedures did not greatly alter endogenous CK levels. The MS-based method facilitated the quantification of all well known CK isoprenoid metabolites in four different transgenic *Arabidopsis thaliana* lines expressing GFP in specific cell populations within the primary root apex. Our results revealed the presence of a CK gradient within the *Arabidopsis* root tip, with a concentration maximum in the lateral root cap, columella, columella initials, and quiescent center cells. This distribution, compared with previously published auxin gradients, implies that the well known antagonistic interactions between the two hormone groups are cell type specific [5].

These technologies have been very usable to understand better the network regulation of hormone action. The following examples are included: we investigated the function of the low-duplicated CYP715 cytochrome P450 gene family that appeared early in seed plants and evolved under strong negative selection. *Arabidopsis* CYP715A1 showed a restricted tissue-specific expression in the tapetum of flower buds and in the anther filaments upon anthesis. *cyp715a1* insertion lines showed a serious defect in petal development, and transient alteration of pollen intine deposition. Flower hormone profiling, indicated a modification of gibberellin homeostasis and a significant disturbance in the turnover of jasmonic acid derivatives. Petal growth was partially restored by the active gibberellin GA3 or the functional analog of jasmonoyl-isoleucine, coronatine. CYP715 appears to function as a key regulator of flower maturation, synchronizing petal expansion and volatile emission [75].

We also identified REPRESSOR OF CYTOKININ DEFICIENCY 1 (ROCK1) as an ER-localized transporter of UDP-GlcNAc and UDP-GalNAc in plants. In contrast to animals, nothing is known about the function of the two respective sugar residues in the plant ER. We demonstrated that ROCK1-mediated transport plays a role in the ER-associated protein quality control and loss of ROCK1 enhances cytokinin responses by suppressing the activity of cytokinin-degrading CKX proteins [92].

To elucidate the link between proteasome function, NO resistance, and patho-

genesis, we screened for suppressors of NO hypersensitivity in a mycobacterial proteasome ATPase mutant and identified mutations in Rv1205. We determined that Rv1205 encodes a pupylated proteasome substrate. Rv1205 is a homolog of the plant enzyme LONELY GUY, which catalyzes the production of hormones called cytokinins. Remarkably, we report that an obligate human pathogen secretes several cytokinins. Finally, we determined that the Rv1205-dependent accumulation of cytokinin breakdown products is likely responsible for the sensitization of *Mycobac-*

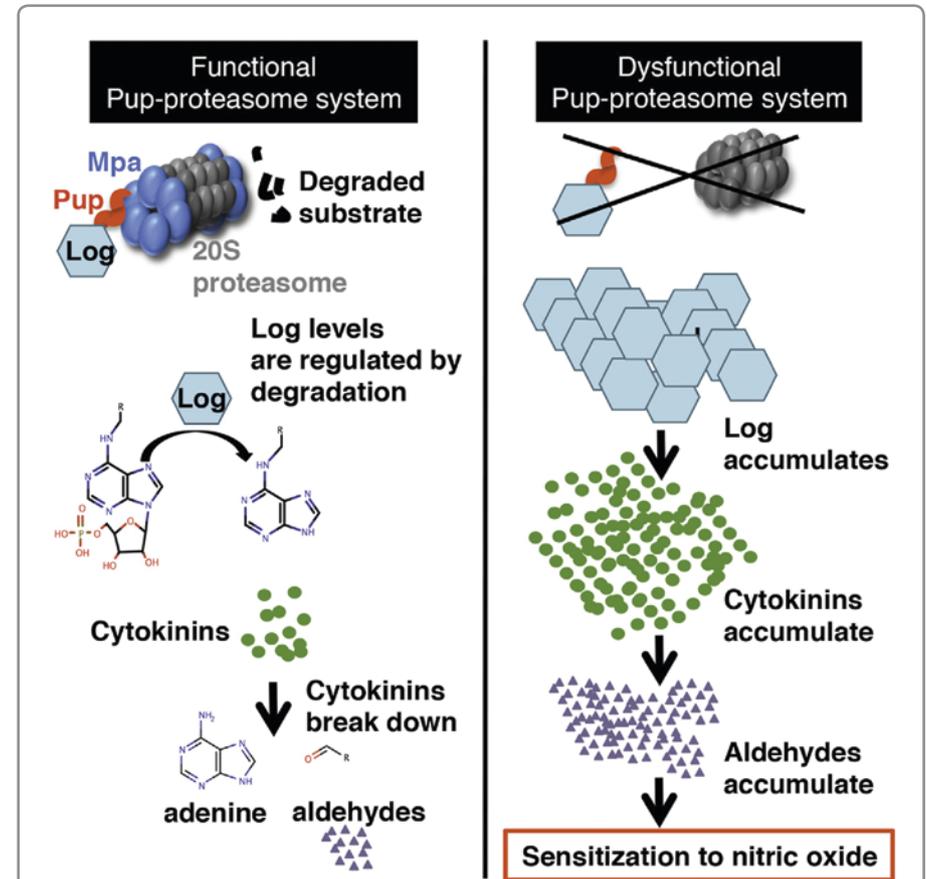


Figure 2: Proteasomal control of cytokinin synthesis protects *Mycobacterium tuberculosis* against nitric oxide.



terium tuberculosis proteasome-associated mutants to NO [115].

We also provided genetic evidence that nematode-derived cytokinin is involved in activating the host cell cycle during infection. Our findings show the ability of an animal to synthesize and secrete a functional plant hormone to establish long-term parasitism [121]. We found that Si-induced brown spot resistance functions independently of the classic immune hormones salicylic acid and jasmonic acid. Our data also rule out a major role of the abscisic acid (ABA) and cytokinin pathways, but suggest that Si mounts resistance to *C. miyabeanus* by preventing the fungus from hijacking the rice ethylene (ET) machinery. Interestingly, rather than suppressing rice ET signaling per se, Si probably interferes with the production and/or action of fungal ET [141]. We also identified the ability of *Pseudomonas fluorescens* G20-18 to efficiently control *P. syringae* infection in *Arabidopsis*, allowing maintenance of tissue integrity and ultimately biomass yield. Microbial cytokinin production was identified as a key determinant for this biocontrol effect on the hemibiotrophic bacterial pathogen. These results demonstrated microbial cytokinin production as a novel microbe-based, hormone-mediated concept of biocontrol. This mechanism provides a basis to potentially develop novel, integrated plant protection strategies combining promotion of growth, a favourable physiological status and activation of fine-tuned direct defence and abiotic stress resilience [193].

We also examined the role played by ABA/GA interactions regulating the formation of arbuscular mycorrhizal (AM) in tomato. ABA attenuates GA-biosynthetic and increases GA-catabolic gene expression leading to a reduction in bioactive GAs. These findings, coupled with the evidence that ABA application leads to reduced bioactive GA1, support the hypothesis

that ABA could act by modifying bioactive GA levels to regulate AM [232]. We also described how regulation of auxin oxidation via transcriptional control of *A. thaliana* gene DIOXYGENASE FOR AUXIN OXIDATION 1 (AtDAO1) expression is important at low to normal auxin concentrations. In contrast, higher auxin levels lead to increased Gretchen Hagen3 expression and auxin conjugation. Integrating this understanding into a multicellular model of root auxin dynamics successfully predicts that the *dao1-1* mutant has an auxin-dependent longer root hair phenotype. Our findings revealed the importance of auxin homeostasis for maintaining this hormone at optimal levels for plant growth and development (Mellor N et al. 2016, PNAS USA 113: 11022-11027).

A hallmark of plants is their adaptability in terms of size and form in response to widely fluctuating environments. The metabolism and redistribution of the phytohormone auxin play pivotal roles in establishing active auxin gradients and resulting cellular differentiation. Newly synthesized auxin moves to the hypocotyl where it induces elongation of hypocotyl cells. We showed that loss of function of VAS2 (IAA-amido synthetase Gretchen Hagen 3 (GH3).17) leads to increase in free IAA at the expense of IAGlu (IAA-glutamate) in the hypocotyl epidermis. This active IAA elicits shade- and high temperature-induced hypocotyl elongation largely independently of 3-IPA-mediated IAA biosynthesis in cotyledons. Our results reveal an unexpected capacity of local auxin metabolism to modulate the homeostasis and spatial distribution of free auxin in specialized organs such as hypocotyls in response to shade and high temperature [302]. In *Arabidopsis* (*A. thaliana*), the cytokinin signal is perceived by three membrane-located receptors named ARABIDOPSIS HISTIDINE KINASE2 (AHK2), AHK3, and AHK4/CRE1. Using a forward genetic approach, we isolated constitu-

tively active gain-of-function variants of the AHK2 and AHK3 genes, named repressor of cytokinin deficiency2 (*rock2*) and *rock3*, respectively. It is hypothesized that the structural changes caused by these mutations in the sensory and adjacent transmembrane domains emulate the structural changes caused by cytokinin binding, resulting in domain motion propagating the signal across the membrane. Detailed analysis of lines carrying *rock2* and *rock3* alleles revealed how plants respond to locally enhanced cytokinin signaling [309].

Early flowering time, a prolonged reproductive growth phase, and, thereby, increased seed yield suggest that cytokinin regulates various aspects of reproductive growth. In particular, it counteracts the global proliferative arrest, a correlative inhibition of maternal growth by seeds, through an as yet unknown activity of the hormone [309]. Maize is the highest yielding cereal crop grown worldwide for grain and silage. We showed that modulating the expression of the maize PLASTOCHRON1 (*ZmPLA1*) gene, encoding a cytochrome P450 (CYP78A1), results in increased organ growth, seedling vigour, stover biomass and seed yield. Transcriptome studies, hormone measurements and expression of the auxin responsive DR5rev:mRFP marker suggest that PLA1 may function through an increase in auxin. Detailed analysis of growth over time demonstrates that PLA1 stimulates the duration of leaf elongation by maintaining dividing cells in a proliferative, undifferentiated state for a longer period of time. The prolonged duration of growth also compensates for growth rate reduction caused by abiotic stresses [431].

Research projects: 8, 22, 26, 30, 32, 36, 54, 64, 68, 70, 98, 100, 118, 121, 122



Laboratory of Hormonal Regulations in Plants

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The Laboratory of Hormonal Regulations in Plants researches mainly two groups of plant signalling compounds (phytohormones), i.e. auxins and cytokinins, and their role in plant interactions with the environment. In 2015–2017, the laboratory investigated quantitative and qualitative aspects of mechanisms involved in their metabolism and transport, their signalling role in plant development and stress responses as well as identification of the intracellular machinery for the regulation of activity and distribution of auxin and cytokinin signalling components.

Auxin

In 2015–2017, the laboratory continued research on auxin, predominantly with respect to mechanisms of auxin action, regulation of auxin homeostasis and the dynamics of auxin carriers. We have contributed



In the picture (from the left):

Ing. Kateřina Malínská, Ph.D. / researcher, Mgr. Petr Klíma, Ph.D. / researcher, Bc. Jiří Danko / technician, Ing. Klára Hoyerová, Ph.D. / researcher, RNDr. Adriana Jelínková, Ph.D. / researcher, Mgr. Roman Skokan / Ph.D. student, Bc. Karolína Holečková / technician, Prof. RNDr. Eva Zažímalová, CSc. / researcher, Ing. Jozef Lacek / Ph.D. student, Ing. Petr Hošek, Ph.D. / postdoctoral fellow, RNDr. Alena Gaudinová / research specialist, Mgr. Sylva Přerostová / Ph.D. student, Eva Kobzová / technician, Mgr. Barbara Kramná / Ph.D. student, RNDr. Radomíra Vaňková, CSc. / researcher, Ing. Petr Skůpa, Ph.D. / researcher, Ing. Václav Motyka, CSc. / researcher, Mgr. Zuzana Vondráková / research specialist, RNDr. Martina Laňková, Ph.D. / research assistant, Ing. Karel Müller, Ph.D. / researcher, Ing. Petre Dobrev, CSc. / researcher, Ing. Eva Pokorná, Ph.D. / postdoctoral fellow, Bc. Marie Korecká / technician, RNDr. Jan Petrášek, Ph.D. / head of the laboratory, Ing. Miroslav Kamínek, CSc. / researcher.

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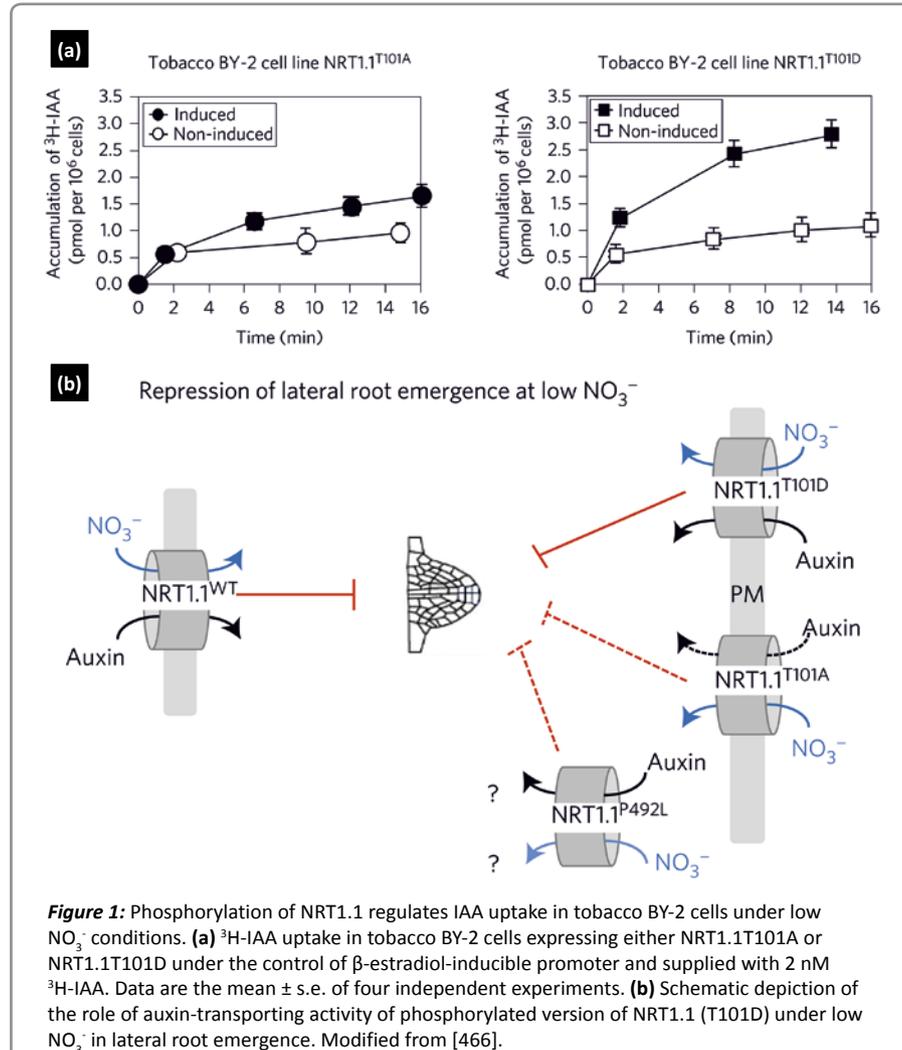
Mgr. Kamil Růžička, Dr. rer. nat. / researcher, Ing. Milada Čovanová, Ph.D., Dr. rer. nat. Katarzyna Retzer, Ph.D. / postdoctoral fellows, Ing. Roberta Filepová / research specialist, Mgr. Markéta Fílová / research specialist (maternity leave), Mgr. Matouš Glanc / Ph.D. student, Bc. Lucie Doležálková / diploma student, Mgr. Nikola Drážná / technician (maternity leave), Bc. Vojtěch Knirsch / technician, Daniel Nedvěd / BSc. student.

fundamentally to the characterization of the non-canonical member of the PIN family of auxin transporters, PIN6 [270]. In contrast to either plasma membrane (PM)-localized PINs with the long central hydrophilic loop facing the cytosol or the membrane of endoplasmic reticulum (ER)-localized PINs with the very short

central hydrophilic loop facing the cytosol, this PIN-type protein has been shown to localize to both the PM and the ER. This dual localization of PIN6 seems to correspond to its medium-size central hydrophilic loop. We have performed transport and auxin metabolic profiling assays in suspension-cultured cells and

revealed that PIN6 is involved in both auxin transport across the PM and control of intracellular auxin homeostasis. These findings indicate a unique position of PIN6 within the PIN family of auxin carriers and show how complex is the mechanism of control by auxins in various developmental processes.

Characterization of the modulation of auxin transport and auxin metabolic



profiles in relation to the mode of action of selected intermediates of the phenylpropanoid metabolic pathway, namely, derivatives of cinnamic acid, was carried out in detail. We have shown that 3,4-(methylenedioxy)cinnamic acid [276], as well as *cis*-cinnamic acid [428], inhibit auxin efflux from cells, thus adding novel compounds to native, endogenous auxin transport inhibitors and shedding light on the allelochemical properties of such substances. Moreover, 3,4-(methylenedioxy)cinnamic acid can also interfere with both phenylpropanoid and auxin metabolism, modifying in this way, the intracellular homeostasis of auxin and relevant phenylpropanoids [276]. In contrast to the above derivatives of cinnamic acid, we have also characterized the action of flavonols on auxin homeostasis and contributed to studies revealing that flavonols rhamnosylated in position 7 interfere with auxin metabolism but not with auxin transport [221].

We have also contributed significantly to further characterization of the nitrate transceptor (transporter&receptor/sensor) NRT1.1. As a continuation of our previous work showing the auxin-transporting activity of NRT1.1, we have characterized the role of NRT1.1 by testing the auxin transport of mutated versions carrying point mutations in the phosphorylation site at amino acid residue T101 [466]. We have shown by ^3H -IAA uptake assays that it is the phosphorylated form of NRT1.1 (T101D) that dominates in its auxin-uptake activity and that this function is needed for the repression of lateral root emergence under low NO_3^- (**Fig. 1**).

Using biochemical approaches, characterization of auxin binding to the putative auxin receptor, Auxin-Binding Protein1 (ABP1) was tested. Using several *abp1* mutants with substitutions in the metal core or in the hydrophobic amino acids of the auxin-binding pocket and neutral mutations, we showed that the ABP1 auxin-binding pocket is crucial for the developmental role of ABP1 and activating the downstream elements of the ABP1 signalling pathway [41].

Pharmacological tools were used to define substrate recognition for the auxin influx carrier AUX1. Using 2,4-D uptake assays and mathematical modelling in tobacco BY-2 cells, IC_{50} values were obtained for a collection of 35 auxinic compounds. This analysis revealed that many tested compounds with previously described herbicide action are not substrates for AUX1. 3D structural modelling of these compounds provided a rationale for these findings that are of commercial and ecological importance, namely for herbicide resistance management [345].

In the field of the cell biology of auxin carriers, we have uncovered their differential dynamics within the PM [225]. Using raster image correlation spectroscopy (RICS) and fluorescence recovery after photobleaching (FRAP) methods in suspension-cultured tobacco cells, we have shown that the mobility of AUX1 auxin influx

carrier is lower than the PIN1 auxin efflux carrier, which has been shown to be dependent on the cytoskeleton in contrast to AUX1 (**Fig. 2**). Introducing methods of super-resolution microscopy into *in vivo* analysis of root-specific PIN2 auxin efflux carrier allowed us to unravel the importance of cysteine residues for their PM localization [417]. The laboratory also identified a new tobacco homolog of adenosine ribosylation factor guanine-nucleotide exchange factor (ARF-GEF), NtGNL1a [55] and showed its role in the endocytosis of PIN1 auxin efflux carrier. In this work, screening of the effect of a spectrum of vesicle trafficking inhibitors was performed. Our data demonstrate the potential of tobacco BY-2 cells for selective mapping of ARF-GEF-regulated endomembrane trafficking pathways.

Cytokinins

In relation to cytokinins, attention was paid to biosynthesis and metabolism in a very wide range of developmental contexts. We performed comprehensive screening of non-vascular plants such as bryophytes [151] and cyanobacteria and algae [464] showing a weak gluco-conjugation of endogenous cytokinins (as well as endogenous auxins) and intensive production of *cis*-zeatin-type cytokinins together with strong oxidative degradation of auxins and we found apparent differences in metabolic strategies in vascular and non-vascular plants. We also participated in the characterization of cytokinin metabolism of the filamentous fungus, *Leptosphaeria maculans*, and reported, for the first time in the fungal kingdom, its two novel components, cytokinin oxidase/dehydrogenase and cytokinin adenosine kinase-related activities [441]. With respect to hormonal homeostasis under the combined influence of beneficial inoculants (arbuscular mycorrhiza and endophyte fungi) and a synthetic

cytokinin analogue thidiazuron, our analyses indicated their close correlations in the whole-plant-microbial context [425].

We revealed distinct priorities in glucosylation of *trans*-zeatin and *cis*-zeatin in feeding experiments in maize [201] demonstrating the preference of maize cytokinin glucosyltransferases for O-glucosylate zeatin in the *cis*-position thus forming *cis*-zeatin-O-glucosides resistant to irreversible cleavage by cytokinin oxidases/dehydrogenases, which may explain the over-accumula-

tion of *cis*-zeatins in maize tissues and organs. We have also characterized two tomato CK biosynthetic genes, SIIPT3 and SIIPT4, in homologous as well as heterologous systems, determined their different spatiotemporal expression patterns and *in vitro* enzymatic activity of their products during tomato plant development (**Fig. 3**) and in response to salt stress. Based on the obtained data, a hypothetical scheme of SIIPT3 and SIIPT4 action during early salt stress was proposed [155].

A large number of studies have concentrated on the

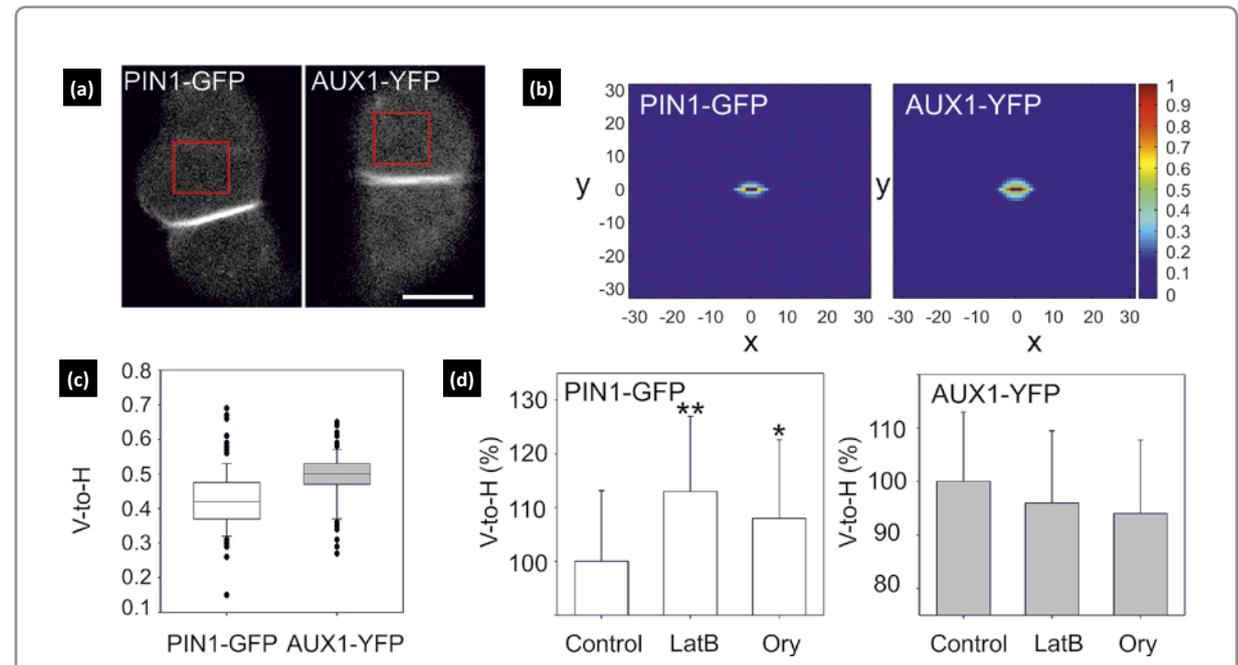


Figure 2: Raster image correlation spectroscopy (RICS) of integral PM auxin carriers. **(a)** Single confocal laser scanning microscopy sections of PM region of PIN1-GFP and AUX1-YFP with highlighted regions for RICS experiment (red square, 256 × 256 pixels), scale bar ~ 5 μm. **(b)** Two-dimensional correlation functions for PIN1-GFP and AUX1-YFP, the extension along y coordinate in AUX1-YFP suggests its slower motion in comparison with PIN1-GFP. **(c)** Box plot of V-to-H ratio between the horizontal and vertical amplitude for PIN1-GFP and AUX1-YFP. Individual outliers are shown as dots, error bars indicate 90th and 10th percentiles. Slower mobility of AUX1-YFP characterized by its higher V-to-H mean values in comparison with PIN1-GFP, values are significantly different ($p < 0.01$, $n = 80$, five biological repeats). **(d)** Mean values of V-to-H parameters showing the sensitivity of RICS measurements to applied cytoskeletal drugs latrunculin B (LatB; 0.5 μM, 30 min) and oryzalin (Ory; 20 μM, 30 min), note significant decrease in the mobility of PIN1-GFP after cytoskeletal drugs. Modified from [225].

role of cytokinin metabolism in plant development. We have participated in studying the mechanisms marking the transition from primary to secondary growth in hemp (*Cannabis sativa* L.) hypocotyls. Detailed analysis of phytohormones together with immunohistochemical and transcriptomic approaches revealed that cytokinins are closely linked to secondary growth and bast fiber development in hemp plants; cytokinin-N- and O-glucosides were involved in these processes at later stages of plant development when their levels were decreased indicating reduced inactivation of the total CK “pool” [165]. We have also contributed to identification and functional characterization of the tomato (*Solanum lycopersicum*) transcription factors SIDREB2 [200] and SIWRKY3 [340] demonstrating their important role in the control of tomato plant development and salinity response. Contrasting phytohormone profiling was demonstrated in relation to osmotic adjustment under salinity in the cultivated glycophyte tomato *Solanum lycopersicum* and its halophyte wild relative species *Solanum chilense* [335] as well as between two representatives of the Brassicaceae family, the salt-sensitive *Arabidopsis thaliana* and the salt-tolerant *Thellungiella salsuginea* [409].

The role of cytokinins and auxins in physiological and morphological processes in spontaneously regenerated centaury (*Centaurium erythraea* Rafn.) plants grown in vitro [288] was studied as well as their involvement in altered cytokinin homeostasis in AtCKX1- and AtCKX2-transformed centaury plants, where the overexpression of the AtCKX1 and AtCKX2 genes resulted in a substantially shifted *trans*-zeatin/*cis*-zeatin ratio [139]. In addition, we made a fundamental contribution to revealing close relationships between de novo organogenic response in two types of kohlrabi (*Brassica oleracea* var. *gongylodes*) explants in vitro and their cytokinin and auxin contents [24].

The Laboratory also collaborated in characterizing natural leaf senescence in common reed (*Phragmites australis*) plants grown in different habitats at a bay in the Baltic Sea [23] as well as in in vitro cultured tobacco (*Nicotiana tabacum*) plants [289]. Levels of cytokinins have been also studied during plant development, namely with respect to the role of new cytokinin response factors [253] and developmentally-regulated up-regulation of cytokinins during autotetraploidization of energy willow *Salix viminalis* [180].

Phytohormones in stress signalling

Apropos stress signalling and the role of phytohormones, main attention was paid primarily to the role

of cytokinins. The study of hormonal dynamics during early heat stress response revealed the crucial role of cytokinins in transient up-regulation of stomata conductance (**Fig. 4**) and subsequent stimulation of transpiration, which enables maintenance of leaf temperature lower than the environment, at least until defence mechanisms are activated [31]. Using transformants with induced over-expression of the cytokinin biosynthetic gene, cytokinin functions in the heat stress response were further characterized, including the effect on proteome [272].

Mechanisms of hormonal regulation of cold stress responses were studied in *Triticum monococcum* [170] and *Triticum aestivum* [356]. Enhanced drought toler-

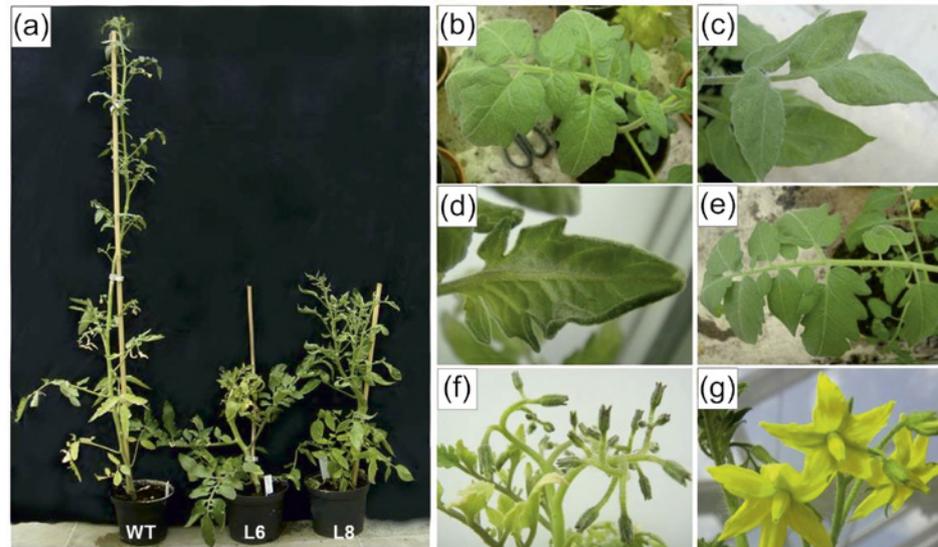


Figure 3: Phenotype of T0-generation 35S::SIIP3 tomato plants. L6 and L8 transgenic lines showed dwarfed aerial part with a branching phenotype (a), modified leaf shape (b, c, d) and flower inflorescence (f) compared to WT plants (e and g). Modified from [155].



ance achieved by over-expression of potato annexin STANN1 was associated with elevation of salicylic acid content and diminished suppression of active cytokinin levels during drought stress and with faster up-regulation of active cytokinins during recovery [128]. Comparison of salt stress response of glycophyte *Arabidopsis thaliana* and halophyte *Eutrema salsugineum* showed better “stress preparedness” of the halophyte, associated with constitutively elevated hydrogen peroxide content, the activity of several antioxidant enzymes [245], and higher levels of abscisic acid and jasmonic acid in the shoot apices [409]. The halophyte response to salt stress was faster and stronger, showing preferential protection of the shoot apex. Interactions between cytokinins, brassinosteroids and light (white and green) were followed in barley [328].

Plant responses to a deficit of nitrogen, an essential plant nutrient, can differ even in closely related species, as shown in *Plectranthus parviflorus* and *Plectranthus ambiguus*. While *Plectranthus parviflorus* responded to the lack of nitrogen by cytokinin suppression and chlorophyll degradation, *Plectranthus ambiguus* accumulated *cis*-zeatin type cytokinins and began gradual leaf abscission [240].

Cytokinins also play an important role in plant-pathogen interactions as well as in reaction to wounding. Transient elevation of isopentenyladenine- and *cis*-zeatin type cytokinins was determined after wounding of *Nicotiana attenuata* and especially during the response to herbivory [119], both in the attacked leaves and to a minor extent systemically. In this system, they were found to play a central role in the establishment

of optimal patterns of defence allocation in plants [315]. Hormonal profiles during plant defence to cyst nematode *Heterodera schachtii* showed that ethylene had a positive effect on nematode attraction, while jasmonic acid triggered the early defence in *Arabidopsis* [59]. Hormonal changes also underlie differences in susceptibility to shoot herbivores after the attack of the belowground pathogen (*Heterodera schachtii*) in *Arabidopsis* [60]. Our data also indicated the participation of auxin in plant-pathogen interactions. Knock-out of the expression of indole-3-acetic acid amido hydrolase IAR3 resulted in a decrease of IAA levels accompanied by enhanced basal defences and higher tolerance to *Phytophthora infestans* infection of *Nicotiana benthamiana* and tomato plants [177].

Other research activities

Our laboratory has also published several invited reviews. Firstly, we summarised current knowledge of native and synthetic inhibitors of plant hormone transport [212] and updated the Encyclopedia of Life Science article on polar auxin transport [481]. Secondly, an overview focused particularly on the discovery and earlier experimental work on cytokinins, namely discoveries of kinetin and *trans*-zeatin by the laboratories of Profs. Skoog and Letham [57]. Thirdly, the important role of *cis*-zeatin in abiotic and biotic stress responses was reviewed [118].

Last, but not least, both advanced microscopical and analytical methods have been further improved and optimized for their use in plants. In the field of microscopy, advanced fluorescence methods based on image correlation techniques [225] and super-resolution techniques [417] were firstly introduced for determination of the mobility of auxin carriers within PM. Analytical determinations of plant hormones utilizing our 2D HPLC/MS unit were used primarily for auxin and

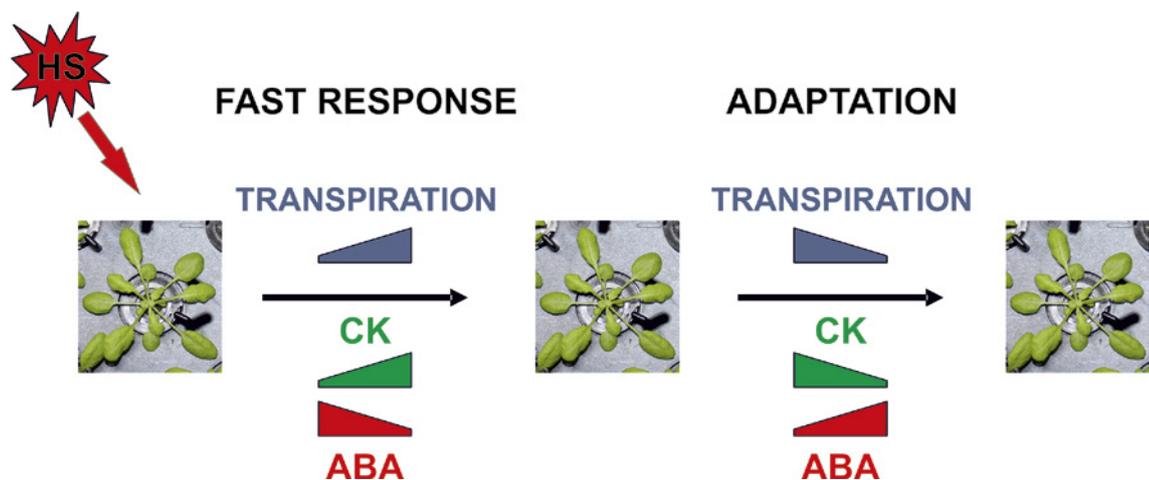


Figure 4: During early heat stress response cytokinins transiently up-regulate stomata conductance, which results in stimulation of transpiration. Activation of this cooling mechanism allows maintaining leaf temperature lower than the environment, until plant defence is activated [31, 272].



cytokinin determination [469], and other plant hormones in a large number of projects. These included hormonal responses to abiotic [31, 128, 155, 200, 245, 272] and biotic [59, 60] stresses, hormonal profiling of bryophytes [151], identification of unknown cytokinin metabolites [201], identification of the action of auxin transport regulators [347, 367] and determination of auxin metabolites in male gametophytes [181]. A new HPLC system obtained in 2015 allowed us to improve hormonal metabolism profiling. Furthermore, using this HPLC, we developed an efficient method for determination of DNA methylation and applied it to study the effect of stress on DNA methylation in white clover [191].

Collaboration with other institutions

In 2015–2017, the Laboratory has been involved in collaborations with a number of foreign and local laboratories, namely:

- Dr. Marc Behr (Luxembourg Institute of Science and Technology, Esch-sur-Alzette, Luxembourg): Role and function of phytohormones in secondary growth and bast fiber development; shared experimental material and joint papers [165].
- Břetislav Brzobohatý (CEITEC-Central European Institute of Technology, Mendel University in Brno, Brno, Czech Republic), shared experimental material and joint papers [31, 272].
- Dr. Kalina Danova (Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria): Interrelationships between endogenous phytohormonal status and secondary metabolites production; joint projects, shared experimental material.
- Dr. Ivana Dragičević, Dr. Milana Trifunović-Momčilov, Dr. Slavica Ninković, Dr. Branka Uzelac, Dr. Dragan Vinterhalter (Institute for Biological Research “Siniša Stanković”, Belgrade, Serbia): Involvement of phytohormones in physiological and morphological processes in *in vitro* cultured plants; shared experimental material and joint papers [24, 139, 288, 289].
- Prof. Jiří Friml (VIB, Univ. Ghent, Belgium and IST Austria, Klosterneuburg, Austria): Transport of auxin and its role in plant development; shared experimental material and joint papers [41, 270, 428].
- Prof. Gabor Galiba (Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Hungary), shared experimental material and joint papers [170].
- Prof. Alain Gojon and Dr. Philippe Nacry (Institut de Biologie Intégrative des Plantes, CNRS/INRA, Montpellier, France): Nitrate transporter NRT1.1 and its role in auxin transport; shared experimental material and joint papers [466].
- Prof. Martin Hof (J. Heyrovský Institute of Physical Chemistry CAS, Prague): Advanced fluorescence microscopy techniques, shared experimental material and joint papers [225].
- Prof. Joseph Kieber (Biology Department, University of North Carolina at Chapel Hill, USA), shared experimental material and joint papers [253].
- Dr. Vít Latzel (Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic), shared experimental material and joint papers [191].
- Prof. Stanley Lutts, Dr. Imène Hichri and Dr. Muriel Quinet (Earth and Life Institute, Université catholique de Louvain, Louvain-la-Neuve, Belgium): Molecular mechanisms involved in hormonal control of plant development and salinity response; joint projects, shared experimental material and joint papers [155, 165, 200, 335, 340].
- Prof. Autar Mattoo (USDA-ARS, Beltsville, MD, USA), shared experimental material, joint USDA project (Plant tissue analysis for quantification of phytohormones in tomato fruits).
- Dr. Stefan Meldau (KWS SAAT AG, Einbeck, Germany), shared experimental material and joint papers [118, 119].
- Prof. Richard M. Napier (School of Life Sciences, University of Warwick, Coventry): Auxin analogs and their interference with auxin transport machinery, structure-function studies of auxin influx and efflux carriers; joint projects, shared experimental material, sabbatical stay in the laboratory, joint papers [276, 345, 417, 428].
- Prof. Christoph Ringli (Institute of Plant Biology, University of Zurich, Switzerland): Auxin transport and metabolism assays for the identification of the role of flavonols; shared experimental material and joint papers [221].
- Prof. G. Eric Schaller (Department of Biological Sciences, Dartmouth College, Hanover, USA), shared experimental material and joint papers [253].
- Dr. Bartel Vanholme (Department of Plant Systems Biology, VIB, Ghent, Belgium): Auxin transport and metabolism assays for the identification of new regulators of auxin homeostasis; shared experimental material and joint papers [276, 428].
- Prof. Krzysztof Wiczeorek (Division of Plant Protection, Department of Crop Sciences, BOKU, UFT Tulln, Austria), shared experimental material and joint papers [59, 60].
- Dr. Lenka Závěská-Drábková (Institute of Botany, Czech Academy of Sciences, Průhonice, Czech Republic), shared experimental material, joint projects and papers [151, 464].

Research projects: 40, 41, 43, 45, 49, 72, 87, 93–95, 101, 113



Laboratory of Mass Spectrometry Service Department

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The main task of the service laboratory is to provide chemical analyses for research groups at the home institution and to collaborate with other research institutes and universities. Currently, we also provide analytical services to other entities in the Czech Republic and abroad.



Figure 1: LC-MS system TSQ Quantum Ultra AM with Rheos 2200 chromatograph and CTC HTS autosampler.



In the picture (from the left):

Ing. Jiří Malbeck, CSc. / head of the laboratory, Ing. Alena Trávníčková / technician, Ing. Bedřich Pešek / technician (till August 2017).

The laboratory was established in 2007 by separation from the larger research division in which the laboratory team worked as an analytical unit. From the beginning, the laboratory was defined as a service centre for carrying out special instrumental analyses and development of new analytical methods. We focus on quantitative analysis of biologically active compounds in plant matrices using chromatographic methods with mass spectrometric detection. Our analytical results are incorporated into the publications of colleagues from our institute as well as external scientific institutions. Included in our work is analysis of cytokinins [24, 31, 124, 139, 288], polyamines [175], auxins, abscisic acid and its metabolites [35] or phenolic acids.

Laboratory instrumentation

The main part of the laboratory equipment consists of two LC-MS systems, one GC-MS system and one HPLC instrument with fraction collector device (see figures). All instruments are equipped with auto-samplers.

For the preparation of samples, we use the rotary vacuum concentrator Christ Alpha RVC and rotary evaporator Büchi Rotavapor R-200. The analytical Balance Mettler XP26 with a sensitivity of 2 µg is ideal for the preparation of expensive analytical standards.



Figure 2: LC-MS system LCQ with Rheos 2000 chromatograph and CTC HTS autosampler.



Figure 3: GC-MS system Polaris Q with Trace GC chromatograph and CTC combi PAL autosampler.



Figure 4: HPLC Agilent 1200 with fraction collector Gilson.



Laboratory of Pathological Plant Physiology

Head of laboratory:

doc. Ing. Lenka Burketová, CSc.

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The Laboratory of Pathological Plant Physiology has been studying plant-microbe interactions for many years. Our main interest is signalling pathways implicated in defence responses and induced resistance to pathogens. Besides academic collaboration, we cooperate with institutes of applied research for effective transfer of our results. Our recent research has been focused on: (1) the role of pathogen effectors and hormonal network interplay in plant-microbe interactions, (2) cytokinin metabolism in a fungal phytopathogen *Leptosphaeria maculans*, (3) phospholipid signalling in biotic stress and (4) induced resistance to plant pathogens.

(1) We have uncovered the function of the effector molecule AvrLm4-7 of a hemibiotrophic fungal pathogen *L. maculans* in its interaction with *Brassica napus*, (2) identified key components of cytokinin metabolism in *L. maculans*, (3) showed interconnection between defence signalling regulated by salicylic acid and the phospholipid signalling system based on phospholipase D activity, and (4) found candidate elicitors of *B. napus* resistance secreted by *L. maculans* *in vitro*.



In the picture (from the left):

Standing: Ing. Barbora Jindřichová, Ph.D. / junior scientist, Ing. Martin Janda, Ph.D. / postdoctoral fellow, Doc. Ing. Lenka Burketová, CSc. / head of the laboratory, Ing. Lukáš Maryška / Ph.D. student, Ing. Daniel Stehlík / Ph.D. student, Ing. Lucie Lamparová / technician.

Sitting: Mgr. Lucie Trdá, Ph.D. / postdoctoral fellow, Mgr. Hana Krutinová / Ph.D. student, Mgr. Tetiana Kalachová, Ph.D. / postdoctoral fellow.

Not in the picture:

Ing. Vladimír Šašek, Ph.D. / postdoctoral fellow (until 2015).

The role of pathogen effectors and hormonal network interplay in plant-microbe interactions

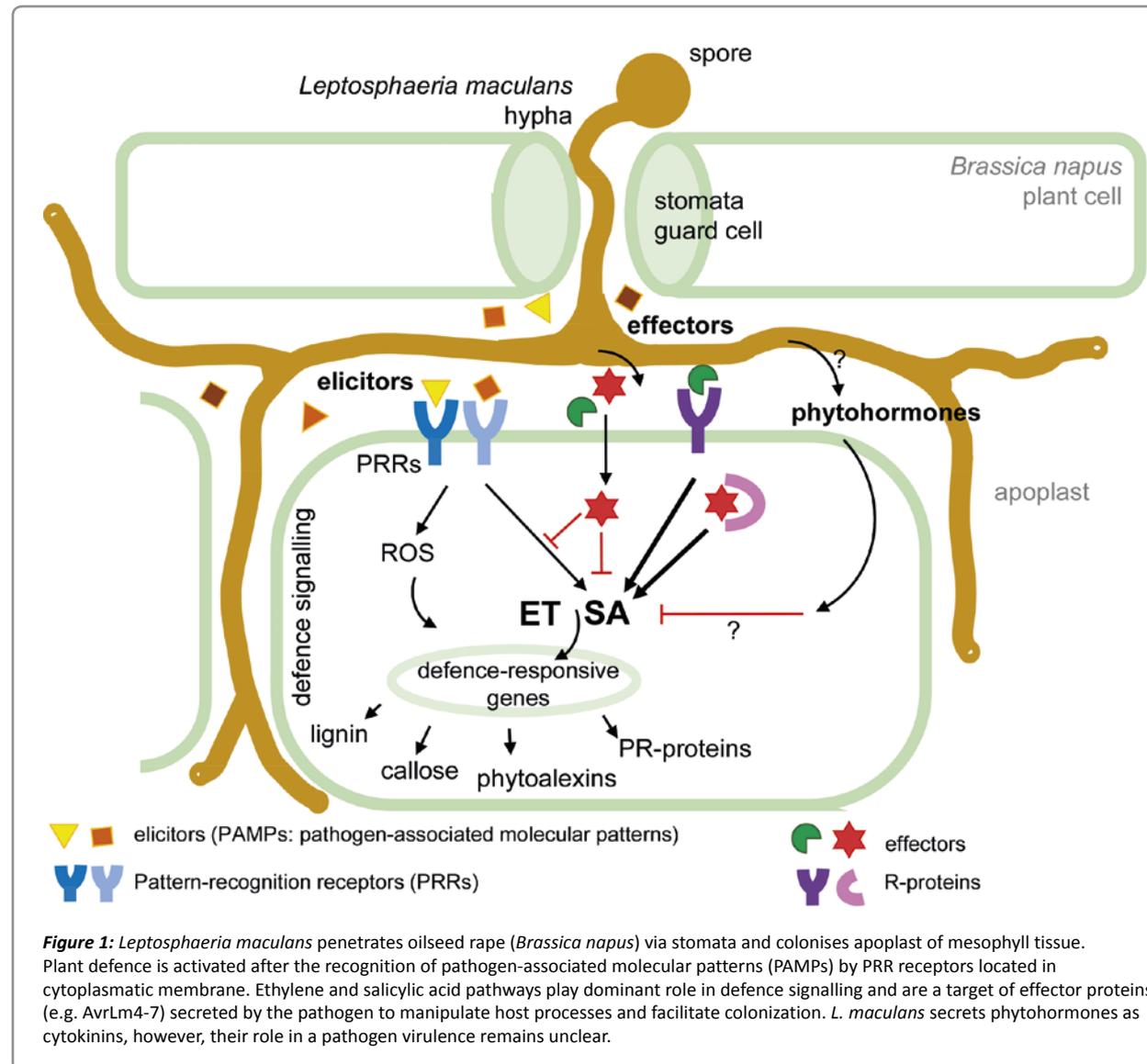
Adapted pathogens have acquired effector proteins for overwhelming basal plant resistance, however, the mode of action of these effectors is incompletely

understood. Since our laboratory has been involved in the study of *B. napus* interaction with its pathogen, the hemibiotrophic ascomycete *L. maculans*, we focused on a widely spread *L. maculans* effector protein AvrLm4-7 that is significantly involved in the

virulence (**Fig. 1**). Using two isogenic lines, we clearly showed that AvrLm4-7 affected various components of the plant immune system such as salicylic acid- and ethylene-dependent signalling, the pathways regulating effective defence against *L. maculans* infection, and reactive oxygen species (ROS) accumulation in infected tissues (**Fig. 2**) [236]. Our results suggest that the increased aggressiveness of the *L. maculans* isolate containing AvrLm4-7 could be caused by defects in ROS-dependent defence and/or linked to suppressed SA and ET signalling. This work is a part of an ongoing complex study on the proteinaceous and hormonal effectors of this pathogen.

Characterization of novel components of cytokinin metabolism in the fungus *Leptosphaeria maculans*

Not only plants but also diverse microorganisms are able to produce phytohormones, including cytokinins (CKs), though knowledge of their biosynthesis and metabolism is still limited. We demonstrated that *L. maculans* produces a wide range of CKs *in vitro* and modifies the CK profile in infected *B. napus* tissues. Using functional genomics, enzymatic and feeding assays with CK bases supplied to culture media, we showed that *L. maculans* contains a functional: (i) isopentenyltransferase (IPT) involved in cZ production; (ii) adenosine kinase (AK) which plays a role in phosphorylation of CK ribosides to nucleotides; and (iii) CK-degradation enzyme cytokinin oxidase/dehydrogenase (CKX). Our data further indicate the presence of *cis-trans* isomerase, zeatin O-glucosyltransferase(s) and N6-(Δ^2 -isopentenyl)adenine hydroxylating enzyme [441]. This research provides deeper insight into the CK metabolism in fungi.



Phospholipid signalling in biotic stress

Our previous work revealed interconnection between the salicylic (SA) signalling pathway and a phospholipid signalling system. In earlier research on *Arabidopsis* suspension cells, we showed that *n*-butanol, which specifically modulates phospholipase D activity, significantly suppresses the transcription of the pathogenesis related (PR-1) gene, generally accepted as the SA pathway marker. In the presented study, we investigated the site of *n*-butanol action in the SA pathway. We were able to show in *Arabidopsis* plants treated with SA that *n*-butanol inhibits the transcription of defense genes (*PR-1*, *WRKY38*). Fluorescence microscopy of *Arabidopsis thaliana* mutants expressing *35S::NPR1-GFP* (nonexpressor pathogenesis related 1) revealed significantly decreased nuclear localization of NPR1 in the presence of *n*-butanol (**Fig. 3**) [50].

The transcriptional regulation of *Brassica napus* phospholipase D (PLD) following treatment with defense-related stimuli was studied. We cloned eight *B. napus* genes encoding members of PLD beta, gamma, and delta isoforms and carried out a phylogenetic analysis with its ancestor species, *Brassica rapa* and *Brassica oleracea*, and with the model plant *Arabidopsis thaliana*. Remarkably, the genes encoding the PLD gamma and PLD beta isoforms were up-regulated by stimuli associated with the SA signalling pathway [51].

This work was done in cooperation with prof. Olga Valentová of the University of Chemistry and Technology Prague (UCT Prague).

Induced resistance to plant pathogens

Considering the demand for alternatives to pesticides, induced resistance to pathogens was a prospective direction for our research [21]. We searched for compounds that induce resistance in plants with a focus on potential fungal elicitors secreted by *L. ma-*

culans into cultivation medium. Their ability to induce resistance in *Brassica napus* against this pathogens was evaluated and the candidate elicitors identified by mass spectrometry analysis following fractionation by either ion-exchange chromatography or isoelectric focusing. The most active fractions obtained by both separation procedures showed predominantly en-

zymes that may be involved in the degradation of plant cell wall polysaccharides [237].

Collaboration with applied research

Long-term collaboration with the Institute of Oil-seed Crops (OSEVA Pro. Ltd.), OSEVA Development and Research Ltd., and the Institute of Plant Production

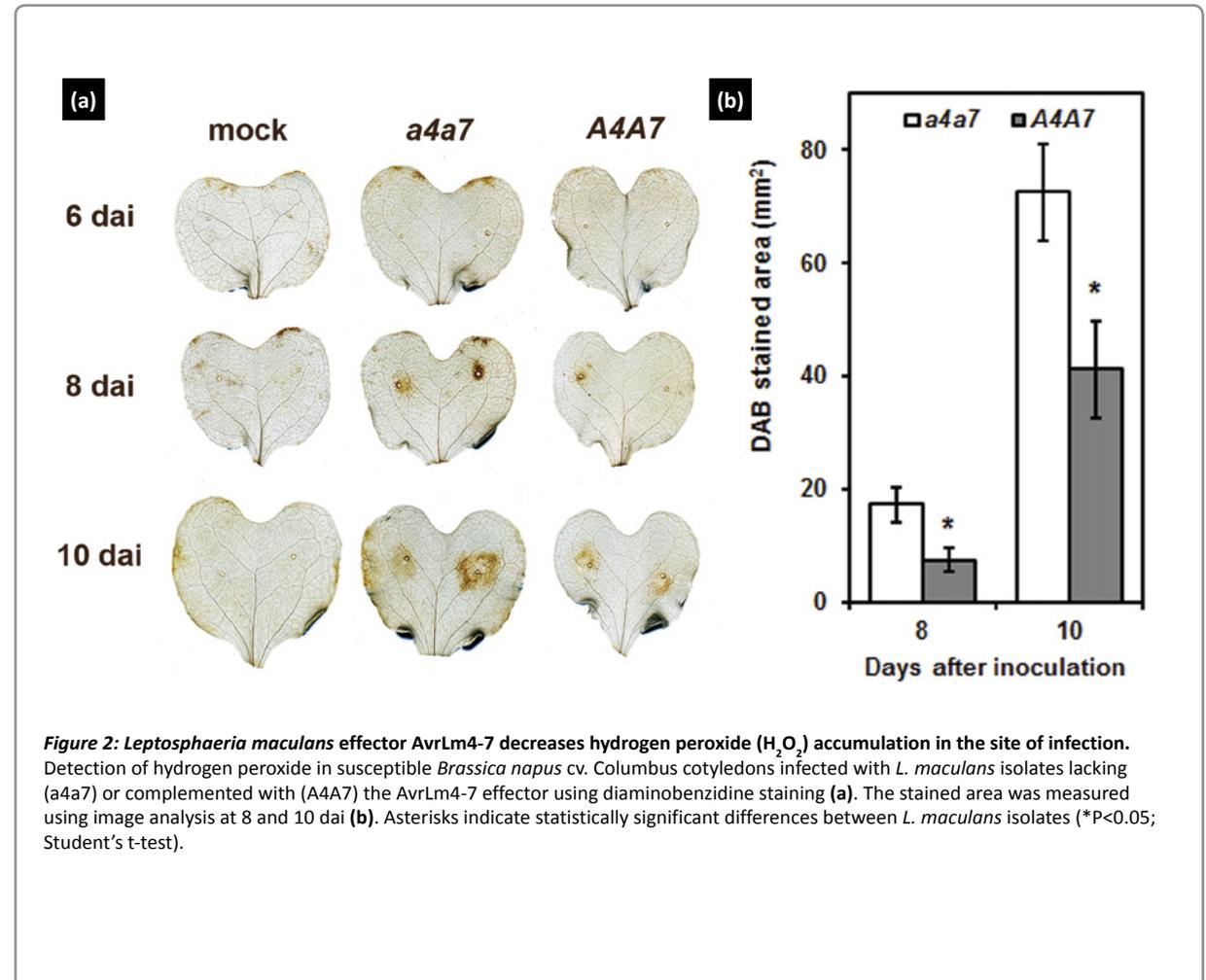


Figure 2: *Leptosphaeria maculans* effector AvrLm4-7 decreases hydrogen peroxide (H₂O₂) accumulation in the site of infection. Detection of hydrogen peroxide in susceptible *Brassica napus* cv. Columbus cotyledons infected with *L. maculans* isolates lacking (a4a7) or complemented with (A4A7) the AvrLm4-7 effector using diaminobenzidine staining (a). The stained area was measured using image analysis at 8 and 10 dai (b). Asterisks indicate statistically significant differences (*P<0.05; Student's t-test).

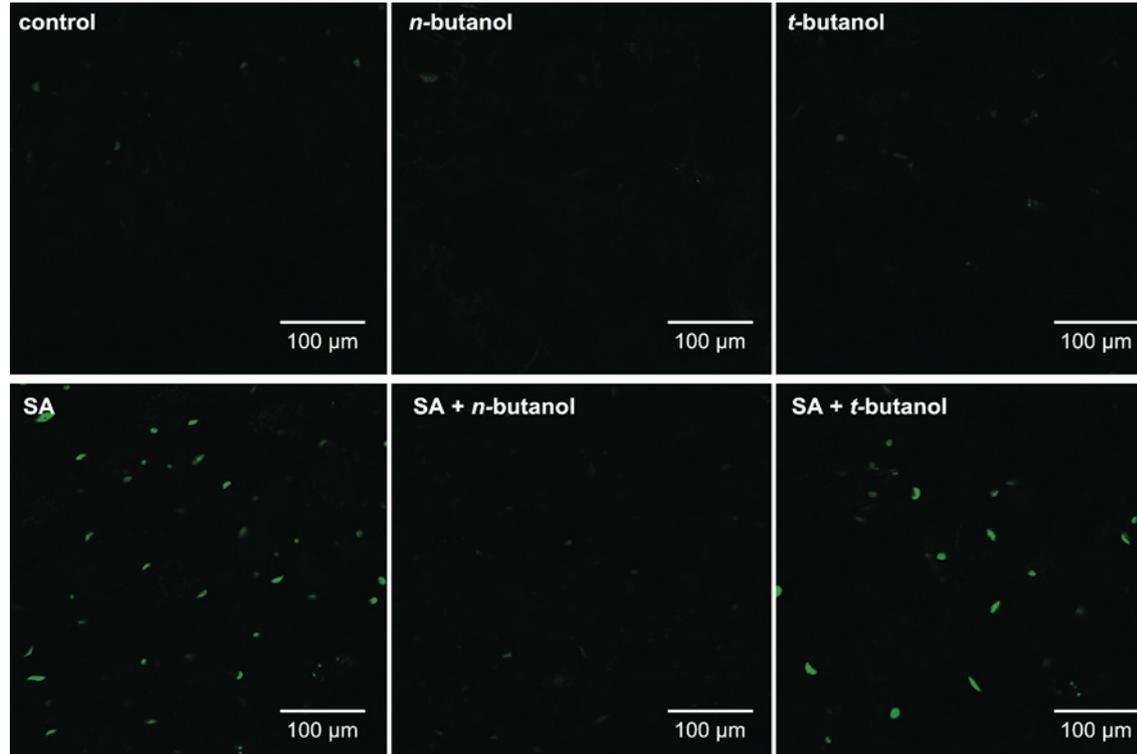


Figure 3: Translocation of NPR1 proteins into nucleus is significantly decreased in the presence of *n*-butanol. SA and *n*-butanol on the localization of NPR1. 10-day-old seedlings of *A. thaliana* 35S::NPR1-GFP mutants were treated for 6h with fresh MS medium (control), 1% *n*-butanol (*n*-but), 1% *t*-butanol (*t*-but), 250 μM NaSA (SA), 250μM NaSA (SA) and 1% *n*-butanol or 1% *t*-butanol.

Prague led to patent applications granted by the Industrial Property Office of the Czech Republic in 2016 [patents 3, 4]. The subjects of these applications are bio-based resistance-inducing compounds/molecules useable as active substances in potential crop-protecting preparations. The results of our laboratory tests were validated in field experiments by our collaborators. The source material for inducer preparations originated in Tomas Bata University in Zlín (protein inducers) or prepared in collaboration with UCT Prague (mycelial elicitors).

International collaboration

International collaboration is of a great importance for the laboratory. Research on phospholipid signalling has been proceeding in close collaboration with Dr. Eric Ruelland from the Université Paris Est-Créteil (UPEC). The work involving *L. maculans* was carried out in collaboration with the laboratory of Dr. Thierry Rouxel, INRA, Centre de recherche de Versailles-Grignon, France, as shown by a joint publication [236]. Other traditional close collaborations of the laboratory have been with the Plant Protection Institute of the Hungarian Academy of Sciences in Budapest (Dr. Jozska Fódor) on ROS, Eötvös Loránd University in Budapest, Hungary (Dr. Károly Bóka) on electron microscopy, on fungal effectors with Dr. Peter Solomon from The Australian National University, Canberra, Australia, and on SA signalling with Dr. Kenichi Tsuda from the Max Planck Institute in Köln, Germany.

Research projects: 4, 19, 46, 52, 79, 102, 116, 122



Laboratory of Plant Biotechnologies

Head of laboratory:

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From 2015 to 2017, this laboratory focused predominantly on plant-xenobiotic interactions and secondary metabolites in plants. The research was funded by 19 projects in total from different grant agencies and included the following.

1) Detailed study of plant metabolism and plant stress responses, enabled by our success in obtaining OPPK grants for purchase of the most sophisticated equipment – 2D GC-MS and LC-MS/MS (Figs. 1, 2). Proteomic and transcriptomic methods became routinely used at this time.

2) Application of our extensive knowledge of the use of plants in environmental protection (phytoremediation) achieved on a laboratory scale for semi-real and real conditions. This is part of our contribution resolving environmental contamination generally. We were able to continue and extend our international cooperations via joint projects, mainly with Israel, the USA, China and other countries. We have taken part in 16 COST Actions, which gave us wide opportunity to cooperate with scientists from other European and COST countries. These cooperations led, among others, to re-election of Tomáš Vaněk as a Board member of the European Plant Science Organization (EPSO). We also took part in the prepara-



In the picture (from the left):

Front row: Mgr. Marcela Dvořáková, Ph.D. / researcher, Ing. Kateřina Mořková / technician, Zdena Hornychová / technician, Ing. Lenka Langhansová, Ph.D. / researcher, Mgr. Radka Podlipná, Ph.D. / researcher, RNDr. Mgr. Tomáš Vaněk, Ph.D. / head of the laboratory, Ing. Šárka Petrová, Ph.D. / researcher, Ing. Jan Rezek, Ph.D. / researcher.

Second row: RNDr. Mgr. Petr Soudek / researcher, Ing. Přemysl Landa, Ph.D. / researcher, Mgr. Petr Maršík, Ph.D. / researcher, Mgr. Daniel Haisel, Ph.D. / researcher, Ing. Miroslav Šíša, Ph.D. / researcher.

Not in the picture:

Mgr. Marie Kvasnicová, Ph.D. / researcher, Bc. Alexandra Solodovnikova, Bc. Jekaterina Ten, Bc. Milan Novák, Bc. Žaneta Antonínová / master students, Mgr. Aneta Hrdinová, Mgr. Eliška Syslová, Ing. Karolína Pumprová / Ph.D. students.

tion of 5 new COST Actions as well as preparation of 3 mutual proposals of Czech-USA cooperation. In total, we published 44 papers in international impacted journals.

In relation to plant-xenobiotic interactions, detailed study of the fate of pharmaceuticals in plants was car-

ried out with the aim of using the results for environment protection.

In the case of the human non steroidal anti-inflammatory drug (NSAID) ibuprofen, we were able to identify more than 300 different metabolites from detoxification phases I and II [379]; see **Fig. 3**.

An integral part of this on going research is evalua-



tion of the fate of xenobiotics in plants at the enzymatic level. We emphasized the environmental importance of plants in the detoxification of veterinary drugs in an extensive review which evaluated the results of studies on the transport and biotransformation of veterinary drugs in plants. The risks and consequences of veterinary drug escape into the environment and the potential role of phytoremediation technologies were considered and future perspectives outlined [15].

Secondary metabolites of plant origin could become starting compounds for the development of new drugs. We compared two widely used pharmacophore modelling and screening software programmes for prediction of the most physiologically effective compounds, as the activities and stability of many plant secondary metabolites can be improved by suitable chemical modification. We also focused on characterization of natural products with anti-inflammatory, anti-proliferative, anti-cancer or anti-microbial properties, which could be used as natural substituents for synthetic pharmaceuticals; see e.g. [434].

The results achieved in this area were utilized, among others, for submission of two international patents in cooperation with the team of D. Schuster from Innsbruck University.

Description of selected results

Plants and environment

The main focus of the Laboratory research has been study of the mechanisms of plant-xenobiotic responses to, enable use of plants for phytoremediation purposes and optimization of individual processes.

The effect of exogenous polyamine (putrescine) on the accumulation of heavy metals in crops was tested. The levels of cadmium, zinc and iron were measured in different vegetables grown in hydroponic medium supplemented with heavy metals +/- putrescine. The



Figure 1: 2D gas chromatography coupled with mass detection will be performed on LECO Pegasus® 4D GCxGC-TOFMS system equipped with Cooled Injection System CIS 4 (GERSTEL), multipurpose sampler (GERSTEL) and Dynamic Headspace with Thermal Desorption Unit (GERSTEL). This equipment is used for precise analysis of predominantly hydrophobic and volatile compounds.

potential daily intake, based on the average daily consumption of various vegetable species, and the influence of polyamines on metal uptake were calculated [273].

To evaluate the impact of different soil amendments on metal toxicity, the effect of toxic metals and three different biochars on seed germination was studied in three sorghum cultivars (*Sorghum bicolor* L.). The toxicity of cadmium, copper and lead was tested using

standard ecotoxicity tests. The results showed that beech tree biochar was the most efficient in reducing the toxicity of the tested heavy metals [427, 496].

The hypothesis that halophytes are more tolerant to Cd and thus very suitable for remediation was tested by comparing Cd accumulation in the halophyte *Juncus gerardii* and the glycophyte *Juncus inflexus* [290]. Utilization of high-yielding crops for phytoextraction of selected metals was also studied in real conditions

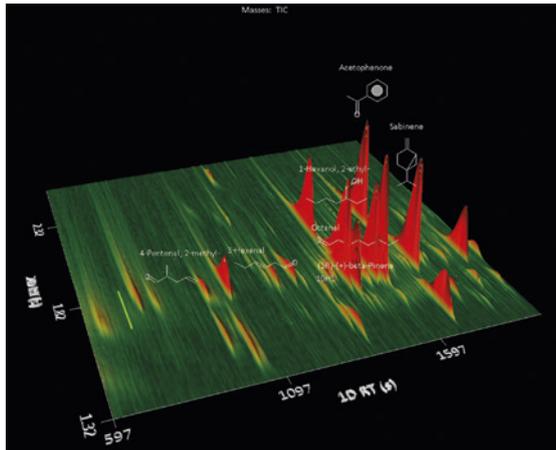


Figure 2: Attack of some pathogens or exposition to certain stresses result in the release of volatile organic compounds (VOC) of signal character. These compounds are spread among plants in a canopy, where they can induce defence in yet non-attacked plants or they can attract natural enemies of pathogens, mainly insects. In this picture you can see stress-depending production of VOC in *Salvia officinalis*.

[382]. Contribution to our knowledge of the mechanisms of stress response in plants to radionuclides was discussed in [383].

Apart from heavy metals, serious soil and water contamination is also caused by organic xenobiotics, especially pharmaceuticals. We have focused on both veterinary and human drugs.

A review covering drugs commonly used in veterinary practice with respect to their environmental impact and a summary of their toxic effect on plants is published [163]. The fate of anthelmintics on plants and the environment has been covered in three other papers [252, 279, 378].

Widely used human pharmaceuticals, such as NSAIDs, are taken up and metabolized by higher plants. The results obtained in laboratory conditions in both *in vitro* and hydroponic experiments [368] were confirmed in real conditions in a constructed wetland for ibuprofen and other widely spread acidic NSAIDs – naproxen, ketoprofen and diclofenac [380, 483]; see **Fig. 4**.

Other compounds studied due to their ubiquity in the environment were dinitrotoluene [110] and flame retardants and PAHs [103, 403].

Given the increasing use of nanoparticles for multiple purposes and their uncontrolled release into the environment, we evaluated the impact of nanosized metal particles on plant behaviour by comparison of the transcriptomic response of *Arabidopsis thaliana* roots to ZnO nanoparticles, bulk ZnO, and ionic Zn²⁺. The similarity of the transcription profiles and the increasing number of transcript changes which correlated with the increased concentration of Zn²⁺ in cultivation medium, indicated that released Zn²⁺ may substantially contribute to the toxic effect of ZnO

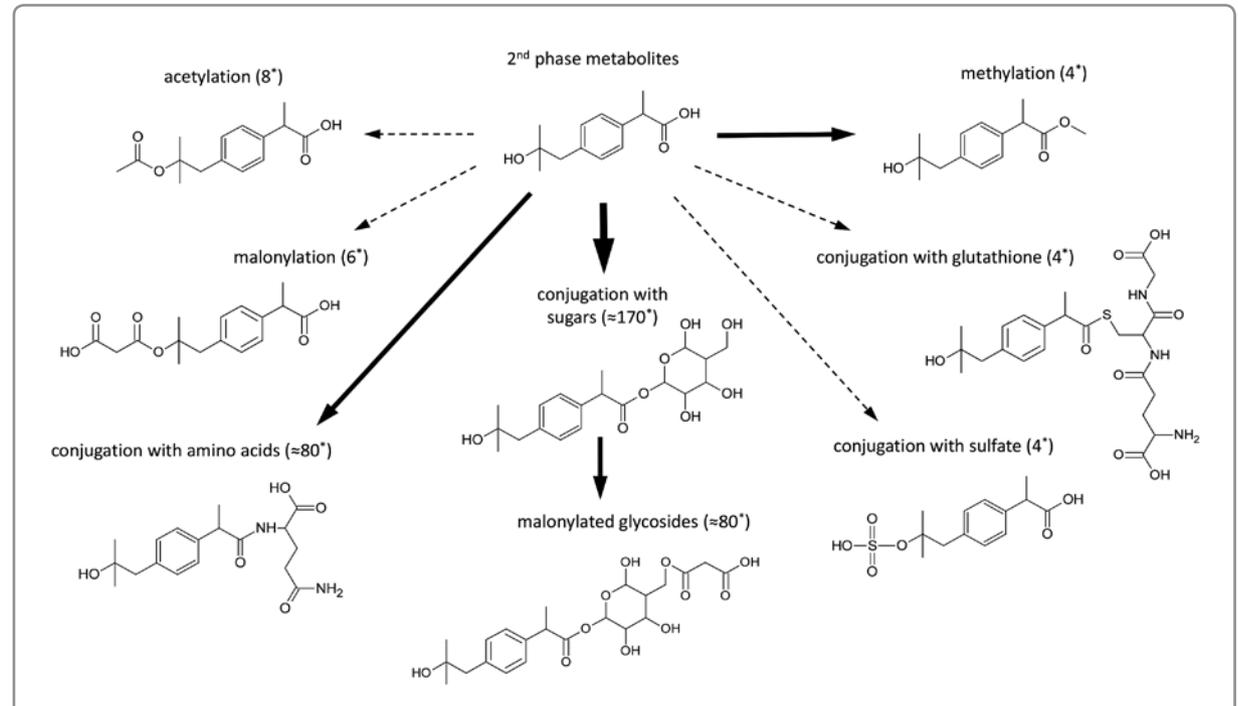


Figure 3: The uptake and metabolism of ibuprofen (IBU) by plants at the cellular level was investigated using a suspension culture of *Arabidopsis thaliana*. Almost all IBU added to the medium was metabolized or bound to insoluble structures in 5 days. More than 300 metabolites were determined by liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis, and most of these are first reported for plants here.

nanoparticles, as the decisive role of particle size has not been demonstrated [73]. The effect of ZnO nanoparticles on the hormonal pool of *Arabidopsis thaliana* and its potential influence on crop plants has been described in [445]; see **Fig. 5**.

The toxicity of various metal oxide ENPs (Al_2O_3 , CuO , Fe_3O_4 , MnO , TiO_2 , and ZnO), including nanowires, their bulk counter-particles and soluble metal salts was tested using germinating seeds of *Sinapis alba* L. Fe_3O_4 , TiO_2 , MnO_2 , and Al_2O_3 ENPs did not negatively affect seed germination at any tested concentrations (up to 100mg/l). However, CuO and ZnO ENPs showed a dose-dependent inhibition of germination. A comparison of ENPs with bulk materials did not reveal significantly higher ENP toxicity [224].

The effect of ENPs on plant physiology was studied at the transcriptome level as well as in a model system of wetland plants [319, 369]. A general overview of the effect of nanoparticles on plants was also published [28].

Some of the above results can be utilized for solving real environmental problems associated with both soil and water contamination. Based on our Laboratory results, we successfully completed a project “Biotechnology system for agricultural waste-waters cleaning and reuse”, supported by the Technology Agency of the Czech Republic. The results raised the possibility of recycling agricultural water and to reuse it with benefits to solving the problem of water shortage in agriculture.

Plant metabolites

Secondary metabolites, including many pharmaceuticals, are of plant origin. They could become the starting compounds for development of new drugs. Pharmacophore modelling has become an integrated tool in drug discovery.



Figure 4: Accumulation and/or degradation of Praziquantel (PZQ) in plants were determined using *Phragmites australis* *in vitro* cultivated plants. In case of initial PZQ concentration 20 mg L^{-1} , 90 % was removed from liquid media within 21 days. Laboratory results were confirmed in real scale using the constructed wetland (CW), where PZQ was completely removed until the first purification pond. This result offers a promising possibility to use CW for PZQ removal from agricultural as well as domestic waste-waters.

We focused our effort on characterization of natural products with anti-oxidant and anti-inflammatory properties which could be used as natural substituents of synthetic pharmaceuticals.

Antioxidant activity, total sulfite content and concentration of 14 phenolic compounds were compared in selected native Georgian red and white wines as well as in wines commonly produced in Europe. Georgian red wines exhibited higher antioxidant capacity and were richer in several polyphenols, while the concentration of *trans*-resveratrol was lower than in European red wines. Our findings showed significant differences among red wines of different origin and cultivar. However, the winemaking technology was the most important factor in the case of white wines [134].

The effect of the quinone anti-oxidants tert-butylhydroquinone and 2,5-di-tert-butylhydroquinone on arachidonic acid metabolism was evaluated in *in vitro* conditions [69].

The impact of dietary stilbenes on 5-lipoxygenase and cyclooxygenases activities was also studied *in vitro*, in order to exclude the effects of potential plant contamination [70].

In connection with cooperation with Zhejiang University and China Tobacco Zhejiang Industrial. Co., Ltd., (Hangzhou, China), the biological activity of the TCM component, *Myrica rubra* and its potential utilization were described in [370]; see **Fig. 6**.

Computational methods can be applied with advantage to drug development for the identification of novel pharmacologically active candidates and also for the prediction of pharmacokinetic properties and potential adverse effects, aiding in this way prioritizing and identifying the most promising compounds. In this respect, several programs, that is, common virtual screening methods, were compared. Our results suggest that the rational selection of method may be

a powerful strategy for maximizing the success of the research project, closely linked to its aims [62].

During the screening of plants as a source of anti-inflammatory compounds, the phenolic compound, miconidin acetate, isolated from flower buds of Brazilian plant *Eugenia hiemalis*, was found to inhibit the pro-inflammatory enzyme 5-lipoxygenase more effectively than the reference inhibitor, zileuton. The results showed that flower buds of *E. hiemalis* are an interesting source of anti-inflammatory compounds, mainly of miconidin acetate [509].

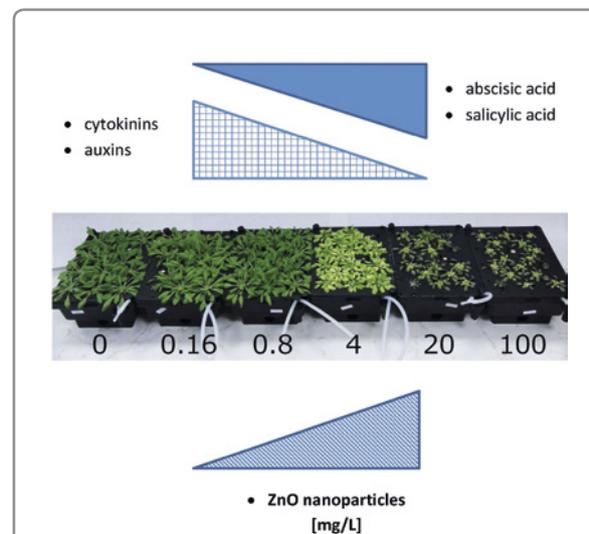


Figure 5: As plant interactions with the environment are mediated by plant hormones, complex phytohormone analysis has been performed in order to characterize the effect of ZnO nanoparticles on *Arabidopsis thaliana* plants. Growth suppression of *Arabidopsis* plants correlates with ZnO nanoparticle content and growth inhibition is associated with decrease of cytokinins and auxins in apices. Nanoparticle stress effects are indicated by abscisic and salicylic acids elevation.

Part of our effort was focused on preparation of compounds with potential anti-cancer effects.

Histone deacetylase inhibitors can be used for this purpose, too. We reviewed the potential of histone deacetylase inhibitors, modifiers of gene expression, in cancer treatment. The focus was cancer stem cells, which are believed to be responsible for tumor relapse, metastasis and resistance to therapeutics [182].

The synthesis of tetracyclic diterpenoids which is relevant for pharmacologic purposes, too, was developed by Dr. Šiša [271].

In terms of the project “LD14127 Synthesis of strigolactone derivatives”, 9 analogues of strigolactones were prepared by M. Dvořáková and some of these will be patented prior to publication. A review of the biological activities of strigolactones, plant secondary metabolites which act as plant hormones and symbiosis inducers, has been published. This covered the functions of strigolactones in the regulation of plant growth, resistance to stress, nutrient uptake, as well as their effect on host recognition by parasitic plants. A following paper described the synthesis of some strigolactone analogues as well as their biological activity [34, 326].

Societal impacts

Our comprehensive results in the area of utilizing plants in environment protection (phytoremediation) enabled us to extend our efforts to semi-real and real conditions. The aim was to contribute to solving environmental problems generally, not only in the Czech Republic.

The Technology Agency funded two of our projects directed to practical tasks. The first was an evaluation of the fate of plastic materials defined as biodegradable in real conditions (Bioplast). The results confirmed, unfortunately, the many caveats about these materials,



which are in fact biodegraded very slowly and release toxic chemicals into the environment. Our results will be utilized by the Ministry of Environment for the development of environmental policy as well as by the company ECO-COM, which is responsible for the collection and separation of waste materials in the Czech Republic.

In the frame of the second project, “Bioclean”, we have demonstrated the ability of constructed wetlands to clean agricultural waste waters and remove not only “standard” contamination but also the residues of veterinary and other pharmaceuticals. We have confirmed the possibility of utilizing this full scale system not only for decontamination but also for recycling of water, which can be used directly at the farm for irrigation, with high economic impact.

Our laboratory also initiated, submitted and was finally funded by the Czech Technology Agency large project “Support for the process of commercializing the results of research and development at the Institute of Experimental Botany AS CR v.v.i.”.

The general problem with water shortage led us to utilize a plant and phytotechnology method to solve it. Our project “Utilization of R&D results of the Institute of Experimental Botany AS CR v.v.i.” was supported by from EU structural Funds and the Prague municipality and started on January 1, 2017.

More than 19 students have been trained in our unit, at Bachelor, Masters and Doctoral levels, within the frame of our research in plant protection as well as plant bioactive compound synthesis. In terms of the Erasmus+ programme, 4 students from the UK, Italy and Poland have worked in our Laboratory.

We have had considerable impact on the environmental policy due to T. Vaněk’s membership in the

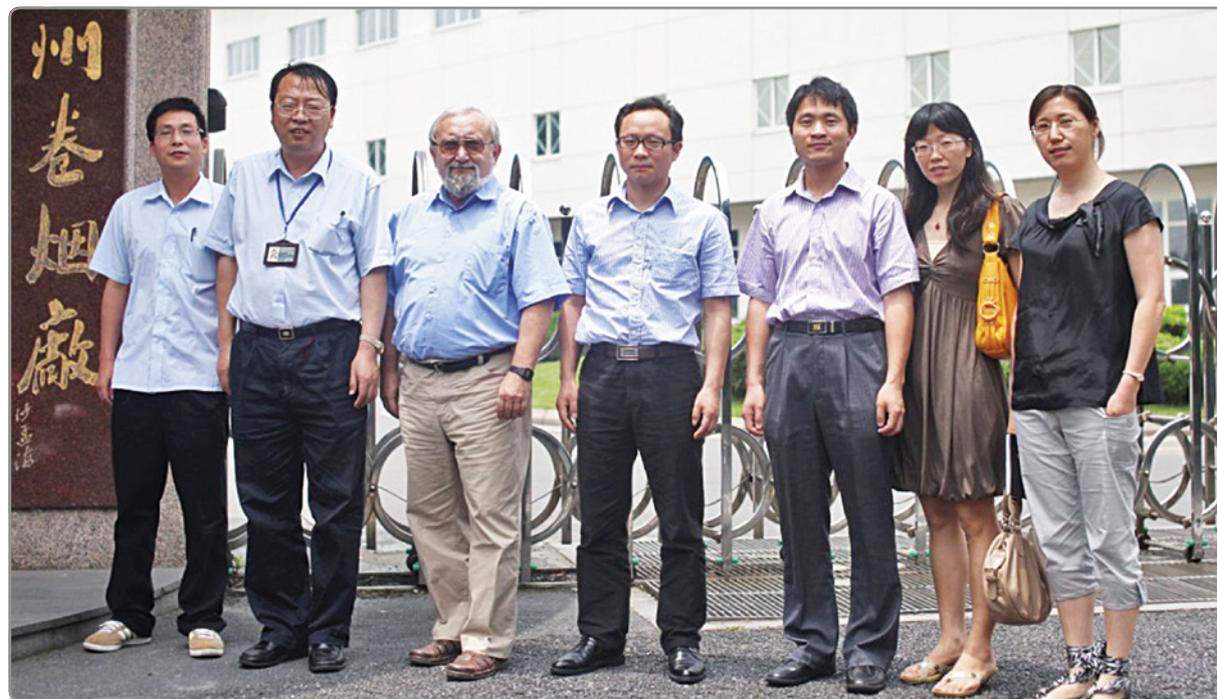


Figure 6: In the frame of cooperation with China, five common projects were solved up to now with three partners – Zhejiang University and China Tobacco Zhejiang Industrial Co., Ltd. in Hangzhou as well as with Institute of Soil Sciences in Nanjing. This picture is from the visit of College of Life Sciences of the Zhejiang University.

Scientific Committee of the Ministry of Environment (till 2015). As a vice-chairman of the Committee for Environment and Agriculture of Central Bohemia Region, T. Vaněk has had the opportunity to influence environmental policy at this level, as has P. Soudek, a member of the Environmental Commission of the City Council Kladno. Last but not least, T. Vaněk has contributed, as a member of the EPSO Board, to the science policy at EU level (Eriksson D et al. 2018, *Nature Biotechnology* 36: 18-19).

Research projects: 6, 7, 10, 23, 27, 37, 50, 73–75, 80–82, 84, 85, 88–91, 104, 106, 108, 110, 112, 119, 120, 122, 123



Laboratory of Plant Reproduction

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The Laboratory of Plant Reproduction was established in 2007. It explores two research areas associated with plant reproduction – cytoplasmic male sterility (CMS) and flowering. Our research is focused on non-model plants – the genera *Silene* and *Chenopodium*. We employ cutting-edge molecular and computational methods to study global organellar transcription patterns in plants [127], to compare female and hermaphrodite mitochondrial transcript levels, and quantify RNA editing in *Silene vulgaris*. We reported the first case of non-coding mitochondrial RNA associated with CMS in plants [429, 436]. We also published the first mitochondrion-wide estimates of the order of editing and splicing, two critical steps in mitochondrial gene expression. Additionally we have constructed a transcriptome of the weedy annual plant *Chenopodium rubrum* and used it to comprehensively search for the *FT/TFL1* genes which govern important aspects of plant development. We discovered a novel phylogenetic clade of *FT* genes most likely involved in embryo development [179]. Our work reveals the species of the genus *Chenopodium* to be remarkably useful and easily manipulated models for the study of floral induction in weedy fast-cycling plants lacking a juvenile



In the picture (from the left):

Ing. Jana Walterová-Drabešová, Ph.D. / postdoctoral fellow, Manuela Krüger, Ph.D. / postdoctoral fellow, RNDr. Helena Štorchová, CSc. / head of the laboratory, Mgr. Helena Hubáčková-Mašterová / Ph.D. student, Ing. Kateřina Haškovcová / research assistant.

Not in the picture:

Ing. Pavla Koloušková / Ph.D. student, Mgr. Lucie Černá, Mgr. Oushadee Abeyawardana / research assistants.

phase. The existence of both long-day and short-day accessions discovered in a weedy plant *Chenopodium ficifolium* provides a unique opportunity to study the transition between these contrasting photoperiodic responses in the same species. *C. ficifolium* is a close relative of *Chenopodium quinoa* [132], and therefore knowledge about floral induction in this diploid species may be utilized when researching flowering in the more complex tetraploid crop.

Cytoplasmic male sterility (CMS) and mitochondrial transcriptomics

CMS represents a textbook example of cyto-nuclear interaction. Despite diverse specific mechanisms in diverse taxa, CMS is generally encoded by mitochondrial genes which interact with nuclear genes, regardless of taxon. This consistent phenomenon highlights CMS' value as a vantage point for understanding cyto-nuclear interactions. CMS is widely exploited in agricultural



crops. Male-sterile plants cannot be self-pollinated and produce heterozygous progeny which often show higher fitness and yield. CMS has been investigated primarily in agricultural plants. Only a few CMS systems have been characterized at the molecular level in natural populations. Mitochondrial CMS genes arise by the rearrangements in angiosperm mitochondrial genomes, which are surprisingly large for eukaryotes and undergo frequent recombination. CMS genes consist of pieces of various mitochondrial regions, most often the *atp* genes, or unknown open reading frames (ORFs). CMS genes encode chimeric proteins which

interfere with mitochondrial function. Nuclear fertility restorer (*Rf*) genes may inhibit CMS gene expression and restore pollen production.

Silene vulgaris (bladder campion or maiden's tear) is one of the most thoroughly explored examples of CMS in natural populations. We have previously identified several candidate CMS genes in the complete mitochondrial genomes of *S. vulgaris*. One of them (*Bobt*) was differentially expressed in male-sterile and male-fertile plants. However, no chimeric gene was found in the KOV haplotype, one which produces both hermaphrodites and females in high frequency. We

have, therefore, selected this haplotype for the first mitochondrial transcriptomic study in *S. vulgaris* [429]. The transcriptomes of both genders were very similar, we found only one genomic region that was highly overexpressed and differentially edited in females relative to hermaphrodites. This region lacks an ORF and thus represents the first case of a non-coding mt RNA associated with CMS in plants.

By comparing editing in spliced and unspliced transcripts, we developed methods that enable us to determine the order of editing and splicing. The majority of transcripts were shown to have been edited prior

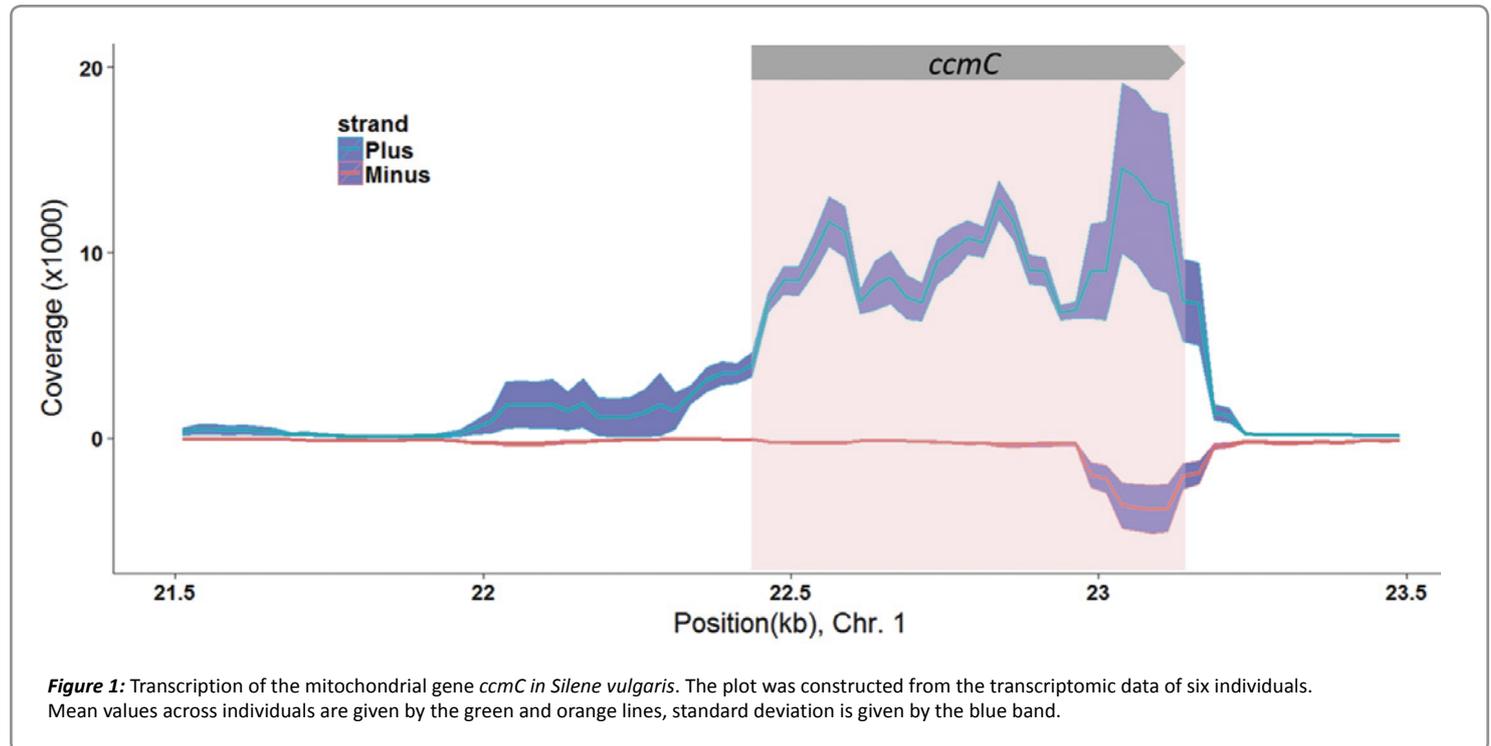




Figure 2: A typical habitat of *Silene acaulis*, a gynodioecious plant with circumpolar distribution. View from Thompson Pass towards Valdez, South Alaska.



to splicing at most positions. However, we found three positions, where splicing obligately preceded editing. The presumed explanation for this sequence of events appears to be that the correct editing recognition motifs are created by splicing in two cases, or a case of the intron interfering with editing prior to splicing in the third position [429].

An extreme variation in the sequence and structure of mitochondrial genomes at within-species level in *S. vulgaris* raises the question: how are the variable regulatory motifs read and interpreted by nuclear genes? To solve this question, we continue with studies of additional mitochondrial transcriptomes in this interesting species, as well as analyses of the complementary transcripts of nuclear genes.

Flowering-related genes in *Chenopodium rubrum*

The family Amaranthaceae includes several important crops – sugar beet, spinach and quinoa. Despite its significance, this family has attracted little attention from researchers. The genetic background to the control of flowering has been studied in sugar beet in our group and by others. In *Chenopodium rubrum*, we have revealed the floral inducer *FLOWERING LOCUS T LIKE 1* (*FTL1*) in previous research. *C. rubrum* was a traditional model plant for the physiological study of flowering in our institute for many years. Next generation sequencing has opened the door for detailed genetic studies in the species previously limited by a lack of sequence information. We utilized 454 pyrosequencing and

Illumina high-throughput sequencing to generate reference transcriptomes of *C. rubrum*. We used these to search for the genes belonging to the *FT/TFL1* family of plant developmental regulators [179]. In this process, we discovered an ancient phylogenetic clade of *FT genes* represented by the *CrFTL3* gene of *C. rubrum*. This gene diverged very early, most likely along with the divergence of Caryophyllales. The *FTL3* gene is expressed only in seeds, which suggests its participation in the control of seed and embryo development. We are now working on a comprehensive atlas of gene expression in *C. rubrum* under various photoperiodic regimes. The recently published genome of *Chenopodium quinoa* will accelerate floral induction research in all the representatives of Amaranthaceae. It paves the way for understanding the adaptation of flowering time control to environmental conditions – not only in crops, but also in many wide-spread weedy species.

Collaborative research

Our laboratory collaborates with Prof. Daniel B. Sloan (Colorado State University, USA) in the field of genomics and transcriptomics of plant mitochondria [14, 429]. We continue productive joint research with Prof. Eric Jellen of Brigham Young University (Utah, USA) [132]. We aim to understand the evolution and adaptation of flowering-related genes across the amaranth family. We helped to introduce transcriptomic approaches at the University of South Bohemia in České Budějovice in the course of collaborative research on the aquatic rootless plant *Utricularia vulgaris* [14].

Research projects: 12, 39, 66, 97

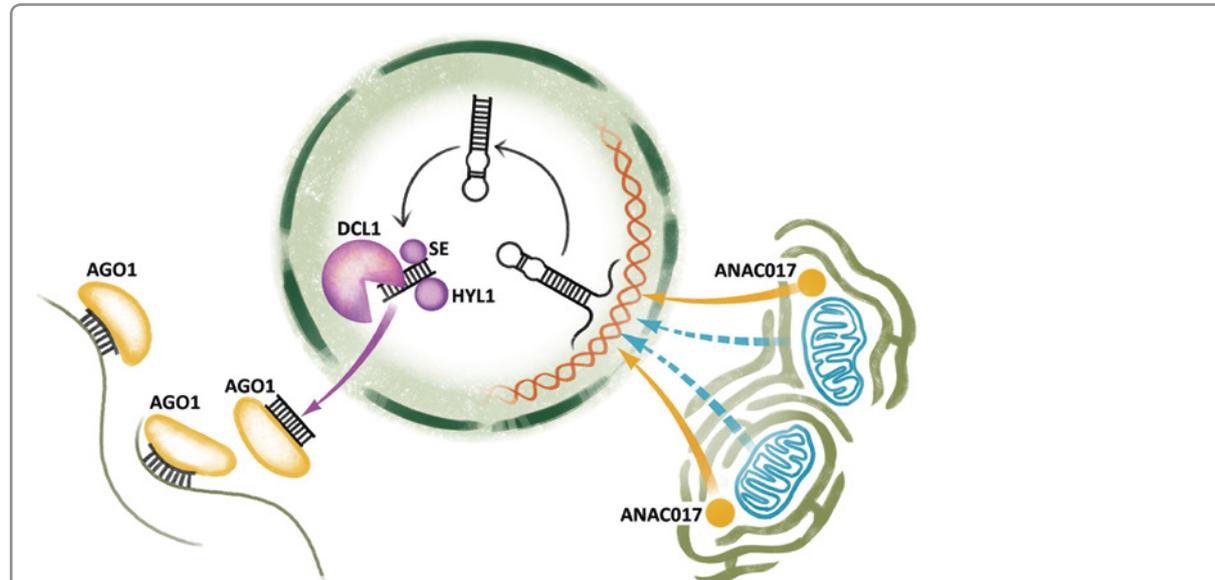


Figure 3: Induction and biogenesis of miRNA during CMS. Mitochondria altered by the action of CMS-associated genes send retrograde signals to the nucleus by means of the transcription factor ANAC017 localized close to endoplasmic reticulum and/or by unknown factors. They trigger miRNA gene expression and the production of pri-miRNA. Afterwards, miRNA duplexes are transported to the cytoplasm, where they join AGO1, find target mRNAs and initiate its cleavage or translation inhibition by RISC.



Laboratory of Pollen Biology

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The Laboratory of Pollen Biology IEB has continuously dedicated its activities to the fundamental research of plant reproductive development, sexual plant reproduction and genome stability. In this area, the laboratory has performed research leading to several pioneering and highly cited publications, namely the priority results on pollen developmental transcriptomics (in a broader sense the first study of effectively single-cell global gene expression profiling and its developmental dynamics in plants).

These results also contributed to the establishment of the current paradigm in male gametophyte research towards asking and answering specific gene-oriented questions. Moreover, novel strategies to manipulate the gametophyte development and function are of current interest in agriculture and breeding. This is related to the introduction of new model species like *Physcomitrella*, hops and tomato. Moreover, we are continuously extending our activities and our research is focused mainly on several aspects of pollen development, pollen communication with female tissues and genome stability.



In the picture (from the left):

Petra Rožnovská / technician, Bc. Božena Klodová / diploma student, Mgr. Alena Náprstková / Ph.D. student, Vesta Aleknavičiute / Erasmus student, Christos Michailidis, Ph.D. / postdoctoral fellow, Said Hafidh, Ph.D. / researcher, Zahra Aghcheh Kahrízi, M.Sc. / Ph.D. student, Mgr. Pavel Bokvaj / research assistant, assoc. prof. RNDr. David Honys, Ph.D. / head of the laboratory, RNDr. Lenka Steinbachová, Ph.D. / postdoctoral fellow, RNDr. Lenka Závěská Drábková, Ph.D. / postdoctoral fellow, RNDr. Jan Fíla, Ph.D. / postdoctoral fellow, Ing. Jana Feciková / research assistant, Micheala Hromadová / BSc. student, Mgr. Katarína Kulichová / Ph.D. student, Mgr. Marcela Holá / postdoctoral fellow, Ing. Radka Vágnerová / Ph.D. student, RNDr. Karel J. Angelis, CSc. / senior researcher, Daniel Sanchez, Ph.D. / postdoctoral fellow, Bc. Peter Darivčák / diploma student.

Not in the picture:

RNDr. Nikoleta Duplákova, Ph.D. / researcher, RNDr. David Reňák, Ph.D. / postdoctoral fellow, RNDr. Zuzana Gadiou, Ph.D., Ing. Iveta Jelínková, Mgr. Filip Linhart / research assistants, Karel Raabe, Helena Kočová / BSc. students.

Regulation of *Arabidopsis* pollen development

Screen and functional analyses of male gametophytic transcription factors

Male gametophyte development leading to the formation of a mature pollen is precisely controlled at various levels, including transcriptional, post-transcriptional and posttranslational. We focused on

the identification and functional characterization of pollen-expressed transcription factors (TF) involved in the regulation of pollen development. For that, we optimized the detailed protocol of the separation and large-scale isolation of the homogeneous populations of *Arabidopsis* developing spores at several stages – uninucleate microspores, bicellular pollen, immature

tricellular pollen and mature pollen [181]. We have continued in our effort to shed light on the regulatory network of the large transcription factors family – dimeric basic leucine zipper (bZIP) – during pollen development. Recently, we have reports the interaction network of six bZIP TFs expressed in *Arabidopsis thaliana* pollen and highlighted the potential functional role for AtbZIP18 in pollen. AtbZIP18 was shown to interact with three other pollen-expressed bZIP TFs – AtbZIP34, AtbZIP52, and AtbZIP61 in yeast two-hybrid assays. AtbZIP18 functional characterization as well as the analyses of *atbzip18*– pollen microarray data pointed towards a potential repressive role for AtbZIP18 and its functional redundancy with AtbZIP34 in pollen [336]. Finally, we performed thorough comparative analyses of callose synthase family proteins from major plant lineages to determine their evolutionary history across the plant kingdom. This analysis identified new evolutionary lineages of callose synthase subfamilies and has established a basis for understanding their functional evolution in terrestrial plants with emphasis on proteins involved in the male gametophyte development [459].

Tobacco pollen as a bicellular model for -omic studies

Tobacco pollen developmental transcriptomics and translomics

The majority of flowering plants produce bicellular pollen. The two cells of the pollen grain are destined for separate fates in the male gametophyte, which provides a unique opportunity to study genetic interactions that govern guided single-cell polar expansion of the growing pollen tube and the coordinated control of germ cell division and sperm cell fate specification.

We performed the first comprehensive developmental transcriptomic analysis of the tobacco male

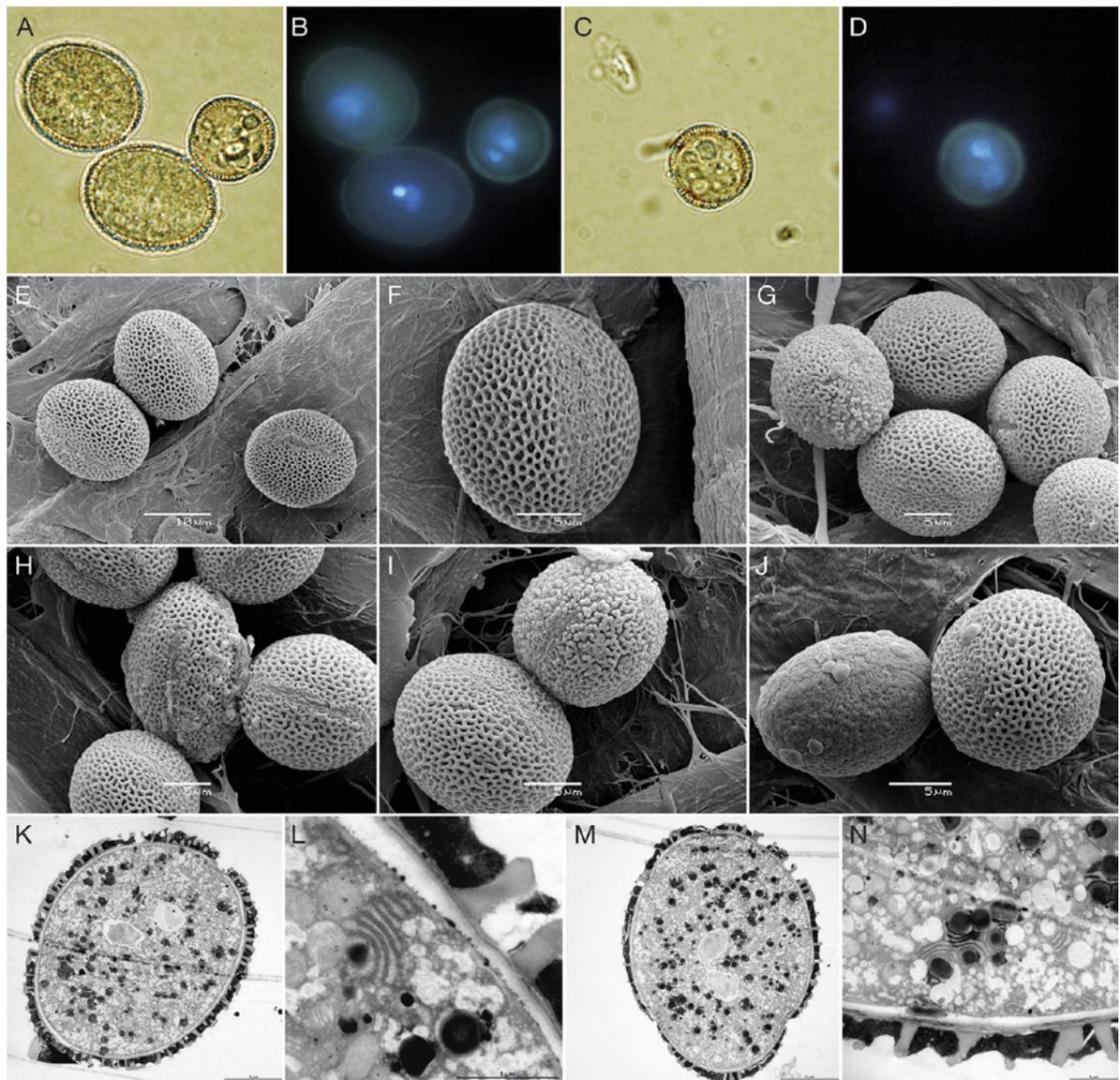


Figure 1: Morphological characterization of *atbzip18* pollen. Phenotypic defects in *atbzip18* pollen (a–d). Bright-field (a, c) and fluorescence images after DAPI-staining (b, d) are shown. Scanning electron micrographs of wild-type (e, f) and *atbzip18* pollen (g–j). Transmission electron micrographs of mature wild-type (k, l) and *atbzip18* (m, n) pollen [336].

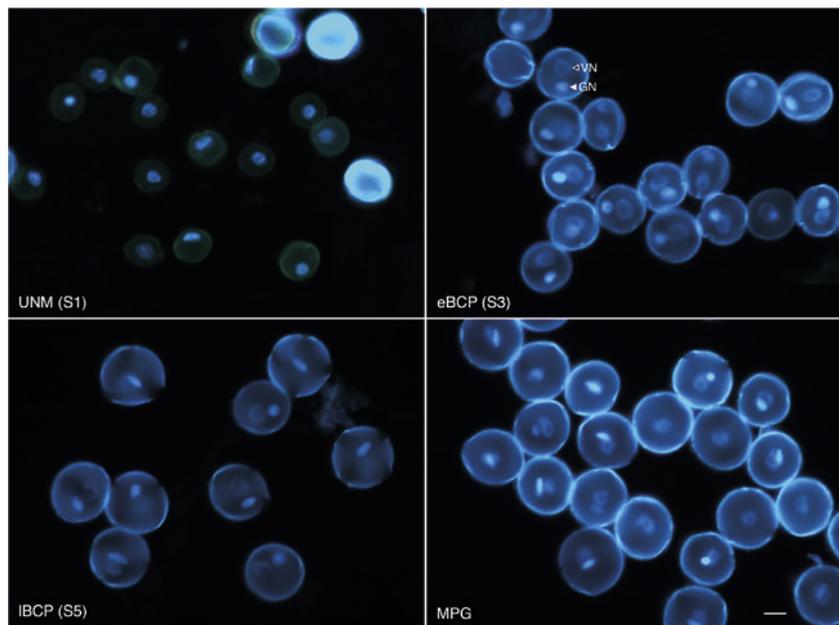


Figure 2: DAPI-stained populations of developing spores used for the transcriptomic analysis: microspores (UNM); early bicellular pollen (eBCP); late bicellular immature pollen (IBCP); and mature pollen MPG. Scale bar = 10 μm . GN, VN stand for generative nucleus and vegetative nucleus, respectively [465].

gametophyte representing the first plant species shedding bicellular pollen [465]. These transcriptomic data sets presented a benchmark for future functional studies using developing pollen as a model. We further showed the complexity of tobacco male gametophyte transcriptome over the period of pollen development. We demonstrated the ongoing transcription activity and specific transcript accumulation in post-pollen mitosis I immature pollen. Previous transcriptomic data from *Arabidopsis* showed massive expression of genes encoding proteins forming both ribosomal subunits that were accumulated in developing pollen, whereas their expression was not detectable in growing pollen tubes (Honys and Twell, 2004). We observed

a similar phenomenon in less advanced bicellular tobacco pollen [465]. Comparison with tobacco pollen tube proteome even revealed that most of these transcripts were not translated (joint effort with Z. Zdráhal, CEITEC MU Brno) that highlighted them as the likely candidates for paternal complement to postfertilization events.

Recently, we have initiated completely new direction of our research, tobacco pollen translomics. It has been well established that both transcription and translation play an important role in global and specific gene expression patterns during pollen maturation. On the contrary, germination of many pollen species has been shown to be largely independent of transcription but vitally dependent on translation of stored mRNAs. We demonstrated that large ribonucleoprotein particles (EPP granules) were formed in immature pollen where they contained translationally silent mRNAs and then served as a long-term storage of mRNA transported along with the translational machinery to the tip region where the translation took place. Such an organization is extremely useful in fast tip-growing pollen tube. Moreover, the asymmetric mRNA distribution is the determinant of protein gradient influencing cell polarity, cell fate and overall patterning during development. We proposed a model outlining the network of posttranscriptional control with a focus on the role of stored RNPs and started the functional characterization of RNA-binding proteins (collaboration with C. Bousquet-Antonelli, INRA Perpignan, France). We have extended our transcriptomic and proteomic analyses to cover three cytoplasmic subfractions containing mRNAs at different translational status and to demonstrate their developmental dynamics – 1) actively translated transcripts associated with polysomes (PS – termed translome), 2) pollen mRNAs bound to pollen stored ribonucleoprotein particles (stored mRNPs/free mRNPs – termed mRNPome) and 3) long-term stored transcripts on EPP granules (EPPs – termed sequestrome [196; Hafidh, submitted to Plant Physiology]).

Tobacco pollen developmental phosphoproteomics

Rapid changes of protein phosphorylation play a crucial role in the regulation of many cellular processes. Being post-translationally modified, phosphoproteins are often present in low abundance and tend to co-exist with their unphosphorylated isoforms within the cell.

Therefore, we first developed the protein extraction protocol suitable for subsequent phosphoprotein enrichment from tough tobacco pollen tissue and selected the appropriate phosphopeptide enrichment procedure (MOAC, metal oxide/hydroxide affinity chromatography) and used it to describe a population of differentially (de)phosphorylated phosphoprotein candidates from both mature and *in*

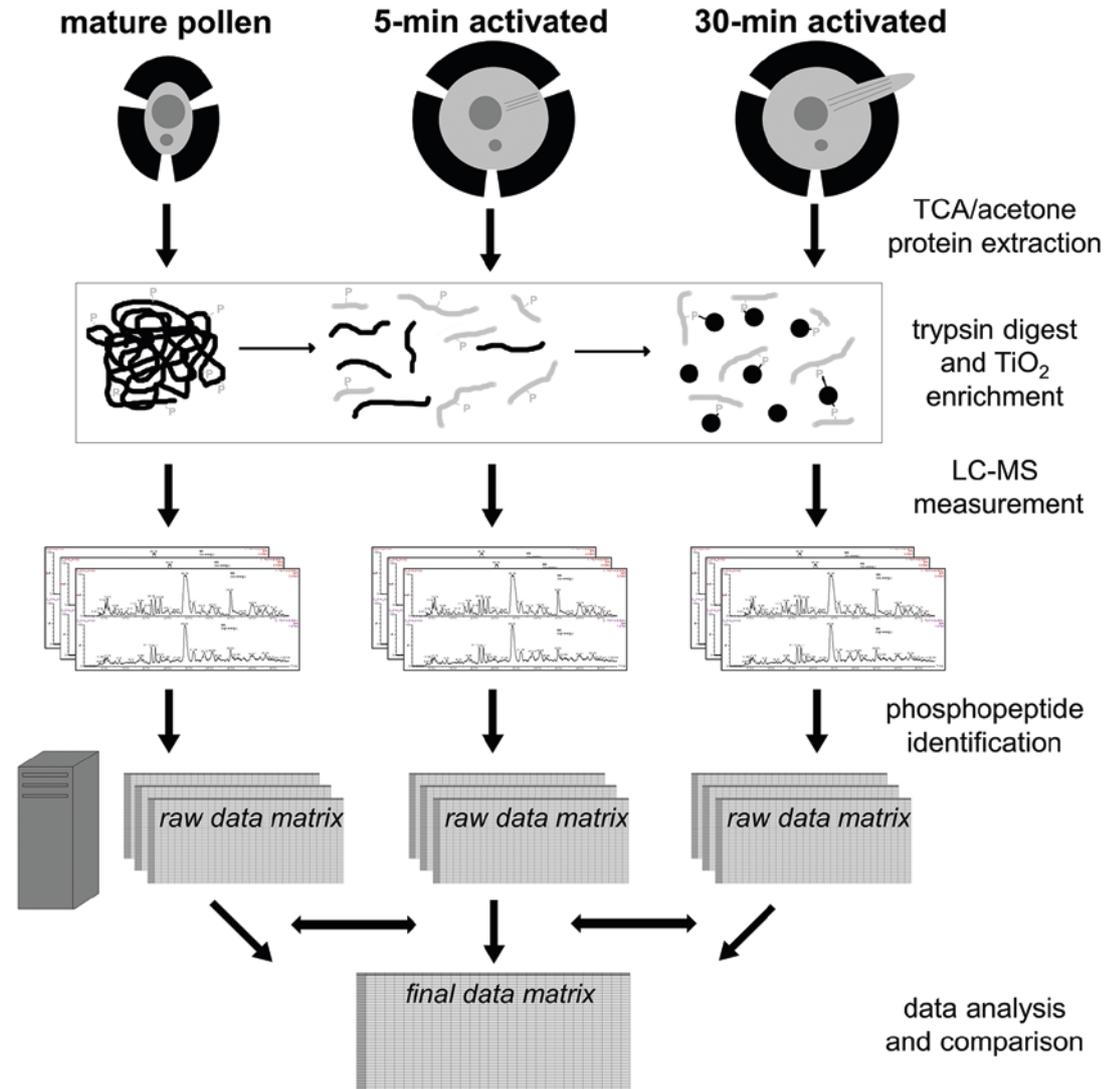


in vitro activated tobacco pollen grains. First, we identified and validated a set of 139 phosphoprotein candidates including the detection of 52 phosphorylation sites. As a joint effort with Dr. H.-P. Mock's (IPK Gatersleben) and Dr. R. Zahedi's (ISAS Dortmund) groups, we showed for the first time the dynamics of protein phosphorylation and dephosphorylation associated with early stages of pollen germination. We further extended this analysis to three time points – mature pollen, 5-min and 30-min-activated pollen. We identified 471 phosphopeptides (301 phosphoproteins) carrying 432 phosphorylation sites, position of which was exactly matched by mass spectrometry. The quantitative data highlighted the regulatory trends; we showed that several phosphopeptides representing the same phosphoprotein underwent different regulation, which pinpointed the complexity and dynamics of protein phosphorylation at the initiation of the progamic phase. Collectively, we showed the first phosphoproteomics data on activated pollen where the position of the respective phosphorylation sites was clearly demonstrated [186].

Tobacco secretomics

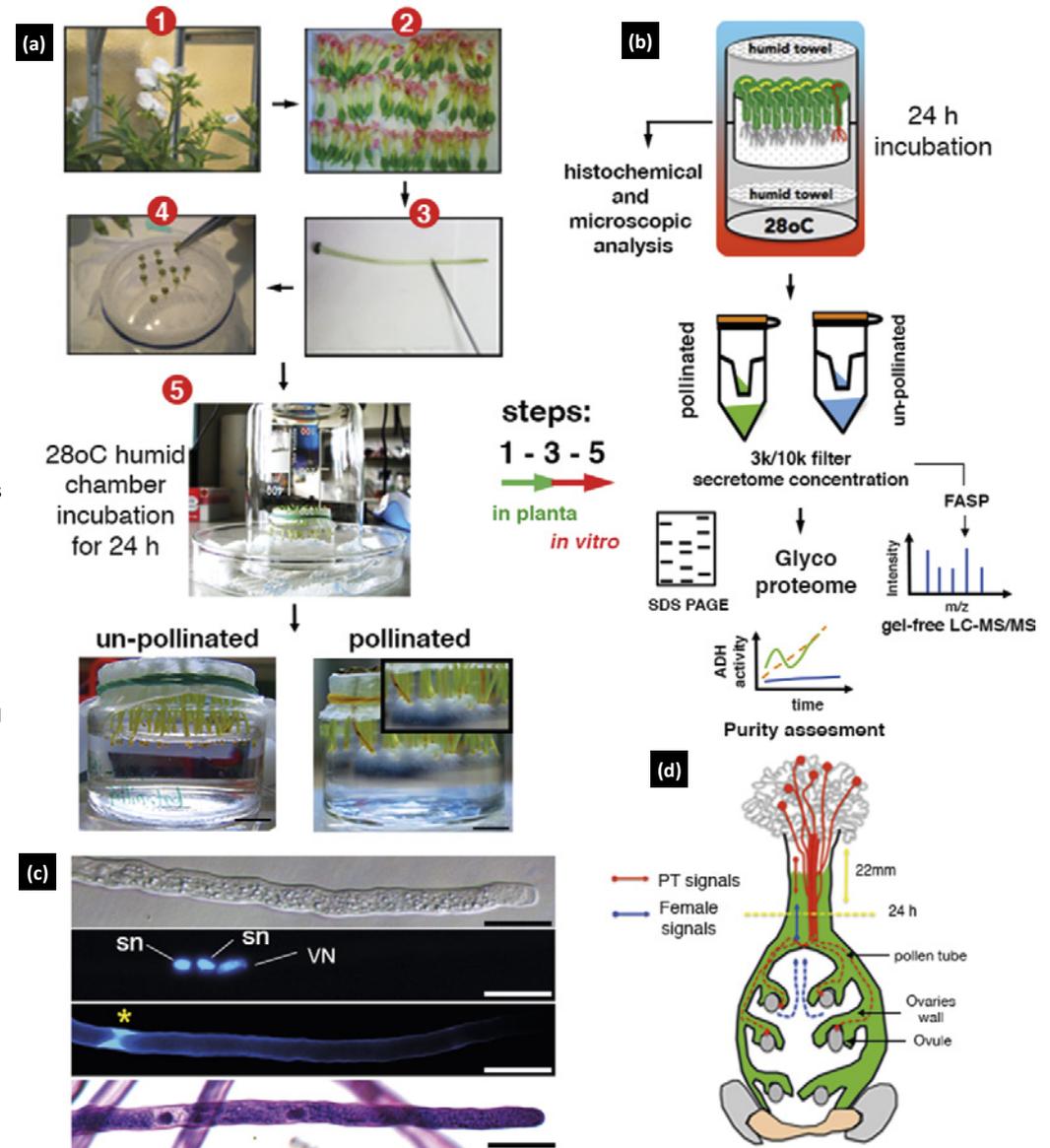
Some of the stored mRNAs encode for secreted proteins required for male-female signalling during pollen tube guidance. To understand the spectrum of translational regulation and mRNA storage, we studied pollen tube secretomics as “bottom-up” approach to link with our sequestrone transcriptome. As a novel approach, we have improvised a modified SIV (semi-*in vivo*) technique, SIV-PS (SIV pollen tube secretome) in collaboration with M. Johnson, Brown University, USA and R. Palanivelu, Univ. of Arizona, USA. As

Figure 3: A schematic workflow of the performed experiments. The three stages of tobacco male gametophyte (particularly mature pollen, pollen activated *in vitro* for 5 min and pollen activated *in vitro* for 30 min) were subjected to TCA/acetone protein extraction, trypsin digest and phosphopeptide enrichment by TiO_2 . The obtained phosphopeptide-enriched eluate was fractionated by nLC and measured by MS/MS. The present phosphopeptides were identified (if possible with the unambiguous position of the phosphosite) and the results further analyzed [186].



a joint effort with Z. Zdráhal's group (CEITEC MU, Brno), we performed gel-free LC-MS/MS for highthroughput analysis of pollen-tube-secreted proteins. Our approach has led to the identification of over 341 protein groups on average (801 accessions). Among them are pollen tube-secreted ligands and receptor proteins representing potential male components in perceiving ovule-emitted cues for guidance. Primarily proteins of $\leq 30\text{kDa}$ (60 %) of which 40 % were $\leq 20\text{kDa}$ dominated the pollen tube secretome. They included Plant defensin subfamily, Cysteine-rich, LORELEI-like GPI-anchored 3 (LLG3), Thionin-like protein, RNAses, lipid transfer proteins (LTPs), pollen Ole-e-allergen, arabinogalactans, pectinases and invertases. The pollen tube secretome comprised vastly of non-classical type of secreted proteins. Intriguingly, we discovered that TCTP1, a non-classically-secreted protein hijacked the classical secretory pathway and co-localized with nanovesicles/exosome marker Ole-e-1. This follow-up study has uncovered novel pistil-dependent pollen tube-secreted proteins critical for establishing male-female signalling interaction map for successful sperm cells delivery and fertilization and as means to overcome interspecies pre-zygotic barriers [197]. The link between pollen tube secretome with the secretome is currently evaluated.

Figure 4: Semi-*in vivo* pollen tube secretome (SIV-PS) approach for identification and quantification of pollen tube-secreted proteins. **(a)** An improvised SIV-PS technique setup from *in planta* (steps 1–3) to *in vitro* incubation of the pollen tubes (steps 4–5). The inset shows emerging pollen tubes from excised pistils. Scale bars = 2 mm. **(b)** Schematic representation of the SIV-PS workflow. **(c)** Micrographs of SIV pollen tubes showing normal pollen tube growth in bright field with streaming organelles (top panel), sperm cell formation (second panel), callose deposition and callose plugs (asterisk, third panel), and pollen tube viability assessed by Alexander stain (bottom panel). sn sperm cell nucleus, VN vegetative cell nucleus. Scale bar = 40 μM . **(d)** A tobacco pollinated pistil showing the site of stylar excision and the presumed peptide signaling flow from male and female gametophytes [197].





DNA repair and chromosome maintenance

Based on previous results we study mechanism of plant response to DNA damage from the indicated point of view that behind sensitive phenotype of repair mutants is rather an effective error prone mechanism inactivating genes throughout genome by inducing mutations during lesion repair or bypass than repair defect itself, leading to accumulation of unrepaired lesions and collapse of procession through the cell cycle. In this respect, we showed that behind strong genotoxic and mutagenic activity of UV radiation is tolerance of photodimers in template DNA by error-prone bypass synthesis by error-prone DNA polymerases leading to C to T transitions throughout genome [47]. To ascertain the role of DNA polymerases in procession of DNA lesions during non-homologous end joining (NHEJ) and base excision repair (BER) pathways we investigated possible role of POLA in cooperation with T. Furukawa and A.B. Britt, UC Davis. We closer characterized DNA Pol λ activities, particularly synthesis of short DNA patches, what is assumed as its main activity during BER, and as we showed is also part of DSB repair NHEJ pathway [39].

Further we started to evaluate plants as potential model system of DNA lesion processing in mammalian cells. The aim is to study effect of cytopreservatives and cytotoxicity boosters used in human medicine as tumor pharmaceuticals. We participated and showed that cytoprotective drug Amifostin besides biochemical protection of irradiated normal vs. malignant cells also stimulates repair of DSB by yet unknown mechanism [202].

Finally, in collaboration with J. Fajkus, Z. Zdráhal (CEITEC MU, Brno) and E. Sykorová (Institute of Biophysics CAS, Brno) we are investigating the role of plant telomerase and telomerase-associated proteins in

telomeres maintenance in *Physcomitrella*, algae, *Arabidopsis* and tobacco. We found in *Physcomitrella* that telomere phenotypes are absent and DSB repair kinetics is not affected in mutants for DSB factors involved in non-homologous end joining (NHEJ). This is compliant with the overall dominance of homologous recombination over NHEJ pathways in the moss, contrary to the inverse situation in flowering plants [37] and that algae strains *Zygnema* sp. 436 and *Zygnema circumcinctatum* TEL 181 are not responsive to DSB induction and repair at all. We also analyzed the transcription of three *Nicotiana tabacum* TERT variants in correlation with telomerase activity in tobacco tissues. High and approximately comparable levels of TERT_Ct and TERT-Cs transcripts were detected in seedlings, roots, flower buds and leaves, while the transcript of the TERT_D variant was markedly underrepresented. A specific pattern of TERT transcripts was found in tobacco pollen with the TERT-Cs variant clearly dominating particularly at the early stage of pollen development. being present in EPP particles encompassing translationally silent mRNA. The existence and transcription pattern of tobacco TERT paralogs indicated its functional significance and *Nicotiana* species again proved to be useful model plants in telomere biology studies [355]. In *Arabidopsis*, we used tandem affinity purification coupled to mass spectrometry to build an interactome of the telomerase catalytic subunit AtTERT, using *Arabidopsis thaliana* suspension cultures. Our results provide an insight into the composition and architecture of the plant telomerase complex and this will aid in delineating molecular mechanisms of telomerase functions [374]. On top of the above research, we also participated at the study of the genetic diversity and interspecific hybridization of the two *Inga* species (*Inga edulis* and *Inga ingoides*) in Peruvian Amazon [257].

Brief list of national and international collaborators (sorted alphabetically)

Dr. B. Banović, University of Belgrade, Serbia; Dr. C. Bousquet-Antonelli, INRA Perpignan, France; Dr. Sofija Božinović, Zemun Polje Maize Research Institute, Serbia; Prof. A.B. Britt and Dr. T. Furukawa, UC Davis, CA, USA; Dr. J. da Costa-Nunes, University of Lisboa, Portugal; Dr. A. Cuming, CPS, University of Leeds, UK; Dr. P. Doerner, University of Edinburgh, UK; Prof. T. Dresselhaus, University of Regensburg, Germany; Prof. J. Fajkus, Assoc. Prof. Z. Zdráhal, Dr. M. Fojtová, CEITEC MU, Brno, Czech Republic; Drs. M. and I. Falk, IBP CAS, Brno, Czech Republic; Dr. Nurit Firon, Volcani Center, Bet Dagan, Israel; Prof. M. Johnson, Brown University, USA; Dr. Yehoram Leshem, MIGAL, Kiryat Shemona, Israel; Prof. A.A. Levy, WIS, Rehovot, Israel; Dr. J. Matoušek, Biological Center, České Budějovice, Czech Republic; Dr. H.-P. Mock and Dr. A. Matros, IPK Gatersleben, Germany; Dr. F. Nogue, INRA Versailles, France; Prof. Ravi Palanivelu, University of Arizona, Tucson, AZ, USA; Dr. R. Peyman-Zahedi, ISAS Dortmund, Germany; D. Scheafer, University de Neuchâtel, Switzerland; Prof. Enrico Schleiff, Dr. Klaus-Dieter Scharf, Goethe University Frankfurt a/Main, Germany; Dr. E. Sýkorová, IBP CAS, Brno, Czech Republic; Prof. D. Twell, University of Leicester, UK; Doc. Dr. Zbyněk Zdráhal, CEITEC Brno.

Research projects: 2, 14, 15, 25, 31, 33, 35, 57, 58, 60, 71, 76, 86, 109, 114



Laboratory of Signal Transduction

Head of laboratory: **RNDr. Jan Martinec, CSc.**

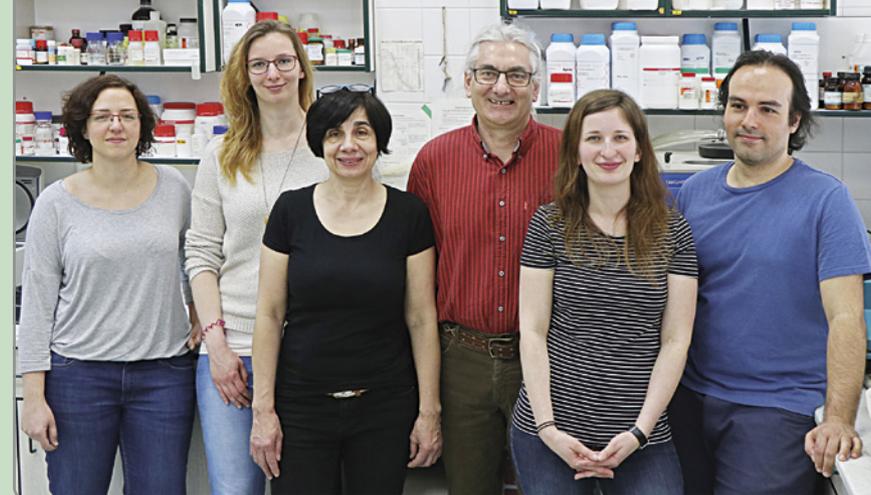
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The Laboratory of Signal Transduction carries out research on developmental and stress signalling in *Arabidopsis thaliana*. Currently, it is mainly investigating the role of the non-specific phospholipase C (NPC) and phospholipase D protein family and flotillins in plant development and stress responses.

Non-specific phospholipase C (NPC) catalyses the hydrolysis of structural phosphoglycerolipids such as phosphatidylcholine (PC) to generate phosphocholine and diacylglycerol (DAG). NPC has a long tradition in animal signal transduction (where it is called PC-PLC) to generate DAG as a secondary messenger in addition to the well-known phosphatidylinositol splitting phospholipase C (PI-PLC). Until 2005, however, there was no information as to a role of NPC in plants at gene level.

During the period 2015–2017, we published a series of articles on functional analysis of NPCs. In these articles, we reported on the role of NPC in responses of plants to aluminium toxicity, heat stress and bacterial attack.

Aluminium ions (Al) are recognized as constituting a major toxic factor in crop production in acidic soils, and for this reason, aluminium's interaction with plants is widely studied. However, the exact molecular mechanism and time sequence of individual changes occurring on Al exposure remains under investigation. We examined the impact of Al on expression, activity, and function of the non-specific phospholipase C4 (NPC4), a plasma membrane-bound isoform of NPC. We observed lower expression of NPC4 using β -glucuronidase assay and decreased formation of labeled diacylglycerol (DAG), a product of NPC activity in *Arabidopsis* wild-type (WT) seedlings treated with $AlCl_3$. In seedlings overexpressing NPC4, the Al-mediated NPC-inhibiting effect was reduced. Second, the growth of tobacco pollen tubes, rapidly arrested by Al exposure was partially rescued by the overexpression of AtNPC4 while *Arabidopsis* knock-out T-DNA insertion line NPC4 (*npc4*) was found to be more sensitive to Al stress during long-term exposure to Al under low phosphate condi-



In the picture (from the left):

Ing. Zuzana Krčková, Ph.D. / postdoctoral fellow, Mgr. Kristýna Kroumanová / research assistant, Kateřina Vltavská / technician, RNDr. Jan Martinec, CSc. / head of the laboratory, Bc. Tereza Podmanická / research assistant, Mgr. Michal Daněk / Ph.D. student.

Not in the picture:

Ing. Daniela Kocourková, Ph.D. / research assistant.

tions. Our observations suggest that NPC4 plays a role in both early and long-term responses to Al stress [101]. We also investigated the mechanism of Al impact on the activity of NPC. Application of the membrane fluidizer, benzyl alcohol, restored the level of DAG during Al treatment. Our observations suggest that the activity of NPC is affected by Al-induced changes in plasma membrane physical properties [490].

Heat stress was another environmental effect that was studied in connection with *Arabidopsis* NPCs in our laboratory [65]. We found that the basal thermotolerance of the NPC1 knock-out line (*npc1*) was impaired compared with WT; *npc1* exhibited significantly reduced survival rates and chlorophyll content on the seventh day after heat stress (HS). Conversely, plants overexpressing NPC1 (NPC1-OE) were more resistant to HS than WT (**Fig. 2**). These findings suggest that NPC1 is involved in plant responses to heat.

Non-specific phospholipase C2 (NPC2) was characterised experimentally for the first time in our article on the response of *Arabidopsis* to biotic stress (Krčková et al. 2018, *Annals of Botany* 121: 297-310). The heterologously expressed NPC2, possessed phospholipase C activity, being able to hydrolyse phosphatidylcholine to diacylglycerol. NPC2 tagged with GFP was predominantly localized to the Golgi

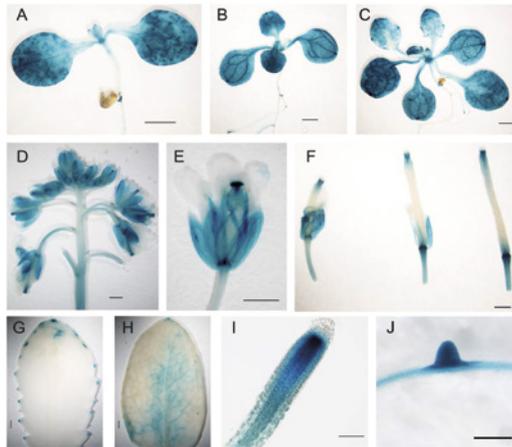


Figure 1: *Arabidopsis* plants stably transformed with putative AtNPC2 promoter:GUS transcriptional fusions were prepared and used for a histochemical GUS assay. GUS staining of plant organs at several developmental stages revealed that NPC2 is expressed in a wide range of plant organs. The intensity of GUS staining was similar throughout seedling development (a–c). In adult plants, GUS activity in the inflorescence and siliques was relatively high, similar to seedlings (d–f). In both young and old leaves of adult plants, GUS activity was weaker than in seedlings (g, h). In the root system, expression of pNPC2:GUS was highest at root tips and lateral roots (i, j). In young leaves, only hydathodes were clearly stained (g). (a) 7-d-old seedling, (b) 10-d-old seedling, (c) 14-d-old seedling, (d) inflorescence, (e) flower, (f) siliques, (g) young leaf, (h) old leaf (d–h, 5-week-old plants), (i) main root (14-d-old seedling), (j) lateral root (10-d-old seedling). Scale bars: a–c, e 1 mm; d, f, g, h 2 mm; i, j 0.1 mm.

apparatus in *Arabidopsis* roots. Transcription of NPC2 decreased substantially after plant infiltration with *Pseudomonas syringae*, flagellin peptide flg22 and salicylic acid treatments and expression of the effector molecule AvrRpm1. The decrease in NPC2 transcript levels correlated with a decrease in NPC2 enzyme activity.

In 2016, we published a review on flotillins, erlins, and HIRs [176] with a view to assessing the current state of knowledge on plant flotillins and HIRs and to assign putative functions to plant flotillins and erlins based on the known functions of their mammalian homologs.

In addition to work on NPCs, flotillins and PLDs, we are involved in a number of other research projects based on collaboration with teams, both within the Institute of Experimental Botany and from other institutes and universities in the Czech Republic. Within the Institute of Experimental Botany, we long have collaborated with the Laboratory of Pathological Plant Physiology. We also collaborate with the Laboratory of Cell Biology [117, 168] and with the Laboratory of Hormonal Regulations in Plants. We have long-enduring collaboration with the research group led by Prof. Olga Valentová from the Institute of Chemical Technology Prague [65, 176; Krčková et al. 2018].

On an international level, we collaborate with the research group led by Dr. Eric Ruelland of the Uni-

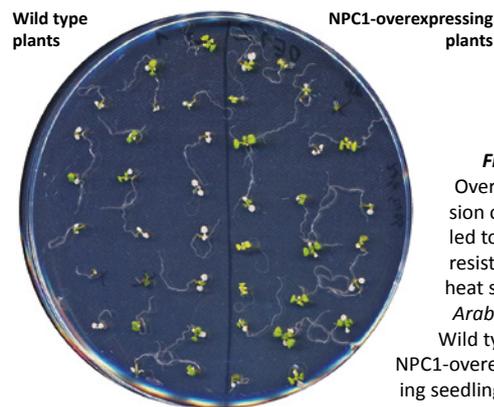


Figure 2: Overexpression of NPC1 led to higher resistance to heat stress in *Arabidopsis*. Wild type and NPC1-overexpressing seedlings were grown on agar plates at 22 °C for seven days. The plates were exposed to heat (42 °C, 45 min), then returned to the control conditions. The picture was taken 7 days after heat stress.

versité Paris Est-Créteil (UPEC), formerly from UPMC Paris [65]. We have also long-lasting collaboration with the group of Dr. Volodymyr Kravets of the Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of Ukraine (Krčková et al. 2018).

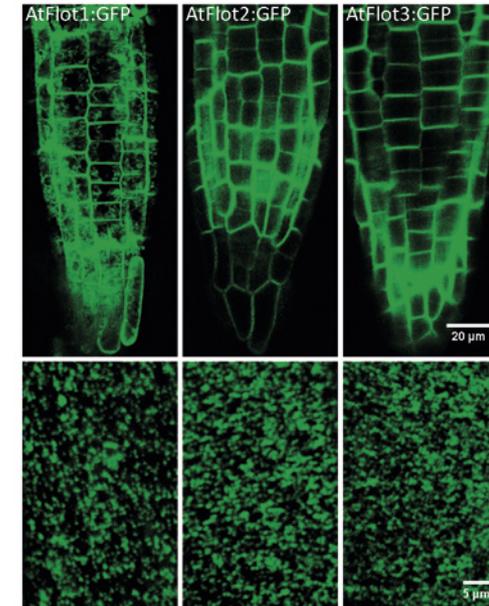


Figure 3: Subcellular localization of single isoforms of *Arabidopsis thaliana* flotillins tagged with GFP in root cells of stable transformants. Upper row: AtFlot2 and AtFlot3 are localized predominantly in plasma membrane whereas AtFlot1 shows also a significant signal in tonoplast and some other endomembranes. Lower row: Within plasma membrane flotillins form discrete foci, i.e. microdomains.

Research projects: 3, 21, 44, 46, 61, 111



Laboratory of Virology

Head of laboratory:

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In 2015 we started a new research topic, namely, the possibilities of modifying the glycosylation pathway of plant seeds to mimic the protein glycosylation observed in mammalian and particularly in human cells. Using the approaches of synthetic biology we are preparing multigene constructs containing the human genes responsible for glycan synthesis in human cells. Our model plant species are *A. thaliana* and *N. benthamiana* and the leguminous crop plants soybean and pea (*G. max* and *P. sativum*). Our goal is to obtain the platform for safe and inexpensive expression and accumulation of authentic human glycoproteins such as antibodies. Our second topic was the understanding and management of plant viral diseases and the development of new and innovative ways to control their incidence. For this purpose, we have generated specific antibodies against plant viruses using various recombinant viral proteins and transgenic plants with increased resistance to viruses. Plant viruses can be used for transient expression of recombinant proteins in plants. We used vectors based on several RNA and DNA plant viruses, namely *Potato virus X*, *Tobacco mosaic virus*, *Bean yellow dwarf virus* and *Cowpea mosaic virus*.

Our laboratory has fruitful international collaboration with teams from Danforth Plant Science Center and USDA, St. Louis, USA; Biopharming Research Unit, University of Cape Town, South Africa; VIB Ghent, Belgium and RWTH, Aachen, Germany.



In the picture (from the left):

RNDr. Naďa Wilhelmov, CSc. / researcher, Mgr. Tomš Moravec, Ph.D. / head of the laboratory, doc. RNDr. Noem eřovsk, CSc. / senior researcher, Ing. Jakub Duřek / Ph.D. student, Ing. Jitka Svobodov / technician, Renata Hadmkov / technician (until 2017), Bc. Kateřina Kratochvlov / diploma student, RNDr. Oldřich Navrtil, CSc. / research assistant, PharmDr. Zuzana Pobořilov, Ph.D. / postdoctoral fellow, Dr.rer.nat. Ing. Helena Plchov / researcher.

Not in the picture:

Mgr. Hana Hoffmeisterov, Ph.D. / postdoctoral fellow (maternity leave), RNDr. Petr Vaculk, Ph.D. / Ph.D. student (until 2015), Lenka Kolabov / technician, Karolína Mullerov / technician (until 2015), Tereza Plouřkov / Bsc. student (until 2017).



During the last two years, we have introduced a novel modular cloning strategy based on the GoldenBraid system that allows us to efficiently design genomic elements and complex plant expression cassettes for metabolic engineering of plant cells. Large numbers of constructs have to be tested prior their use in plant transformation. To that end, we developed a novel *in planta* transient assay in leguminous seeds. Since soybean is not as common as *Arabidopsis* or tobacco in growth chambers, we started with finding optimal growth conditions for rapid seed production. Part of these observations were published in [53]. This research project is executed in cooperation with Agritec, Šumperk.

In 2016 in collaboration with the group of Prof. Rybicki (BRU, University of Cape Town, South Africa) we modified the geminiviral DNA vector based on BeYDV to conform to the GoldenBraid convention and combined its strengths with the advantages of *Cowpea mosaic virus* based expression system of Prof. Lomonosoff, John Innes Centre, Norwich, UK. Using this new viral vector, we are now able to obtain extremely high levels of antibody expression in various plant species. On top of this, the vector is modular and versatile allowing expression of up to three genes from one replicon. We plan to use it for gene editing and homologous recombination in plants as well.

In our effort to characterize genomes of plant viruses, we sequenced several *Potato virus M* isolates [108]. We have also produced polyclonal antibodies against recombinant proteins of potato-infecting viruses. One of the main tools for eradicating viral diseases is early detection. Besides antibody based assays, we also develop methods based on viral genomic DNA and RNA (LAMP, RT-PCR).

Plant viral vectors based on PVX are widely used as protein expression and peptide presentation systems.

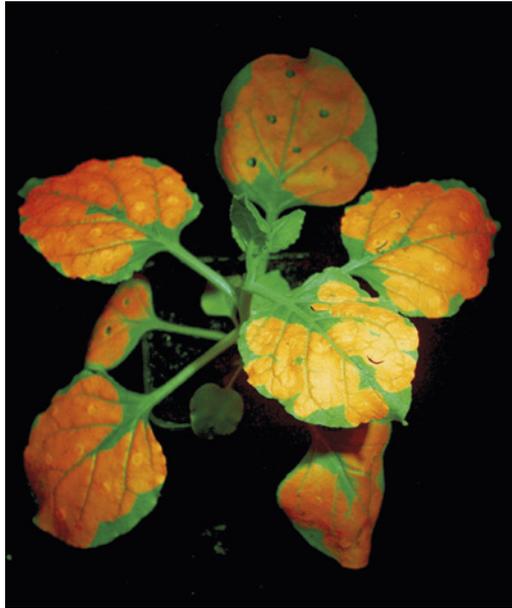


Figure 1: *Nicotiana benthamiana* plant infected with modified geminiviral DNA vector based on BeYDV that expresses coral red fluorescent protein (DsRed).

The 3D-structure of coat protein and viral particle is unknown, however based on the predicted model, we have identified additional loops for antigenic peptide presentation [140, 503].

One of our research branches is also plant responses to stress. Here we cooperated with Faculty of Sciences, Charles University. A comparative analysis of various parameters that characterize plant morphology, growth, water status, photosynthesis, cell damage, and antioxidative and osmoprotective systems together with an iTRAQ analysis of leaf proteome was

performed in two inbred lines of maize (*Zea mays* L.) differing in drought susceptibility and their reciprocal F1 hybrids.

The results clearly showed that the four examined genotypes have completely different strategies for coping with limited water availability and that the inherent properties of the F1 hybrids, i.e. positive heterosis in terms of morphological parameters (or, more generally, a larger plant body) becomes a distinct disadvantage when the water supply is limited. However, although a greater loss of photosynthetic efficiency was an inherent disadvantage, the precise causes and consequences of the original predisposition towards faster growth and biomass accumulation differed even between reciprocal hybrids. Our study also confirmed that the strategy for leaving stomata open even when the water supply is limited (coupled to a smaller body size and some other physiological properties), observed in one of our inbred lines, is associated with drought-resistance not only during mild drought as we showed previously but also during more severe drought conditions [343].

Recently, we have cooperated with the Department of Population Ecology of the Institute of Botany AS CR in the project “The role of genetic and epigenetic changes and trait variation in adaptation of a clonal plant to changing climate”.

Research projects: 28, 122



Station of Apple Breeding for Disease Resistance

Head of laboratory: **Ing. Radek Černý**

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In 2016, it was exactly 50 years since the establishment of our Station which is dedicated to research on breeding apple varieties with resistance to scab, the most widespread and harmful disease in apples, caused by *Venturia inaequalis*. The usual protection against scab requires dozens of chemical sprayings during vegetation. This is financially demanding, labor intensive and has an adverse effect on the environment. Utilization of new apple varieties with both desired growth and economical characteristics and durable resistance, substantially decreases the use of fungicides, has a beneficial effect on the environment and as a result, increases the profitability of apple tree growth through cost reduction of crop treatment.

The fungus *Venturia inaequalis* usually causes symptoms such as brown to black spots on leaves and grey-black scabs on the fruit rendering them unsaleable with economic loss to apple growers.

Resistance to scab in the majority of apple varieties is conditioned by a single gene, *Rvi6* (formerly known as *Vf*). The first source of scab resistance was found in a crab apple, *Malus floribunda* and since then it has been commonly used in breeding. The gene *Rvi6* can be transferred to the progeny by crossing. Its presence in offspring can be identified using molecular markers. By repeated crossing for many generations, we managed to combine resistance against diseases with growing/bearing characteristics and fruit qualities that fulfilled the properties required by growers and consumers.

However, monogenic resistance conferred by *Rvi6* is unstable in nature – in some locations it has already been overcome by new races of fungus. Our breeding program is, for this reason, focused on searching for new genetic sources of protection against scab by breeding apple varieties with stable resistance on a polygenic basis in combination with the *Rvi6* gene. Efficient use of polygenic



In the picture (from the left):

Standing: RNDr. Ludmila Říhová / research assistant, Ing. Jan Zima / research assistant, Dagmar Švestková / technician, Ing. Miloslav Juříček, CSc. / research scientist, Ing. Ludmila Neubauerová / research assistant, Ing. Radek Černý / head of the laboratory, Mgr. Veronika Schaabová / research assistant, Ing. Otto Louda / research assistant, Naděžda Nováková / guest, Květa Rabochová / technician.

Sitting: Zdeněk Mikula / technician, Zdeněk Haleš, DiS. / dipl. technician.

resistance in apple breeding will require the development of genetic markers to identify the genes involved, using molecular biology methods. As new fungus races always find ways to adapt and overcome plant defenses, breeding is a continuous process.

Apart from scab our research group is also working on tolerance to another fungal disease – powdery mildew, caused by the ascomycete *Podosphaera leucotricha*, which causes tree damage such as a whitish coating on leaves and shoots, eventually russetting on fruits which occurs particularly in dry regions.

Our research team forms an interface between basic research employing molecular methods of identification and genetic analysis of scab resistance and, applied research where the knowledge of basic research is used to breed resistant apple varieties with commercial potential.

The basic research involves elucidating the mechanisms of *Riv6* gene mediated resistance as well its breakdown by new races of *V. inaequalis*. The recent completion of the first draft of the genome sequence of *Malus x domestica* 'Golden Delicious' as well as rapid advances in the next generation sequencing (NGS) technology has given us very promising tools to study plant pathogen interactions in non-model species such as the apple tree.

In the past, we had isolated and maintained *V. inaequalis* conidia races that cannot infect *Rvi6* resistant apple varieties and also conidia of those new races that have the ability to overcome *Rvi6* mediated resistance. We have also developed an “in house technique” for artificial inoculation of apple seedlings with maintained conidia as well as monitoring the causal infection. The use of an artificial infection system allows us to evaluate the response of the host plant in detail by performing full quantitative transcriptome sequencing. It also enables us to evaluate differential RNA expression in *Rvi6* cultivar infected with two isolates of *V. inaequalis* in comparison with an uninfected one. Differentially expressed genes may represent various induced plant defense reactions leading to scab resistance.

So far, we have identified hundreds of up/down-regulated genes in apple transcriptomes of infected and uninfected samples. Highly differentially expressed genes were then characterized for their function by homology to *A. thaliana* and/or other known plant genes in relation to genes known to be involved in plant defense pathways. As a result, we found that several

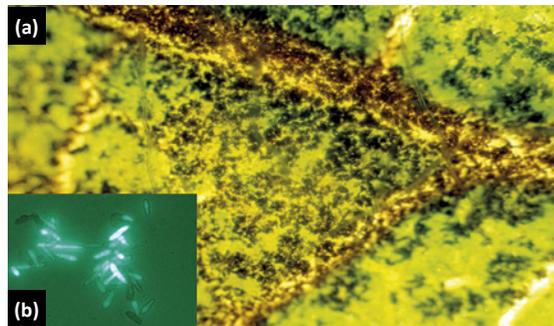


Figure 1: The susceptibility test (a) of *Rvi6* cultivar to *Venturia* inoculum prepared from ten one-conidial isolates (b). Clearly visible fields covered by large amount of newly formed conidia can be observed on the leaves 3 weeks post inoculation.



Figure 2: (a) Opal® in supermarket Globus in the Czech Republic, 2015. (b) Advertising of variety Opal® at the international professional fair Interpoma, Italy 2016. (c) Illustration of plantings of variety Opal® in Washington State, USA. (d) Advertising of variety Opal® by supermarket Tesco, Watford, Great Britain, 2016.

gene groups are involved in *Rvi6* resistance. These are pathogen related proteins (PR-proteins) that play a role in chemical defense against fungi (such as glucanases, chitinases, proteases, protease inhibitors and others), genes involved in signaling pathways and transmembrane transport. Further analyses, identification and characterization of other genes are now in progress.

Apart from disease resistance, new varieties must

meet stringent growth requirements to be commercially successful. These include growing characteristics, high and regular productivity, good storability and good fruit quality (appearance, flavor, firmness, crispness, juiciness and also durability during transfer and manipulation). In these respects, chosen IEB new selections are tested in the Czech Republic, in foreign research centers and by potential business partners



such are nurseries, producers and marketing companies. Commercially perspective varieties resulting from our breeding are legally protected, generally by Community Plant Variety Rights in the EU and by United States Plant Patent in the USA. These varieties are planted predominantly in organic orchards or eventually in integrated production. Propagation and sales are based on concluded License Agreements.

One of the most commercially successful varieties of the IEB breeding program is 'Topaz' and its red mutation 'Red Topaz'. This has been the best-selling European variety with resistance to scab over the last two decades with annual sales of nearly 400,000 trees. 'Topaz' is very popular among organic fruit growers; it is planted mainly in Europe on an area larger than 1,000 ha. 'Topaz' is also very frequently used as a good gene source for further breeding, mainly abroad.

Very popular, mainly in the USA, is the variety 'UEB 32642' known under the trademark Opal® which is registered in more than 40 countries worldwide. Fruit of this variety are characterized by a bright yellow skin, crunchy flesh and an aromatic honey sweet flavor. The variety is protected by the Plant Patent in the USA, by Community Plant Variety Rights in the EU and lately it has been applied for plant protection in a number of countries such are Australia, Brazil, Chile, South Africa, Canada, Morocco, Mexico and New Zealand. In years 2015–2017, more than 400,000 trees of variety Opal® were sold in Europe and in total, almost 1.7 million Opal® trees worldwide since its release onto the market.



Figure 3: Varieties of the new 3rd generation of apple trees with columnar type of growth 'Lambada' (on the left) and 'Rumba' (on the right).

In the USA, Opal® is grown at the farm of Mr. Ralph Broetje in Washington State and Opal® fruits are exclusively sold by the company First Fruits Marketing of Washington which dedicates a part of its total income to charity. For example, they donated 150,000 \$ to a charity in 2015. The Vista Hermosa Foundation, which was established by Ralph and Cheryl Broetje, supports the education and development of youth, a community building and also leadership development, small farmer training and resource development in East Africa, India, Haiti and Mexico.

Among the latest results of the IEB breeding program is an apple variety named 'Bonita'. The meaning of 'Bonita' (from Portuguese "*bonito*" = beautiful, pretty) suggests that the variety is characterized by very attractive, homogenous, bright red colored fruit, along with high and regular productivity and other positive growing characteristics. The exclusive license agreement for propagation and sale of this variety in the territory of the EU was signed with Konsortium Südtiroler Baumschuler from Italy in 2016. Almost 600,000 trees of 'Bonita' intended particularly for ecological orchards were propagated in the very first three years of the commercial production in South Tirol in 2015–2017. Sublicense agreements with other business partners were subsequently signed and these presume the growing of this variety in the territory of Austria, the Czech Republic, Hungary and France under conditions of an integrated production with reduced needs for chemical spraying. 'Bonita' is also at an advanced level of testing with very promising results particularly in the USA and South Africa.

With the example of 'Bonita' variety, the assumption that today's quality of new apple varieties with disease resistance is fully equivalent to and sometimes even better than traditional non-resistant varieties, was fully confirmed. This significantly increases the demand for resistant apple varieties not only for an organic production, representing in practice only about 5–15 % of the total production area, but also opens up new possibilities for integrated production with minimization of plant protection management.

Apart from dessert apple varieties, our program also includes breeding of varieties with a compact columnar growth habit. As a result, the global marketing contract for scab resistant varieties with this type of growth was concluded with an American company Varieties International LLC for the worldwide territory. Released columnar varieties are legally protected in the EU and in the USA. This type of tree is suitable mainly for home gardens as a beautiful solitaire or for planting of hedges. Columnar apple varieties may contribute to the return of fruit trees to smaller home gardens and allow observation of individual tree variability at the time of flowering, fruit formation and in an interesting growth habit. The new third generation

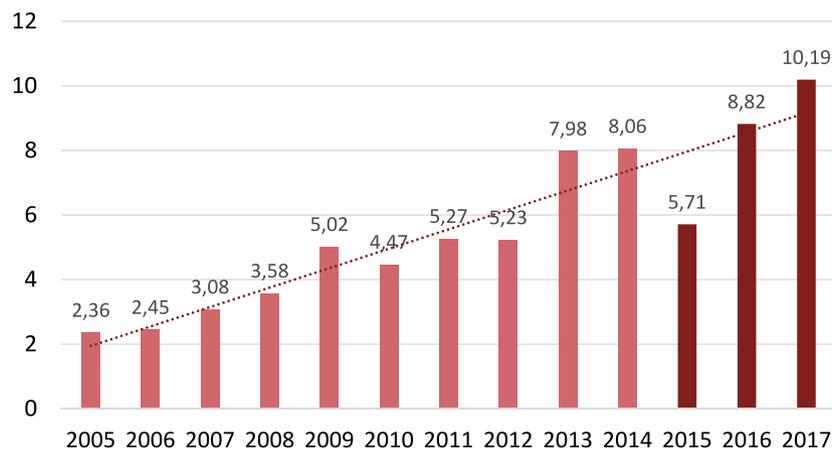


Figure 4: Income from royalties of licensed varieties of apple trees (in millions of Czech crowns – CZK)

this technology it is possible to graft selected seedlings on M9 rootstocks with weak vigor already after one year. This rootstock is characterized by a quick start to crop productivity and thus it shortens the evaluation of fruit quality by several years.

The Station now also possesses a cooling hall with regulated temperature and ventilation, including ULO technology boxes (Ultra Low Oxygen). The ULO technology slows down after-harvest fruit ripening by both regulating the temperature and especially by decreasing oxygen content and increasing carbon dioxide concentration in the atmosphere. This new equipment will permit us to precisely evaluate the storability of fruits and maintain chosen varieties in excellent condition until spring. It will allow us to present them to our business partners in a great quality and participation in professional degustation in late terms of apple storage will be possible as well.

New advanced technologies will make the process of breeding faster and more effective.



Figure 5: Modernized apple breeding station in Střížovice equipped with a new greenhouse and a storage hall including ULO technology.

columnar apple trees of the IEB breeding program was introduced onto the market in 2016 and is represented by varieties 'Lambada' and 'Rumba'.

In the years 2015–2017, the total annual tree sales of IEB varieties with columnar tree type in the Netherlands, Germany, the USA, Switzerland and the Czech Republic rose from 50,000 to almost 80,000. Approximately 550,000 trees have already been sold since 2008 representing the IEB breeding program in home gardens in the Czech Republic and abroad. There is progressive growth especially in the USA, where selected columnar IEB apple varieties are sold at almost 200 stores. Chosen varieties are newly tested in Australia, with potential for commercial production.

The total number of IEB apple tree varieties sold reached over 1.3 million in 2017. The annual income from royalties for licensed varieties was over 10 million CZK in 2017.

In the years 2016–2017, the breeding station in Střížovice was modernized thanks to the generous financial support of the Czech Academy of Sciences and the Institute of Experimental Botany CAS. In particular, a new greenhouse and storage rooms were built.

The modern greenhouse with automatic temperature, light, irrigation and humidity regulation will accelerate the choice of new selections with prospective stable scab resistance. It will allow us to sow apple seeds as early as January, with the aim of artificially inoculating young seedlings with scab conidia and thus pre-select the most promising resistant individuals before planting them in the orchard. Thanks to

Research projects: 122







Research Projects 2015–2017

1. 7AMB15FR038, TriAnnot
2. 7AMB16AT036, Metabolome dynamics in growing pollen tubes following their interaction with female tissues
3. 7AMB17FR005, Role of plasma membrane microdomains and flotillins in the control of the dynamics of the IRT1 iron transporter in *Arabidopsis*
4. 7AMB17FR006, Selection of rhizobacterial communities associated with differences in plant resistance to pathogens
5. 746253, Widening the gene pool of bread wheat by interspecific variation from *Aegilops biuncialis* using advanced genetic and chromosome-genomic resources and tools (AEGILWHEAT)
6. 8G15003, Uptake of engineered nanoparticles (ENPS) by plants and its implications for potential remediation of contaminated water and soil
7. CZ.2.16/3.1.00/21519, Modern equipment for plant research
8. ED3.1.00/14.0327, New biotechnological products of IEB ASCR
9. EF16_013/0001775, Modernization and support of research activities of the national infrastructure for biological and medical imaging Czech-Biolmaging
10. FR-TI3/778, Wastewaters reclamation in integrated biotechnology system
11. FV10599, Gels based on plant triterpenoid acids
12. GA13-02290S, The role of hybridization and polyploidization on the evolution in *Chenopodium album* aggregate: From biosystematics to gene expression
13. GA13-04454S, Foreign genetic material in *Elymus repens* and other Triticeae grasses: its nature, origin, and evolutionary implications
14. GA13-06595S, Telomeres and genome stability in lower plants
15. GA13-06943S, Structural and functional components of plant telomeres
16. GA13-08786S, Chromosome arm 3DS of bread wheat: its sequence and function in allopolyploid genome
17. GA13-11101S, Following elusive low molecular weight organochlorine compounds of natural and anthropogenically affected ecosystems
18. GA13-19073S, Multiscale analysis of signalling phospholipids and their interaction protein partners in the regulation of plant tip growth
19. GA13-26798S, Fungal effectors manipulating plant defence system
20. GA14-07164S, Cloning and molecular characterization of wheat QPm-tut-4A gene conferring seedling and adult plant race nonspecific powdery mildew resistance
21. GA14-09685S, Flotillin: a novel player in plant stress signaling
22. GA14-19590S, Modulation of cyclin-dependent kinases in haematological malignancies
23. GA14-22593S, Metabolism of selected non-steroidal anti-inflammatory drugs in plants and its environmental consequences
24. GA14-28443S, Dark matter in plant cell nuclei – characterization of nuclear proteins
25. GA14-32292S, Widespread translation repression and function of paternally stored transcripts nurturing early stages of embryonic patterning
26. GA14-34792S, New analytical approaches in phytohormone analysis
27. GA15-05325S, Anthelmintics in plants – uptake, biotransformation and transcriptional response
28. GA15-10768S, Impact of humanised glycosylation pathway on protein accumulation and trafficking in plant seeds

29. GA15-14886S, Plant exocyst complex function in autophagy-related membrane transport
30. GA15-15264S, Targeted transport of purine cyclin-dependent kinase Inhibitors into cancer cells
31. GA15-16050S, RNA-binding proteins controlling translational repression of stored transcripts in developing male gametophyte
32. GA15-22322S, Molecular modulation of cytokinin metabolism in model plants *Physcomitrella* and maize focused on function of nucleosidases
33. GA15-22720S, Signalling during pollen-pistil interaction preceding fertilization; the role of short- and long-distance secreted signals of fertilization
34. GA15-24711S, Regulation of pollen tube growth by the exocyst vesicle-tethering complex: functions of multiple isoforms of the EXO70 exocyst subunit
35. GA16-01137S, Factors of genome stability in moss and flowering plants
36. GA16-04184S, Study of the intracellular distribution of cytokinins and their transport to vacuoles
37. GA16-07193S, Anti-inflammatory activity of selected stilbenoids, 2-arylbenzofuranones and their metabolites
38. GA16-08698S, Origin and evolution of sex chromosomes in the dioecious plant *Rumex acetosa*
39. GA16-09220S, Tower of Babel: Mitochondrial-nuclear interactions in the gynodioecious species *Silene vulgaris* investigated with transcriptomics
40. GA16-10948S, Establishment and regulation of auxin homeostasis on a single cell level: Metabolic and transport processes
41. GA16-14649S, Inactivation of cytokinin-type phytohormones via N- and O-glucosylation – phylogeny and significance in evolution of hormonal homeostatic mechanism
42. GA16-16992S, Chromosome genomics of *Agropyron cristatum*, a wild relative of wheat
43. GA16-19557S, Specificity and regulation of auxin transport by nitrate transporter NRT1.1 in plants
44. GA17-00522S, A new insight into the role of phospholipase in leaf senescence
45. GA17-04607S, Light – cytokinin interactions in contrasting *Arabidopsis thaliana* ecotypes during cold acclimation and their impact on freezing stress responses
46. GA17-05151S, Phospholipid metabolizing enzymes as new components of salicylic acid signalling pathway
47. GA17-05341S, Physical map of Ph2 region in hexaploid wheat
48. GA17-06548S, Foreign DNA in barley (*Hordeum* spp.) – are there any genomic enablers of horizontal gene transfer in grasses?
49. GA17-06613S, Phytohormone cross-talk during sub-zero acclimation
50. GA17-10280S, Variability in plant traits as a tool to cope with climate change – from phenotypes to genes and back again
51. GA17-10591S, Definition of physiological, metabolic and adaptation processes in the fern *Pteris cretica* growing on soils contaminated with arsenic
52. GA17-10907S, Environmental impact of noble metal nanoparticles
53. GA17-13853S, Nuclear architecture in interspecific plant hybrids
54. GA17-14007S, Modulation of CDK and related molecular targets in aggressive non-Hodgkin lymphomas
55. GA17-14048S, Spatial and temporal characterization of DNA replication in phylogenetically related plant species with contrasting genome sizes
56. GA17-17564S, Dynamics and evolution of multigene ribosomal RNA loci in Triticeae
57. GA17-23183S, Revealing pollen bZIP transcriptional regulons in *Arabidopsis thaliana*
58. GA17-23203S, mRNA inheritance as a mechanism of parental control over zygotic development
59. GA17-27477S, Multifaceted analysis of diacylglycerol kinase family in plants
60. GAP305/12/2611, The role of bZIP proteins in the control of lipid metabolism and transport during male gametophyte development
61. GAP501/12/1942, Nonspecific phospholipase C: Molecular, cellular and functional characterisation of novel plant enzyme
62. GAP501/12/2220, Sex chromosome evolution – chromosome-specific genomics in genus *Silene*
63. GAP501/12/2554, Physical map of wheat chromosome arm 7DS and its use to clone a Russian wheat aphid resistance gene
64. GAP505/11/1163, Anti-inflammatory activity of extracts isolated from selected Indonesian plants and their effect on opportunistic parasitoses
65. GAP506/12/1320, Will orchids reshape our understanding of genome-wide processes? Solving the enigma of progressively partial endoreduplication
66. GAP506/12/1359, The control of flowering in *Chenopodium* investigated by the transcriptomic approach
67. GBP501/12/G090, Evolution and function of complex plant genomes
68. GJ15-08202Y, Synthesis of new brassinosteroids and study of their interaction with plant and animal receptors
69. GJ16-07155Y, Characterization of meiotic recombination regions in bread wheat
70. GJ17-21581Y, Auxin homeostasis on subcellular level
71. GP13-41444P, The role of auxin and auxin-amino acid conjugate hydrolases during male gametophyte development of *Arabidopsis thaliana*
72. GPP501/12/P951, The role of auxin binding protein 1-mediated signaling in the control of vesicle trafficking in plant cells
73. LD13013, Production of anticancer polyacetylenes by elicited ginseng cultures
74. LD13028, Ecotoxicity of novel flame retardants and their degradation products
75. LD13029, The utilization of charcoal for immobilization of heavy metals



76. LD13049, Localisation of translation of cell wall components in growing pollen tube, an effectively single-cell model system
77. LD13050, Effect of drought and heat stress on polyamine metabolism and contents of phenolics, auxin and abscisic acid in Norway spruce somatic embryos
78. LD13057, Gels produced through supramolecular self-assembly for medicinal chemistry applications
79. LD14056, Mechanism of activation of plant defence against pathogens using protein inducers
80. LD14078, Metabolic interactions between ash tree and its new invasive fungal pathogen *Hymenoscyphus pseudoalbidus*
81. LD14079, Plant non-wood forest products as a source of biologically active substances
82. LD14100, The toxicity of nanoparticles for wetland plants
83. LD14105, Development of marker panel for genotyping and molecular characterization of *Blumeria graminis* f.sp. *hordei* isolates
84. LD14106, Remediation of urban sites using energy plants
85. LD14107, Remediation of urban brownfields using plants
86. LD14109, Role of microRNAs in the regulation of cell wall biosynthesis – implications for the fertility of crop plants
87. LD14120, The impact of phosphorus nutrition on strigolactone, cytokinin and auxin cross-talk
88. LD14125, Phytotoxicity of nanofibres
89. LD14127, Synthesis of strigolactone derivatives
90. LD14128, Synthesis of sirtuin inhibitors
91. LD15006, Synthesis of Hh/SMO inhibitors as a potential anticancer drugs
92. LD15012, Triterpenoid acids in supramolecular chemical biology
93. LD15088, Regulation of transport of auxins and their metabolism by strigolactones
94. LD15093, Comparison of the impact of biotic and abiotic stresses on plant hormonal pool
95. LD15137, Nitrate transceptor NRT1.1 as a tool of plant adaptability to the changing nutrient conditions
96. LG15017, Collaboration with Bioversity International on global analysis and conservation of genetic diversity of banana
97. LH15075, Comparative genomics of plant mitochondria
98. LK21306, Targeted metabolite profiling of plant growth regulators
99. LM2015062, National infrastructure for biological and medical imaging
100. LO1204, Sustainable development of research in the Centre of the Region Haná
101. LTAUSA17081, Hormonal mechanisms of plant acclimation to heat and cold stresses
102. LTC17013, Plant defence system in multiple parallel biotic stresses on the model interaction: *Brassica napus* – *Leptosphaeria maculans* – insect pests
103. LTC17030, Contribution of superresolution microscopy and image analysis to the study of plant in vitro cultures with the emphasis on somatic embryogenesis of conifers
104. LTC17033, Differences between conifers and deciduous trees in metabolomic and physiological responses to selected pesticides and their utilization for environment protection
105. LTC17034, The profile of carotenoids in selected apple varieties in relation to storage conditions
106. LTC17035, Search for new sources of valuable and biologically active carotenoids in rarely used plant species
107. LTC17036, The role of polyamines in the process of plant autophagy
108. LTC17046, Possibilities of phytosorption and phytoextraction of REE from contaminated water and soil by plants
109. LTC17047, The use of plants in monitoring of human DNA damage
110. LTC17048, Synthesis of kinase inhibitors aiming at autophagy
111. LTC17084, The role of lipids and lipid-metabolizing enzymes in plant autophagy
112. LTV17010, Participation at EPSO meetings
113. MSM200381701, AtAUX1: molecular mechanism of its action and the relationships between its structure and transport function
114. PIRSES-GA-2013-612587, Plant DNA tolerance – Plant adaptation to heavy metal and radioactive pollution
115. QK1710302, Improvement of common wheat tolerance to drought, frost, *Phytophthora infestans* and *Fusarium* head blight using genomics and proteomics approaches
116. QK1710397, Characterization of compatibility of relations between agents causing blackleg and oilseed rape varieties as a basis for increasing of growing rentability of this crop in the Czech Republic
117. R20038142, Studium faktorů ovlivňujících rozvoj vodního květu na přehradě Seč (*Investigation of factors affecting algal bloom dynamics in the Seč water reservoir*)
118. TA01010861, Research, testing and production of targeted growth regulators, new fertilizers and combined formulations for crop production
119. TA01020573, Biotechnology system for agricultural waste-waters cleaning and reuse
120. TA01020744, Biodegradable polymers in waste management
121. TA04020547, Progressive biotechnology based on new synthetic cytokinin derivatives to obtain doubled haploid lines of caraway, linseed and pea
122. TG03010009, Support for the process of commercializing the results of research and development at the Institute of Experimental Botany AS CR v. v. i.
123. UH0109, Technological transfer and commercialization of R & D outputs in the Institute of Experimental Botany AS CR





Publications 2015–2017

Impacted Publications

2015

1. Akpinar BA, Lucas SJ, **Vrána J, Doležel J**, Budak H* (2015) Sequencing chromosome 5D of *Aegilops tauschii* and comparison with its allopolyploid descendant bread wheat (*Triticum aestivum*). PLANT BIOTECHNOLOGY JOURNAL 13: 740-752.
2. Akpinar BA, Magni F, Yuce M, Lucas SJ, **Šimková H, Šafař J**, Vautrin S, Bergès H, Cattonaro F, **Doležel J**, Budak H* (2015) The physical map of wheat chromosome 5DS revealed gene duplications and small rearrangements. BMC GENOMICS 16: 453.
3. Akpinar BA, Yuce M, Lucas S, **Vrána J, Burešová V, Doležel J**, Budak H* (2015) Molecular organization and comparative analysis of chromosome 5B of the wild wheat ancestor *Triticum dicoccoides*. SCIENTIFIC REPORTS 5: 10763.
4. Amoo SO, Aremu AO, Moyo M, Sunmonu TO, **Plíhalová L, Doležal K**, Van Staden J* (2015) Physiological and biochemical effects of a tetrahydropyranil-substituted meta-topolin in micropropagated *Merwillia plumbea*. PLANT CELL, TISSUE AND ORGAN CULTURE 121: 579-590.
5. Antoniadou I, **Plačková L**, Simonovik B, **Doležal K**, Turnbull C, Ljung K, **Novák O*** (2015) Cell-type-specific cytokinin distribution within the *Arabidopsis* primary root apex. PLANT CELL 27: 1955-1967.
6. Aremu AO, **Plačková L, Grúz J, Bíba O, Šubrtová M, Novák O, Doležal K**, Van Staden J* (2015) Accumulation pattern of endogenous cytokinins and phenolics in different organs of 1-year-old cytokinin pre-incubated plants: implications for conservation. PLANT BIOLOGY 17: 1146-1155.
7. Aremu AO, Masondo NA, Rengasamy KRR, Amoo SO, **Grúz J, Bíba O, Šubrtová M, Pěňčík A, Novák O, Doležal K**, Van Staden J* (2015) Physiological role of phenolic biostimulants isolated from brown seaweed *Ecklonia maxima* on plant growth and development. PLANTA 241: 1313-1324.
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9. Aremu AO, Stirk WA, Masondo NA, **Plačková L, Novák O, Pěňčík A, Zatloukal M, Nisler J, Spíchal L, Doležal K**, Finnie JF, Van Staden J* (2015) Dissecting the role of two cytokinin analogues (INCYDE and PI-55) on *in vitro* organogenesis, phytohormone accumulation, phytochemical content and antioxidant activity. PLANT SCIENCE 238: 81-94.
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20. **Burešová V, Kopecký D*, Bartoš J**, Martinek P, Watanabe N, Vyhnanek T, **Doležel J** (2015) Variation in genome composition of blue-aleurone wheat. THEORETICAL AND APPLIED GENETICS 128: 273-282.
21. **Burketová L*, Trdá L**, Ott PG, Valentová O (2015) Bio-based resistance inducers for sustainable plant protection against pathogens. BIOTECHNOLOGY ADVANCES 33: 994-1004.
22. **Cápal P, Blavet N, Vrána J, Kubaláková M, Doležel J*** (2015) Multiple displacement amplification of the DNA from single flow-sorted plant chromosome. PLANT JOURNAL 84: 838-844.
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27. Cvrčková F*, **Oulehlová D**, **Žárský V** (2015) Formins: linking cytoskeleton and endomembranes in plant cells. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 16: 1-18.
28. **Cyrusová T**, **Podlipná R**, **Vaněk T*** (2015) The effect of nanoparticles on plants. CHEMICKÉ LISTY 109: 276-280.
29. **Čížková J**, **Hřibová E**, **Christelová P**, Van den Houwe I, Häkkinen M, Roux N, Swennen R, **Doležel J*** (2015) Molecular and cytogenetic characterization of wild *Musa* species. PLoS ONE 10: e0134096.
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35. **Eliášová K**, **Pešek B**, **Vondráková Z*** (2015) Storage compounds, ABA and fumarase in *Fagus sylvatica* embryos during stratification. DENDROBIOLOGY 74: 25-33.
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37. Fojtová M, Sýkorová E, Najdekrová L, Polanská P, Zachová D, **Vágnerová R**, **Angelis K**, Fajkus J* (2015) Telomere dynamics in the lower plant *Physcomitrella patens*. PLANT MOLECULAR BIOLOGY 87: 591-601.
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Non-impacted Publications

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2015

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Issue date: 02.01.2015. **Patent No. KR101478727**
2. **Nisler J**, Zatloukal M, **Spíchal L**, **Doležal K**, **Strnad M**. Derivatives of 1,2,3-thiadiazol-5-yl urea, their use for controlling plant senescence and formulations in which these derivatives are comprised.
Issue date: 09.12.2015. **Patent No. CZ305649**

2016

3. Valentová O, **Burketová L**, **Šašek V**, Phuong DK, Fajmonová J. Plant protection formulation, preparation and use thereof.
Issue date: 06.04.2016. **Patent No. CZ305950**
4. **Burketová L**, **Šašek V**, Kolomazník K, Havel J, Věchet L. Plant biostimulator.
Issue date: 20.04.2016. **Patent No. CZ305975**
5. **Nisler J**, Zatloukal M, Koprna R, **Spíchal L**, **Strnad M**, **Doležal K**. Use of N-furfuryl-N'-1,2,3-thiadiazol-5-yl urea for inhibition of senescence, stress and/or oxidative damage.
Issue date: 04.05.2016. **Patent No. CZ306009**
6. **Szüčová L**, Zatloukal M, **Spíchal L**, Fröhlich L, **Doležal K**, **Strnad M**, Massino FJ. 6,9-disubstituted purine derivatives and their use for treating skin.
Issue date: 10.05.2016. **Patent No. CA2657516**
7. **Szüčová L**, Zatloukal M, **Spíchal L**, Fröhlich L, **Doležal K**, **Strnad M**, Massino FJ. 6,9-disubstituted purine derivatives for cosmetic use.
Issue date: 07.09.2016. **Patent No. EP2043630**

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Issue date: 8.11.2016. **Patent No. CA2710981**
9. **Voller J**, **Zahajská L**, **Plíhalová L**, Komárková J, Burget D, **Kryštof V**, Zatloukal M, **Doležal K**, **Strnad M**, Rosel D, Brábek J, Pataki AC. 2,6-disubstituted purines for use as pharmaceuticals, and pharmaceutical preparations.
Issue date: 07.12.2016. **Patent No. CZ306434**

2017

10. **Dvořáková M**, **Vaněk T**, **Tarkowská D**. Paclitaxel derivatives, the method of production and the use.
Issue date: 08.03.2017. **Patent No. CZ306653**
11. **Zahajská L**, **Nisler J**, Kadlecová A, Zatloukal M, **Grúz J**, **Voller J**, **Doležal K**, **Strnad M**. 6,8-disubstituted-9-(heterocyclyl)purines, preparations containing these derivatives and their use in cosmetic and medical applications.
Issue date: 20.09.2017. **Patent No. CZ306984**
12. **Voller J**, **Zahajská L**, **Plíhalová L**, Komárková J, Burget D, **Kryštof V**, Zatloukal M, **Doležal K**, **Strnad M**, Rösel D, Brábek J, Pataki AC. 2,6-disubstituted purines for use as pharmaceuticals and pharmaceutical preparations containing them.
Issue date: 20.09.2017. **Patent No. CZ306987**
13. **Havlíček L**, Štunc A, **Kryštof V**, Jorda R, Pospíšil T, Zahler S, Vollmar A, **Strnad M**. 5-Substituted 7-[4-(2-pyridyl)phenylmethylamino]-3-isopropylpyrazolo[4,3-d]pyrimidines, their use as pharmaceuticals, and pharmaceutical preparations.
Issue date: 27.12.2017. **Patent No. CZ307147**





Apple Varieties 2015–2017

2015

1. **Tupý J, Louda O, Zima J.** Variety of *Malus domestica* Borkh., **UEB 32642**.
Issue date: 15.01.2015. Identification No. N° 013/15.
A new and distinct, late, dessert, diploid apple variety characterized by medium vigor of trees, scab resistance based on *Vf* gene, high tolerance to mildew, medium sized, round-conical fruits, gold-yellow ground color of the fruit skin with slight orange blush and russeting around the stem cavity, yellow, firm and crisp fruit flesh with a very good sweet aromatic flavor, and very good keeping quality and long shelf life of the fruits. The best fruit quality of the variety is achieved under an intensive growing system in dry and warm wine growing climate.
2. **Tupý J, Louda O, Zima J.** Columnar apple tree named “**ROSALIE**”.
Issue date: 05.05.2015. Identification No. US PP25,501.
A new and distinct, apple tree variety with columnar tree type with moderate growth vigor, *Vf* resistance against scab, ornamental fruits and late maturing. The fruit color is red and the fruit size is large relatively to other ornamental apples.
3. **Tupý J, Louda O, Zima J.** Variety of *Malus domestica* Borkh., **ALLEGRO**.
Issue date: 31.5.2015. Identification No. CH 15.2567.
A new and distinct, healthy and friendly, early ripening apple variety with attractive bicolor appearance, good fruits quality, sweet taste and resistance against scab on assumed polygenic basis, the variety is suitable for organic production as a home garden.

2016

4. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **UEB 112**.
Issue date: 24.02.2016. Identification No. CZ 9/2016.
A new and distinct, late, dessert apple variety which is characterized by fruits with outstanding red coloration on yellow ground color and scab resistance based on *Vf* gene.
5. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **UEB 481**.
Issue date: 24.02.2016. Identification No. CZ 10/2016.
A new and distinct, late, dessert apple variety which is characterized by globose, yellow colored fruit with sweet aromatic taste and scab resistance based on *Vf* gene.
6. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **BONITA**.
Issue date: 24.02.2016. Identification No. CZ 11/2016.
A new and distinct, late, dessert apple variety which is characterized by very attractive bright red colored fruit with globose shape, high and regular productivity and scab resistance based on *Vf* gene.

7. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **RUBELIT**.
Issue date: 23.03.2016. Identification No. CZ 12/2016.
A new and distinct, late, dessert apple variety which is characterized by medium to large, red colored fruits with strongly defined stripes, very good eating qualities and scab resistance based on *Vf* gene.

2017

8. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **UEB 1851**.
Issue date: 16.01.2017. Identification No. CZ 3/2017.
A new and distinct, late, dessert apple variety which is characterized by medium to large, red colored fruits with strongly defined stripes and medium to large sized lenticels; Variety is resistant against scab based on *Vf* gene.
9. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **LUCY**.
Issue date: 23.01.2017. Identification No. CZ 4/2017.
A new and distinctive late dessert apple variety with *Vf*-resistance against scab, medium vigor, drooping tree habit and medium sized globose dark red fruits with weak defined stripes. Fruits have an aromatic sweet-sour flavor. Thinning to achieve regular crop is recommended.
10. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **BONITA**.
Issue date: 31.01.2017. Identification No. CH 17.2667.
A new and distinct late dessert apple variety with good growing characteristics, heavy and regular crop without necessity of thinning. It has a very attractive bright red fruits of good shape and size with a good acidic taste. This variety is suitable particularly for intensive production.
11. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **BONITA**.
Issue date: 06.02.2017. Identification No. EU 45354.
A new and distinct late dessert apple variety with good growing characteristics, heavy and regular crop without necessity of thinning. It has a very attractive bright red fruits of good shape and size with a good acidic taste. This variety is suitable particularly for intensive production.
12. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **LAMBADA**.
Issue date: 10.03.2017. Identification No. CZ 14/2017.
A new and distinct medium to late apple variety characterized by a columnar tree type, presence of *Vf*-resistance against scab and yellow fruits of a very good taste, with eating maturity immediately or shortly after picking. The variety is suitable for home gardens in narrow hedges and for production of dessert apples.

13. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **SWING**.
Issue date: 10.03.2017. Identification No. CZ 15/2017.
A new and distinct late apple variety characterized by a columnar tree type, presence of Vf-resistance against scab and purple red to brown red fruits with firm flesh, very good eating quality, good storability and late eating maturity.
14. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **RUMBA**.
Issue date: 10.03.2017. Identification No. CZ 16/2017.
A new and distinct late apple variety characterized by a very narrow columnar tree type, presence of Vf-resistance against scab and attractive bright red fruits of a good quality and storability. The variety is suitable for home gardens as a solitaire or for narrow hedges, as well as for dessert apples production and juice industry.
15. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **RUBELIT**.
Issue date: 20.03.2017. Identification No. EU 45798.
A new and distinctive early winter dessert apple variety with Vf-resistance against scab, medium to high vigor, bigger slightly ribbed red fruits with strongly defined stripes of a very good, slightly acidic flavor. The variety is suitable for intensive production and for home gardens as it is easy to manage.
16. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **UEB 112**.
Issue date: 20.03.2017. Identification No. EU 45799.
A new and distinctive late dessert apple variety with Vf-resistance against scab, attractive fruits with a big area of red blush on yellow ground color and firm flesh of acidic flavor. Thinning to achieve regular crop is recommended.
17. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **UEB 481**.
Issue date: 20.03.2017. Identification No. EU 45800.
A new and distinctive late dessert apple variety with Vf-resistance against scab and medium to big globose yellow fruits with a flushed pink blush, russet free, firm flesh of very good, aromatic sweet flavor.
18. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **UEB 1851**.
Issue date: 20.03.2017. Identification No. EU 45801.
A new and distinctive late dessert apple variety with Vf-resistance against scab, characterized by medium to high vigor and obloid purple red fruits with stripes and visible lenticels. Fruits have very late eating maturity, sweet-sour taste and a good storability. The variety is suitable to warm areas where it matures better and is suitable especially for ecological production.
19. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **LUCY**.
Issue date: 20.03.2017. Identification No. EU 45802.
A new and distinctive late dessert apple variety with Vf-resistance against scab, medium vigor, drooping tree habit and medium sized globose dark red fruits with weak defined stripes. Fruits have an aromatic sweet-sour flavor. Thinning to achieve regular crop is recommended.
20. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **LUCY**.
Issue date: 30.04.2017. Identification No. CH 17.2674.
A new and distinctive late dessert apple variety with Vf-resistance against scab, medium vigor, drooping tree habit and medium sized globose dark red fruits with weak defined stripes. Fruits have an aromatic sweet-sour flavor. Thinning to achieve regular crop is recommended.
21. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **LAMBADA**.
Issue date: 30.04.2017. Identification No. CH 17.2675.
A new and distinct medium to late apple variety characterized by a columnar tree type, presence of Vf-resistance against scab and yellow fruits of a very good taste, with eating maturity immediately or shortly after picking. The variety is suitable for home gardens in narrow hedges and for production of dessert apples.
22. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **RUMBA**.
Issue date: 30.04.2017. Identification No. CH 17.2676.
A new and distinct late apple variety characterized by a very narrow columnar tree type, presence of Vf-resistance against scab and attractive bright red fruits of a good quality and storability. The variety is suitable for home gardens as a solitaire or for narrow hedges, as well as for dessert apples production and juice industry.
23. **Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **TOPAZ**.
Issue date: 10.07.2017. Identification No. No 216.
A new and distinct winter dessert apple variety with Vf-resistance against scab, characterized by medium vigor, heavy and regular crop of obloid yellow fruits with strongly defined orange stripes on large area of over color and very good acidic flavor. The variety is suitable particularly for intensive production and for home gardens.
24. **Černý R, Zima J, Tupý J, Louda O.** Variety of *Malus domestica* Borkh., **ALLEGRO**.
Issue date: 17.07.2017. Identification No. EU 47325.
A new and distinct, healthy and friendly, early ripening apple variety with attractive bicolor appearance, good fruits quality, sweet taste and resistance against scab on assumed polygenic basis, the variety is suitable for organic production as a home garden.



Science Outreach

The institute actively promotes its research, as well as the field of plant sciences, among the general public. The primary focus is on Czech citizens of all ages – from preschoolers to seniors. We participate in several regular events organized by the Czech Academy of Sciences or by other institutions. We also organize our own events and cooperate with schools, research institutes and science centers on several projects.

Public relations and science outreach activities of our institute are coordinated by a group of professional science popularizers and PR specialists. At the end of 2017, this team had three members:

- **Mgr. Jan Kolář, Ph.D.** (mainly press releases, media relations, web pages for the public, social media and other PR services),
- **Mgr. Markéta Fílová** (event management, workshops, science club for young children, etc.),
- **Ing. Radoslava Kvasničková** (PR services and event management for the Centre of Plant Structural and Functional Genomics).

These professionals are assisted by a team of mostly young researchers and students who develop various science outreach activities, show them at public events and help with event management. In addition, many more scientists participate in the annual Open Door Days or have public lectures on various topics from plant sciences.

Core activities and regular events

The institute's website has a section for journalists and the general public (www.ueb.cas.cz/cs/pro-verejnost). Here we inform about important discoveries and interesting projects of our scientists, upcoming events, media coverage of our research, etc. To reach an even broader audience, we also operate social media accounts on Facebook (facebook.com/UEBavcr) and Twitter (twitter.com/uebavcr).



Figure 1: Artist Federico Díaz in front of his work, *Subliminal*, at EXPO 2015.

Our institute is involved in many outreach activities. Their complete list would be rather long, so here we will just briefly review some selected examples.

We participate in several annual events that promote general interest in science and present a broad array of scientific fields. The largest and most important one is Week of Science and Technology organized by the Czech Academy of Sciences (CAS). During this week, our researchers promote plant biology mainly by lectures and interactive exhibitions. In 2017, a whole day was dedicated to the research program Food for the Future. This program focuses on breeding, agriculture, food processing, biotechnologies and food waste reduction. It involves teams from seven CAS institutes and is coordinated by professor Jaroslav Doležel from our Centre of Plant Structural and Functional Genomics. The program's day at the Week of Science and Technology featured several lectures, a panel discussion and an interactive exhibition.

During the Week of Science and Technology, our institute holds its Open Door Days. School groups, university students and other people interested in plant biology can visit laboratories, talk to scientists and get involved in hands-on activities. We attract almost one thousand visitors every year.

The Czech Academy of Sciences also organizes the Science Fair – a big three-day event at a fairground in Prague. Nearly all CAS institutes present their research here. Our program typically features microscopes, (bio)chemical experiments, a small exhibition of *in vitro* plants and workshops where visitors can learn how to operate basic laboratory equipment (e.g. automatic pipettes).

Other annual activities include Science Festival in Prague, Researchers' Night in Olomouc and Prague Museum Night. We also participate in the Fascination of Plants Day, held biennially by the European Plant



Figure 2: At the Open Door Days, visitors really get in touch with plant biology.

Science Organisation. This worldwide event is focused on plant biology and its importance for humanity.

Our Centre of Plant Structural and Functional Genomics (which coordinates the research program Food for the Future mentioned above) is located in the Moravian city of Olomouc. This centre, as well as the program it coordinates, promote their work at two major events in the city. One of them is Flora Olomouc, a large exhibition of ornamental plants and crops. The second one is Academia Film Olomouc (AFO), an international festival of science documentary films. From 2017, Food for the Future awards a prize at AFO for the best film popularizing global food security issues or related topics.

Major exhibitions

The Czech Academy of Sciences invited several of its institutes to participate in the universal exhibition EXPO 2015 in Milan, Italy. Their research topics were presented in the Czech pavilion, mostly by artworks made by renowned Czech artists directly for EXPO

2015. The Institute of Experimental Botany was among those invited and the artist Federico Díaz created a set of reliefs named *Subliminal* for us. These reliefs were inspired by the wheat genome which was being sequenced at our Centre of Plant Structural and Functional Genomics. A short video explaining the international wheat sequencing program was also prepared for EXPO.

In 2015, our institute and the Institute of Botany CAS also prepared an exhibition “Botanical Stories: World of Plants from Knowledge to Use”.

In 2016, the Czech Academy of Sciences organized an exhibition named “Echoes of EXPO 2015” in downtown Prague. It featured the artworks from Milan inspired by CAS research (including *Subliminal*), supplemented with interactive exhibits.

“Meet Our Crazy Scientists” was an exhibition that Markéta Fílová prepared for summer 2017. It was held in Průhonice near Prague, in a visitor centre of a large natural park which is a UNESCO World Heritage Site. The exhibition presented various hobbies of our employees – from photography and painting to wood carving and amateur theatre.

The Centre of Plant Structural and Functional Genomics cooperates with Fort Science, an interactive science centre of Palacký University in Olomouc. Together they prepared a new permanent exhibition that explains genetics, DNA, plant breeding and related topics. It was officially opened in Fort Science in March 2018.

Other outreach activities

In order to give children, teenagers and adults a direct personal experience with science, we organize various hands-on workshops in our laboratories. For example, participants can learn the basics of plant *in vitro* cultivation or become familiar with laboratory equipment.

In 2017, Markéta Fílová started a science club for young schoolchildren. By hands-on activities, simple experiments and games, they learn about plants and their biology in a playful way.

Scientists from the institute also wrote three popular science brochures for the series *Science around Us*. These are published in Czech by Academia, the publishing house of the Czech Academy of Sciences. Contributions by our authors were *Somatic Embryogenesis in Conifers* (2015), *ScholarOne System: Peer Review and Editing in the Digital Era* (2016) and *Phytoremediation and the Perspectives of Its Application* (2017).



Figure 3: “Principles of plant *in vitro* cultivation” is our most popular workshop.

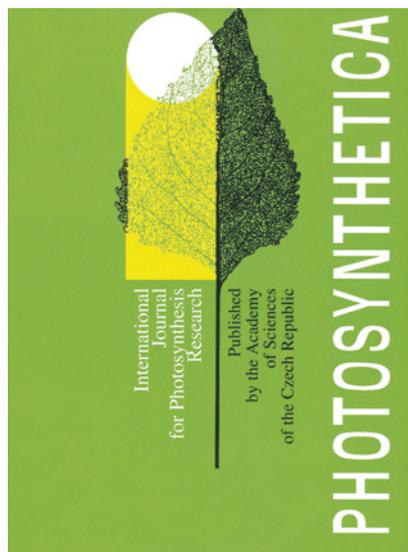
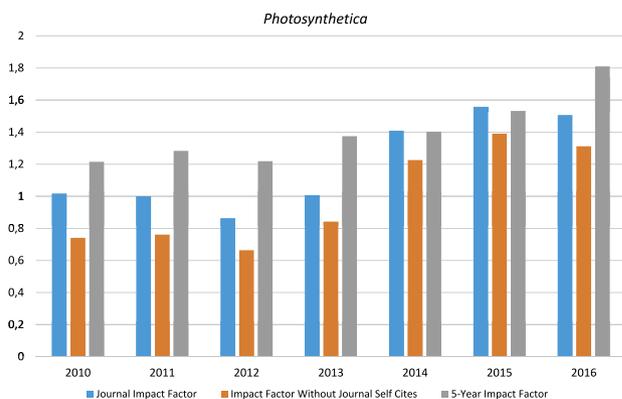
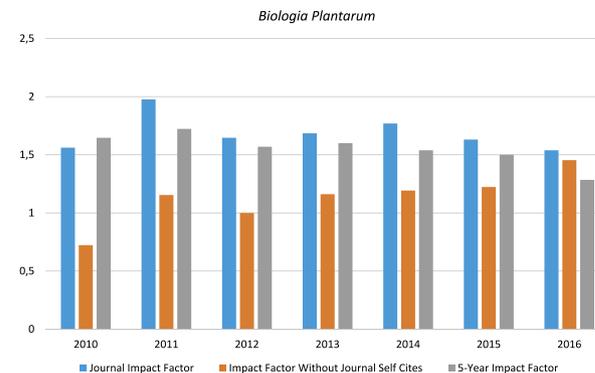
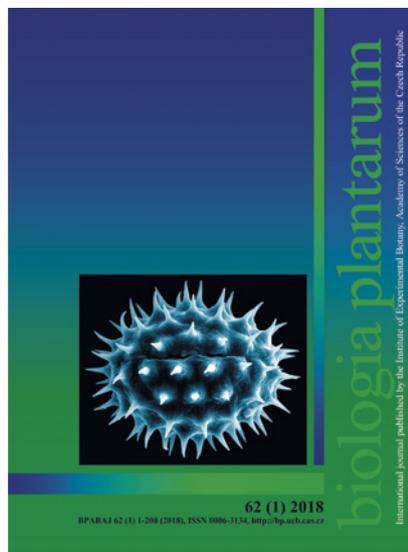


Journals

Institute of Experimental Botany publishes two scientific journals, both with impact factor. The journals are distributed by Springer Nature. Since 2019, both journals are going to be published in open-access mode only.

Biologia Plantarum, an international journal of experimental botany, publishes in English original research reports, review articles and brief communications ranging across all fields of plant physiology, molecular biology, biochemistry, biophysics, biotechnology, genetics, structural botany and pathology. The journal also regularly presents reviews of books dealing with topics within the general scope of the journal.

The Editor-in-Chief of *Biologia Plantarum* is RNDr. Jana Pospíšilová, CSc., Institute of Experimental Botany, Czech Academy of Sciences, Prague.



Photosynthetica is devoted to the investigation of photosynthesis, combining biochemical, biophysical and ecological approaches to the study of photosynthesis in plants. The journal carries specialized reviews on various aspects of photosynthesis research and presents papers on the structure of the photosynthetic apparatus; chloroplast pigments (both *in vivo* and *in vitro*); biochemical and biophysical mechanisms of photosynthetic reactions; measurements of photosynthesis and photosynthetic production by techniques ranging from laboratory gas-exchange measurements to growth analysis, etc. *Photosynthetica* is directed by an international editorial board. The articles are written in English.

The Editor-in-Chief of *Photosynthetica* is RNDr. Helena Synková, CSc., Institute of Experimental Botany, Czech Academy of Sciences, Prague.



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2015–2017

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