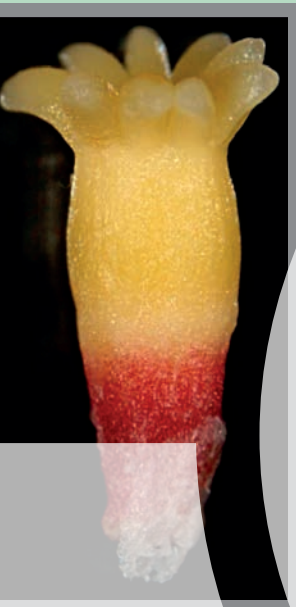
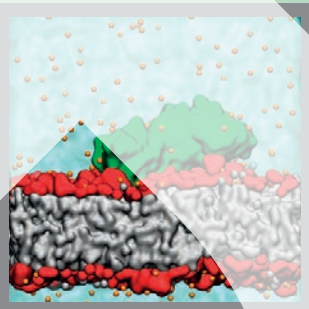
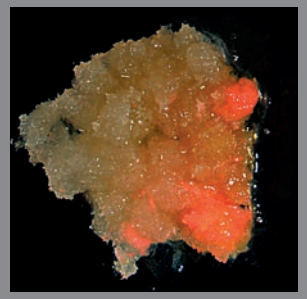
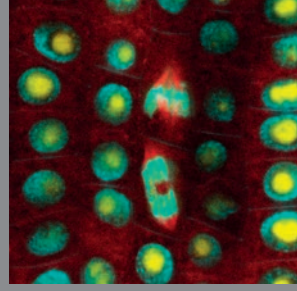
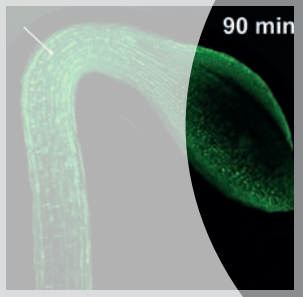
Institute of Experimental Botany
of the Czech Academy of Sciences

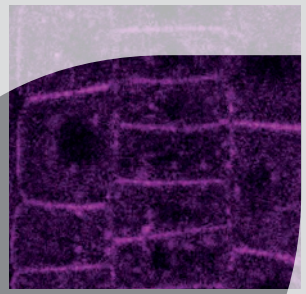


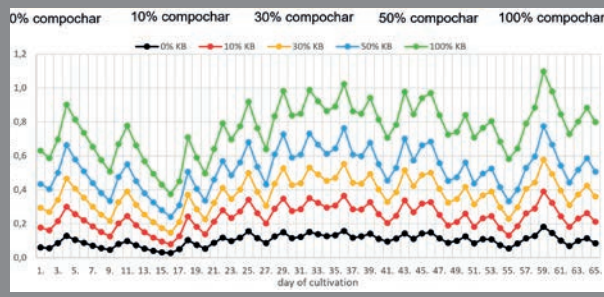












Scientific Report
2021–2023

Contents

Discovering the World of Plants

Foreword from the IEB CAS Director

Dear colleagues,

This scientific report outlines the most significant research activities and other pursuits of the Institute over the period from 2021 to 2023. However, during this three-year span, the field of science was significantly influenced by external events.

In 2020, a year still covered by the last scientific report, the Covid-19 pandemic broke out. In June 2021, the Institute's director at the time wrote that, as far as Covid-19 was concerned, the worst was probably over. But it was not. In November 2021, Covid-19 cases began to increase again in the Czech Republic, and on 25 November 2021, a state of emergency was declared for 30 days. By the end of January 2022, a record number of infections had been recorded. The situation finally returned to normal in early May 2022. Covid was not yet over when Russia's invasion of Ukraine began on 24 February 2022. As a result, energy prices began to rise, the Institute's energy supplier went bankrupt, inflation increased, and a wave of Ukrainian refugees arrived. All of this is related to the mood and mental state of each of us and of society as a whole. We do research, write publications, and submit grant proposals. But a Ukrainian colleague is standing next to you and is asking whether the support of the Academy of Sciences will continue next year. It will continue, and that's good news. However, times are dynamic and are changing rapidly, and that's not an ideal situation for long-term, focused basic research.

So how has this season affected the results of our Institute? Interestingly, 2021 was the best year yet in terms of number of publications. IEB authors published 217 impacted articles, surpassing the previous record of 197 publications set in 2020. It is possible that the lengthy period of home office during the Covid-19 pandemic led to increased publication activity and, conversely, the following year it resulted in a relatively significant drop in the number of papers. In 2022, 162 impacted articles were published. And this trend continued in 2023, when 147 articles were published. The laboratories had not been working for a long time, and everything that had been measured and completed by then had already been published. A new executive board was also elected in 2022, and a new director has been heading the Institute since mid-2022. One of the priorities of the new management is to emphasise the quality of publications. This trend could be another factor that has led to a decline in the total number of publications.

Between 2022 and 2023, two large projects from the Operational Programme Research, Development and Education, which had given many of the Institute's laboratories at least nominal funding security for five years, came to an end. Thankfully, we had success with a major new project in 2023 under the Jan Amos Komenský Operational Programme. The project "Towards Next Generation Crops", led by Prof. Jarek Doležel, will again bring partial but very important financial stability to the Institute's five laboratories until 2028.

The Institute's national and international collaboration is also excellent. Together with the Faculty of Natural Sciences at Palacký University in Olomouc, the Institute runs the very successful Laboratory of Growth Regulators. Our scientists teach at ten Czech universities and train a number of bachelor's, master's, and doctoral students. Teaching is an essential and important part of our scientific work.

Cutting-edge basic research is our goal and our priority. However, we are never far from practice. Our Station of Apple Breeding for Disease Resistance uses modern methods to breed promising new apple varieties with high commercial potential. The apple varieties bred by us are selling well in Europe and the U.S., and the profits from licencing contracts are an important contribution to the Institute's budget. Another commercially successful and promising product is a substance called MTU, patented by our Institute, which serves as a base for preparations that stimulate crop growth and protect them from drought. The licence to produce and sell the substance was sold in 2022, and the products containing MTU are manufactured by the British company IntraCrop.

We continue a more than 50-year tradition of publishing two scientific journals, *Biologia Plantarum* and *Photosynthetica*. Both are among the best scientific journals published in the Czech Republic. However, competition is strong in this field. Prof. Viktor Žárský, the new editor-in-chief of *Biologia Plantarum* as of 1 June 2023, should bring new life into the journal.

Finally, and with great pleasure, I must thank all of my colleagues, who together form an institute that is among the top in the field of experimental plant biology. All around me I see enthusiasm, new ideas, effort, and great dedication to science and to the work for our Institute. But at the same time, the Institute manages to maintain a friendly and supportive environment. My sincere hope is that it will stay that way.

Sincerely,

Jan Martinec

Institute Representatives

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DEPUTY DIRECTOR:

RNDr. Martin Vágner, CSc.

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Ing. Petra Janečková – IP CAS, Prague
Ing. Hana Štěpánková – UCT, Prague

Secretary:

Ing. Alena Trávníčková – IEB CAS

Abbreviations

Institutions of the Czech Academy of Sciences (CAS):

IEB – Institute of Experimental Botany
IOCB – Institute of Organic Chemistry and Biochemistry
IP – Institute of Physiology

Others:

FS CU – Faculty of Science, Charles University
FS UP – Faculty of Science, Palacký University in Olomouc
FS USB – Faculty of Science, University of South Bohemia in České Budějovice
RIFC Ltd. – Research Institute for Fodder Crops Ltd.
UCT – University of Chemistry and Technology Prague

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Prof. Dr. Stanislav Kopřiva – Cologne Biocenter, Germany
dr. Kirsten Leiss – Wageningen University, The Netherlands
Prof. Moritz Nowack, Ph.D. – Ghent University, Belgium
Prof. Robbie Waugh, Ph.D. – University of Dundee, United Kingdom

All positions are as of December 2023

Organisational Structure of the Institute

INSTITUTE OF EXPERIMENTAL BOTANY, CZECH ACADEMY OF SCIENCES

PRAGUE
Lysolaje, Rozvojová 263

- Laboratory of Cell Biology
- Laboratory of Hormonal Regulations in Plants
- Laboratory of Biologically Active Compounds
- Laboratory of Integrative Structural Biology
- Laboratory of Pollen Biology
- Imaging Facility
- Transcriptomics and Cloning Facility
- Bioanalytical Service Laboratory *(from 1 January 2024)*

PRAGUE
Lysolaje, Rozvojová 313

- Station of Apple Breeding for Disease Resistance
- Laboratory of Plant Reproduction
- Laboratory of Virology
- Laboratory of Plant Biotechnologies
- Laboratory of Pathological Plant Physiology
- Journals (editorial office)

PRAGUE
Dejvice, Na Karlovce 1a

- Laboratory of Pollen Biology (DNA Repair Group)

PRAGUE
Krč, Vídeňská 1083

- Isotope Laboratory

STŘÍŽOVICE

- Station of Apple Breeding for Disease Resistance (experimental station)

OLOMOUC
Holice, Šlechtitelů 27, 31

- Laboratory of Growth Regulators
- Centre of Plant Structural and Functional Genomics

- location, address
- research laboratory
- core facility
- experimental field station
- editorial office

The organisational structure is as of December 2023, except for the Bioanalytical Service Laboratory

Buildings of IEB

Rozvojová 263, Prague 6 – Lysolaje (7 laboratories)

Šlechtitelů 31, Olomouc (Centre of Plant Structural and Functional Genomics)

Rozvojová 313, Prague 6 – Lysolaje (5 laboratories)

Šlechtitelů 31, Olomouc (Centre of Plant Structural and Functional Genomics).
A new extension opened in April 2023 that houses the Application Laboratory for Agricultural Research.

Šlechtitelů 27, Olomouc (Laboratory of Growth Regulators)

Střížovice 20, 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 the laboratory:

Mgr. Jan Bartoš, Ph.D.

Phone: +420 585 238 711
E-mail: bartos@ueb.cas.cz

Our research focuses on elucidating the evolution, organisation, and functions of the plant nuclear genome. We aim to unravel the molecular mechanisms of inheritance and the mechanisms by which various plant phenotypes are determined, in order to provide knowledge and concepts that support plant breeding. We also put emphasis on the phenomena connected with polyploidisation and interspecific hybridisation. Other research activities are oriented towards understanding the principles of non-Mendelian inheritance and the molecular mechanisms ensuring genome stability. To this end, we employ a wide range of plant models, including agronomically important Triticeae cereals, forage and amenity grasses, maize, banana, and the model plant Arabidopsis. An integrated part of our work is the operation of the Application laboratory for agricultural research, which cooperates with the breeders, provides them with genomic and bioinformatics services, and participates in the transfer of the latest knowledge and technologies into the practice.

In the picture (from the left):
Front row: Bc. Jitka Weiserová / technician, Mgr. Alžběta Doležalová, Ph.D. / postdoctoral fellow, Mgr. Marek Szecówka, Ph.D. / researcher, Mgr. Jana Szecówka, Ph.D. / postdoctoral fellow, Mgr. Pavla Navrátilová, Ph.D. / researcher, Mgr. Kateřina Holušová, Ph.D. / researcher, Mgr. Lucie Hloušková / Ph.D. student, Mgr. Tereza Bojdová / Ph.D. student, Mgr. Jana Zwyrtková, Ph.D. / postdoctoral fellow.
Second row: Mgr. Simona Martikánová / Ph.D. student, Ing. Radoslava Kvasničková / programme manager, Mgr. Adam Lampar / Ph.D. student, Mgr. Anna Katarzyna Nowicka, Ph.D. / researcher, Fen Yang, Ph.D. / postdoctoral fellow, Jovanka Vladejić / Ph.D. student, Mgr. Iva Ilíková, Ph.D. / research assistant, Mgr. Beáta Strejčková, Ph.D. / postdoctoral fellow, Mgr. Zuzana Tulpová, Ph.D. / postdoctoral fellow, Mgr. Zuzana Korchanová / Ph.D. student, Doc. Mgr. Aleš Pečinka, Ph.D. / deputy head of the centre.
Third row: Mgr. Jaroslav Fiľo / Ph.D. student, Mehrdad Shahbazi / Ph.D. student, Mgr. Eva Hřibová, Ph.D. / researcher, Eliška Čamková / secretary, Mgr. Maciej Majka, Ph.D. / postdoctoral fellow, Mgr. Tereza Šlajsová / assistant, Mgr. Dominika Čmielová / Ph.D. student, Mgr. Magdaléna Stejskalová / assistant, Ing. Hana Šimková, CSc. / researcher, Mgr. Veronika Koláčková, Ph.D. / postdoctoral fellow, Helena Tvardíková / technician, Mgr. Šimon Pavlů / Ph.D. student, Ing. Petr Navrátil / IT technician, Doc. RNDr. David Kopecký, Ph.D. / researcher.
Fourth row: Mgr. Jana Čížková, Ph.D. / researcher, Mgr. Martin Kovačik / Ph.D. student, RNDr. Jan Šafář, Ph.D. / researcher, Ing. Petra Neplechová / accounting officer, Mgr. Eva Dvořák Tomaštíková, Ph.D. / researcher, Prof. Ing. Jaroslav Doležel, DrSc. / researcher, Mgr. Jan Bartoš, Ph.D. / head of the centre.

Not pictured:
Nicolas Blavet, Ph.D., Ing. Radim Čegan, Ph.D., RNDr. Roman Hobza, Ph.D., Mgr. Miroslava Karafiátová, Ph.D., Mgr. Helena Toegelová, Mgr. Miroslav Valárik, Ph.D. / researchers, Mahmoud Said, Ph.D. / research assistant, Mgr. Denisa Beránková, Ph.D., Mgr. Eva Janáková, Ph.D., Mgr. Joanna Majka, Ph.D., Mgr. Hana Stromšíková, Ph.D. / postdoctoral fellows, Mgr. Lucie Bílková, Mgr. Gabriela Majzlíková / assistants, Mgr. Jakub Juračka, Mgr. Kateřina Kaduchová, Mgr. Petr Urbiš / Ph.D. students, Zdeňka Dubská, Eva Jahnová, Ing. Marie Seifertová, Radomíra Tušková / technicians.

The scientists at the Centre of Plant Structural and Functional Genomics (CSFG) continued pioneering and applying unique experimental approaches, such as chromosome genomics to facilitate the analysis of complex genomes, connecting plant phenotypes with gene functions, and exploring the molecular and genetic basis of specific phenomena. Our broad expertise made it possible to perform the studies at a wide range of levels of the organisation, extending from the DNA sequence level up to the molecular composition of chromosomes and interphase nuclei and the spatial organisation of their chromatin. By studying genome organisation and its dynamics at all complexity levels, we strove to obtain an increasingly comprehensive and complex picture of the plant genome. This work benefited from our long-term experience in genetics, microscopy, molecular cytogenetics, flow cytometry, and sorting, the latter enabling the preparation of unique materials for performing the analyses on well-defined samples and at a high resolution.

By combining multiple diverse, complex, and unique experimental approaches, CSFG occupies a unique position within the international plant research community and collaborates intensely with many leading groups around the globe. We contributed as a strategic partner to many projects focusing on the analysis of and resource development for important crops with large genomes, such as wheat, barley, rye, and cowpea. Further collaborative activities included the identification of genomic loci associated with agronomic traits of interest, studies of nuclear organisation, epigenetic regulation, and genome instability. We also used our international network of contacts to provide extraordinary training possibilities for our students and scientists, which led to the establishment of several of the latest technologies at our Centre.

To foster the application of basic knowledge into practice, we transformed our long-term collaboration with and support for the plant breeding sector into the first Application Laboratory for Agricultural Research at the Czech Academy of Sciences. This unit helps plant breeders and farmers with consultations, methodological support, services and contract based analyses.

The CSFG was highly active in engaging the public, particularly the youngest generation, in the plant sciences by organising various outreach activities, trainings, and excursions. Part of our mission was also communication with political representatives about the future of plant breeding and advocating for the use of new breeding technologies and genome editing in the EU.

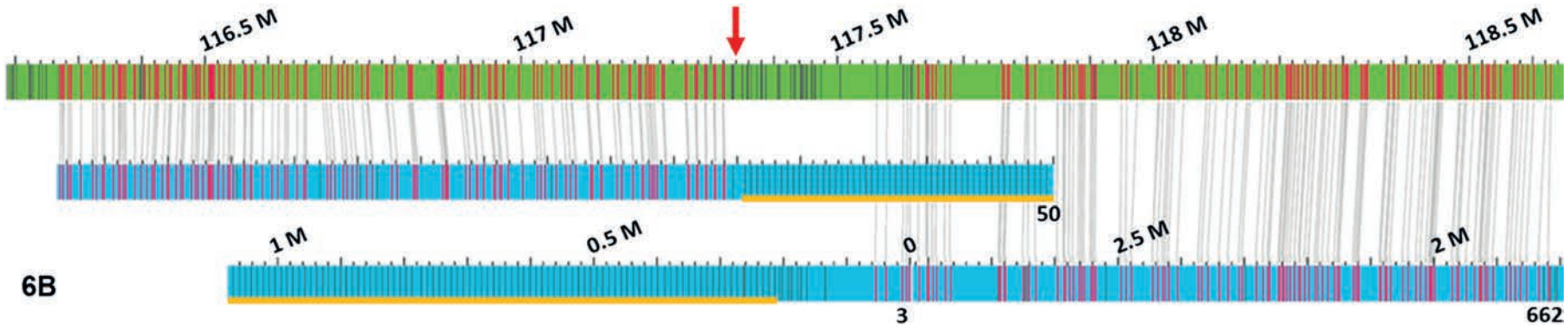

Figure 1. The optical map places a missing 45S ribosomal DNA array on the pseudomolecule. Optical mapping utilises the labelling of specific sequence motifs, typically CTTAAG, on DNA molecules. Tandem arrays of 45S rDNA can be recognised in the maps (blue bars) as a regular label pattern (highlighted yellow). The alignment of these maps to the pseudomolecule of bread wheat chromosome 6B (green bar) showed the precise position of the array (red arrow) and a local sequence misassembly. Adopted from Tulpová et al. (2022).

Advanced assemblies of plant genomes

High-quality genome assemblies are a prerequisite of advanced genomic analyses and a valuable resource for gene cloning. To achieve high contiguity and accuracy of the sequence, next-generation sequencing technologies are complemented by next-generation physical mapping techniques, such as optical mapping. Capitalising on flow sorting of chromosomes and nuclei, we developed efficient protocols for optical mapping in plants, which has so far helped to assemble genomes of 26 plant species. The latest assembly of the barley reference genome, MorexV3, has reached the "nearly-finished" level. We searched for the causes of the remaining sequence gaps and found them associated with long stretches of satellite repeats: almost all centromeric sequences and 45S ribosomal DNA repeat arrays were absent from the pseudomolecules (Navrátilová *et al.* 2022). The 45S rDNA loci were also missing in the bread wheat genome. To fill in these gaps, we coupled chromosome genomics with optical mapping and reconstructed individual rDNA arrays (**Fig. 1**), which enabled locus-specific analyses of transcription activity and DNA methylation status (Tulpová *et al.* 2022).

Deciphering the genetic basis of agronomically important traits

The advances in wheat and barley genomics coupled with our chromosome-based experimental approaches (Zwyrtková *et al.* 2021) offered opportunities for collaborative projects concerning the rapid mapping of agronomically important genes. The targeted chromosome-based cloning via long-range assembly (TACCA) approach was instrumental in discovering that the wheat stripe rust resistance locus Rps8 on chromosome 4H of barley comprises a genetic module including *Pur1* and *Exo70FX12* genes (Holden *et al.* 2022).

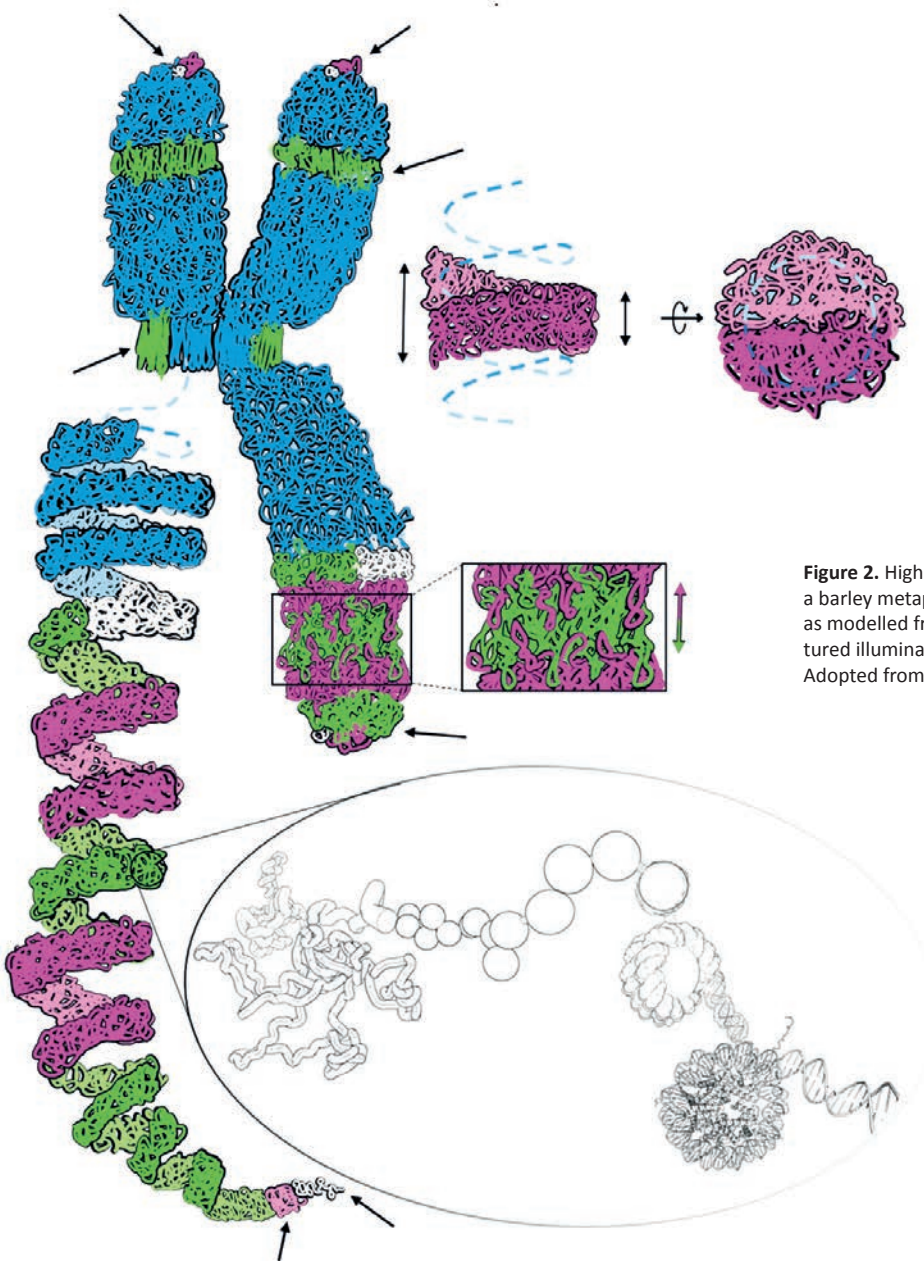

Figure 2. Higher-order structure of a barley metaphase chromosome as modelled from Hi-C data and structured illumination microscopy images. Adopted from Kubalová *et al.* (2023).

Further, we extensively employed the MutChromSeq approach to isolate several resistance genes in bread wheat. This included *Pm4* and *Lr14a* race-specific resistance genes to powdery mildew and leaf rust resistance encoding for a putative chimeric serine-threonine kinase with a unique domain architecture and a non-selective cation channel membrane protein, respectively (Sánchez-Martín *et al.* 2021; Kolodziej *et al.* 2021). The leaf rust resistance genes *Lr9* and *Lr58* from *Aegilops umbellulata* and *Ae. triuncialis*, respectively, were introgressed into bread wheat and mapped to the unusual tandem kinase fusion proteins (Wang *et al.* 2023). Stem rust resistance gene *Sr43* introduced from *Thinopyrum elongatum* to bread wheat was found to encode kinase protein containing two domains of unknown function (Yu *et al.* 2023). Finally, positional cloning and functional validation of *Pairing homoeologous 2* (*Ph2*) revealed the wheat DNA mismatch repair protein MSH7-3D to be a key inhibitor of homoeologous recombination (Serra *et al.* 2021). We also identified a new source of total resistance against powdery mildew disease in a bread wheat landrace from a mountain region of southwest Slovakia (Korchanová *et al.* 2022). QTL analysis identified two resistance loci on chromosomes 7A and 2A with the 2A locus limited to 0.99 cM, representing only 4.3 Mb of the reference genome and comprising 55 predicted genes.

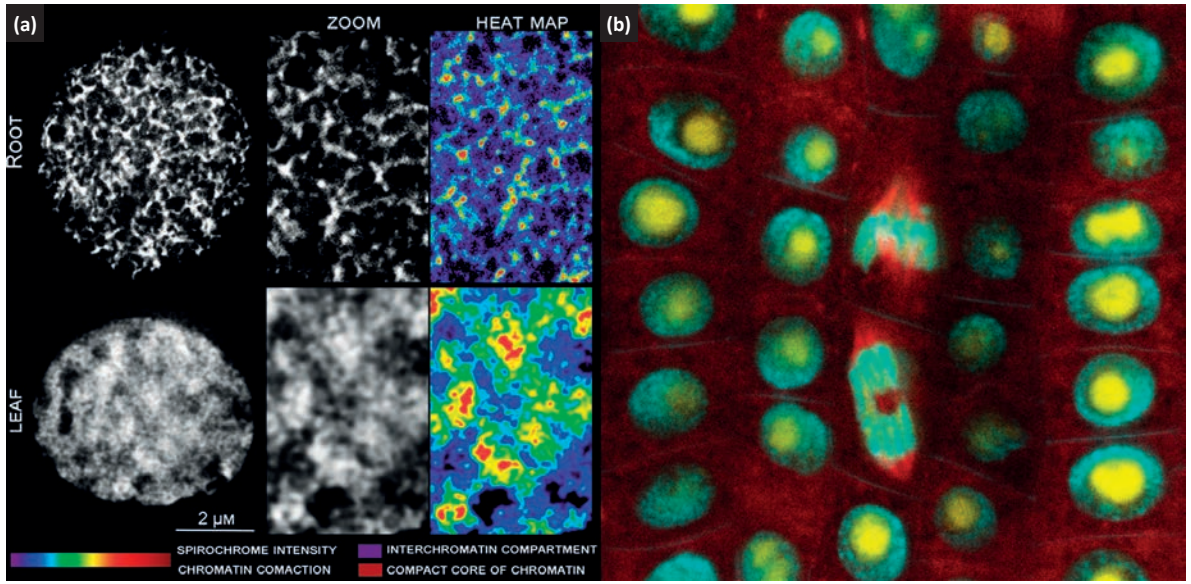

Figure 3. Advanced microscopy to study 3D genome and its dynamics. **(a)** STED analysis of chromatin in G1 nuclei of young leaves and root meristem from rice. DNA was stained with spirochrome (white) and its density was visualised via heat maps. Adopted from Doležalová *et al.* (2023). **(b)** Live confocal laser scanning microscopy of barley roots with chromatin visualised by CFP-H2B (blue), nucleoli by eYFP-FIB1 (yellow), and microtubules by mCherry-TUA3 (red). Cell walls were visualised by the autofluorescence in CFP spectra. The central part of the image shows mitotic divisions with chromosomes in the metaphase (upper) and anaphase (lower) stages. Adopted from Kaduchová *et al.* (2023).

Three-dimensional genome architecture and dynamics

Chromosomes undergo marked structural metamorphoses during the cell cycle. The transcriptionally active interphase requires a different mode of chromatin packaging than the transfer of genetic information during mitosis. The novel molecular techniques based on chromatin conformation capture, Hi-C in particular, have enabled us to study the 3D chromatin structure and its dynamics on the DNA level. We took barley as a model and coupled Hi-C with flow sorting of metaphase chromosomes and polymer modelling, which revealed helical folding of the chromatin with ~30 Mb DNA sequence per turn (Kubalová *et al.* 2023). The turn size varied along the chromosome axis, correlating with the chromatin and gene density. Structured illumination microscopy confirmed a 400 nm chromatin thread forming barley mitotic chromatids. Chromatin from adjacent turns of the helix intermingled due to the stochastic positioning of chromatin loops (**Fig. 2**). Comparison with previously published animal studies suggested minor differences in chromatin organisation between plants and animals.

Furthermore, we continued analysis of the principle of 3D genome organisation and dynamics in cereals using advanced microscopy. Our study, based on the stimulated emission depletion (STED) nanoscopy, re-

vealed detailed chromatin ultrastructure and differences in chromatin compaction between G1 nuclei of rice leaf and root meristems (Doležalová *et al.* 2023). The root meristem nuclei were characterised by a more relaxed chromatin and ultra-structures compared to a more compact chromatin and fewer inter-chromatin compartments in leaf nuclei (**Fig. 3a**). 3D-FISH with oligo painting probes revealed variability in the mutual positioning of chromosome territories during interphase in leaf and root meristem cells of rice. We also demonstrated that Rabl chromosome configuration is not a universal pattern in barley embryos and endosperm tissues (Nowicka *et al.* 2023). It is reinforced by mitotic divisions and fades away in endoreduplicated nuclei.

We produced a series of fluorescent marker lines for chromatin, nucleolus, and microtubules (**Fig. 3b**). In combination with live confocal microscopy, these lines allowed us to measure the duration of barley mitosis and showed that a fraction of nuclei maintains partially condensed chromosomes during interphase, and that mitotic chromosome condensation continues progressively until telophase (Kaduchová *et al.* 2023).

Wheat recombination sites: precise delimitation and characterisation

Meiotic recombination plays a crucial role in speciation and the generation of new haplotypes, and it is a prerequisite for successful breeding. During the mapping of a wheat powdery mildew resistance gene, we identified genomic regions with strikingly increased meiotic recombination rates. To precisely estimate the locus-specific recombination frequencies, a new method of digital droplet PCR pollen genotyping was developed and applied to over 62,000 gametes (**Fig. 4**). The main recombination hotspot (*H1*) was delimited to two subregions of 574 bp and 593 bp and showed a 6-fold higher recombination frequency compared to an average recombination region and was *Ph1* control independent (Majka *et al.* 2023a). The *H1* was marked by open chromatin and DNA hypomethylation (except in the CHH context). The hotspot was strongly suppressed by larger genomic rearrangements but not single nucleotide polymorphisms. For the first time, we studied recombination at a high resolution in wheat, and shed light on chromatin marks associated with particular recombination sites. Understanding the regulation of meiotic recombination is a base for efficient wheat gene pool enrichment and breeding.

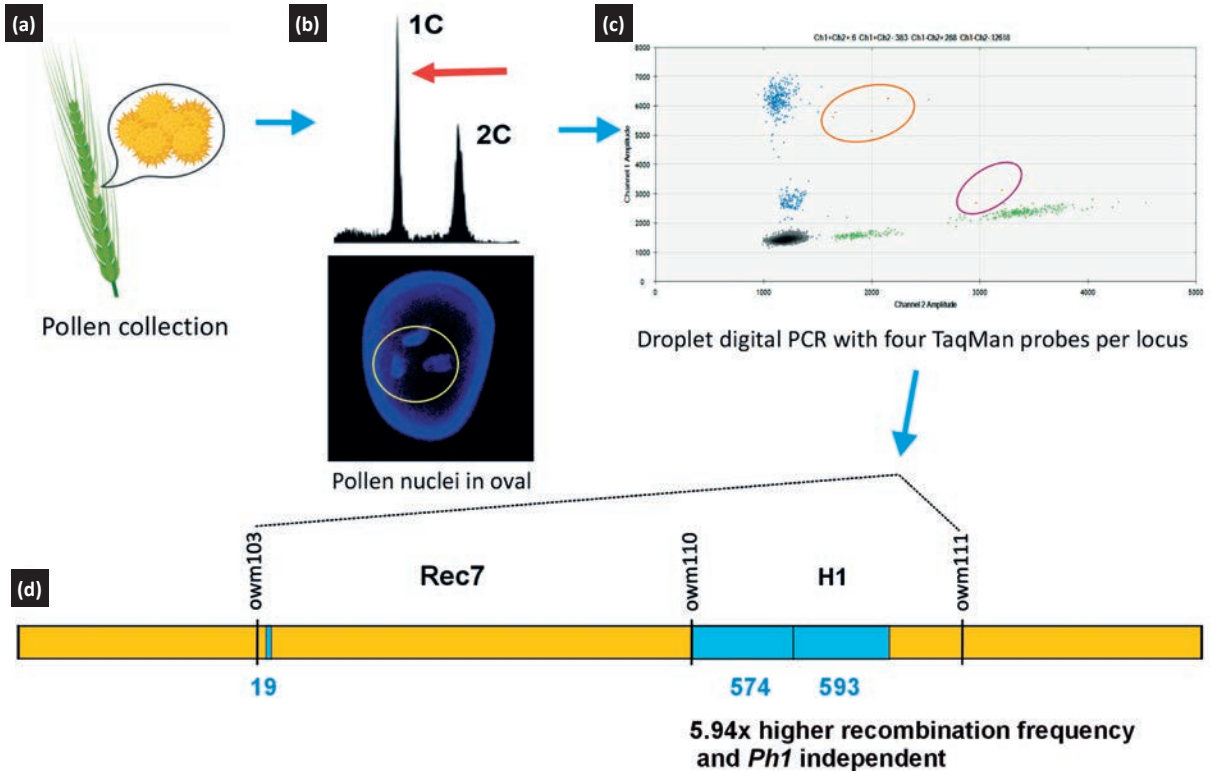

Figure 4. High-resolution characterisation of the wheat recombination sites. **(a)** Pollen was collected, **(b)** the haploid pollen nuclei were purified by flow cytometry (red arrow), and **(c)** recombination events were identified using the ddPing method (ovals). **(d)** The 8 kb genomic region with the regular Rec7 (2.5 kb) and H1 hotspot (1.7 kb) crossing over loci. Blue numbers represent the smallest delimitation of recombination sites under the *Ph1* control (in base pairs).

Genome dominance in plant hybrids

Whole genome duplication and interspecific hybridisation play a pivotal role in plant speciation, adaptation, and breeding. Wide hybrids can display extraordinary heterosis and adaptation to the environment, thus merging the agriculturally valuable traits of their parents (Ferreira *et al.* 2021). We delved into the mechanisms of genome evolution and their stability using *Festuca* × *Lolium* and *Allium cepa* × *A. roylei* hybrids. We found that one parental genome often becomes dominant, while the other one is submissive and can be prone to elimination. In male meiosis, *Lolium* univalents are transmitted through both meiotic divisions more frequently than those of *Festuca* (**Fig. 5**). This is influenced by the silencing of specific *Festuca* genome regions (including kinetochore genes) in hybrids during meiosis (Majka *et al.* 2023b). During female meiosis, chromosomes from the dominant parental genome more frequently transmit through egg cells (Majka *et al.* 2023). Genome dominance is also evident at the level of gene expression. In reciprocal hybrids of *Festuca x Lolium*, we found a significantly higher expression of genes from *Lolium* over the *Festuca* parent. This genome dominance is heritable through successive generations with little influence from the environment and/or plant age (Glombik *et al.* 2021).

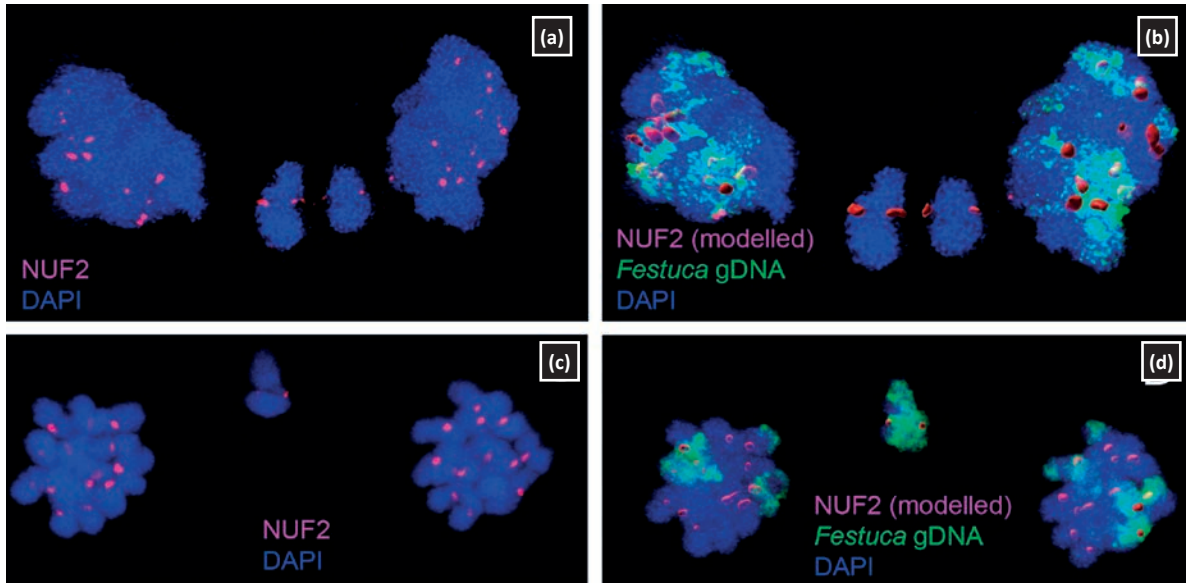

Figure 5. Outer kinetochore assembly to univalents of different parental origin in *Festuca × Lolium* hybrids. The volume of immunostaining signal of antibody for outer kinetochore protein NUF2 to meiotic parental chromosomes in *Festuca × Lolium* hybrids was much smaller on *Festuca* univalents **(c–d)** compared to *Lolium* univalents **(a–b)**. Adopted from Majka *et al.* (2023b).

Non-mendelian inheritance of B chromosomes

A significant group of species has their genomes enriched with dispensable genetic units called B chromosomes. An irregular manner of inheritance, which ignores the Mendelian laws, is the most striking feature of these special chromosomes. Abnormal segregation of B chromosomes into the daughter cells is frequently caused by sister-chromatid non-disjunction, which is driven by the B chromosome itself. The molecular mechanisms behind this process remain unknown.

We sequenced the B chromosome of maize to a high-quality reference level (Blavet *et al.* 2021), and narrowed down the group of candidate genes for non-disjunction, thus making a significant leap forward towards elucidating Bs' enigmatic behaviour. We also published the first molecular study of the B chromosome in wild sorghum (Karafiátová *et al.* 2021), providing the first glimpse into its genetic code, an essential prerequisite for exploring the astonishing beauty of these supernumerary chromosomes.

Maintenance of genome stability

An important line of our research focused on understanding the maintenance of genome stability. We showed that the cytidine analog zebularine, traditionally used as a DNA methylation inhibitor, causes DNA protein crosslinks (DPCs) in Arabidopsis (Procházková *et al.* 2022). DPCs represent a mechanical barrier for transcription and replication, thus triggering a DNA damage response. To identify molecular players involved in the repair of zebularine-induced DPCs, we performed a forward-directed screen HYPERSENSITIVE

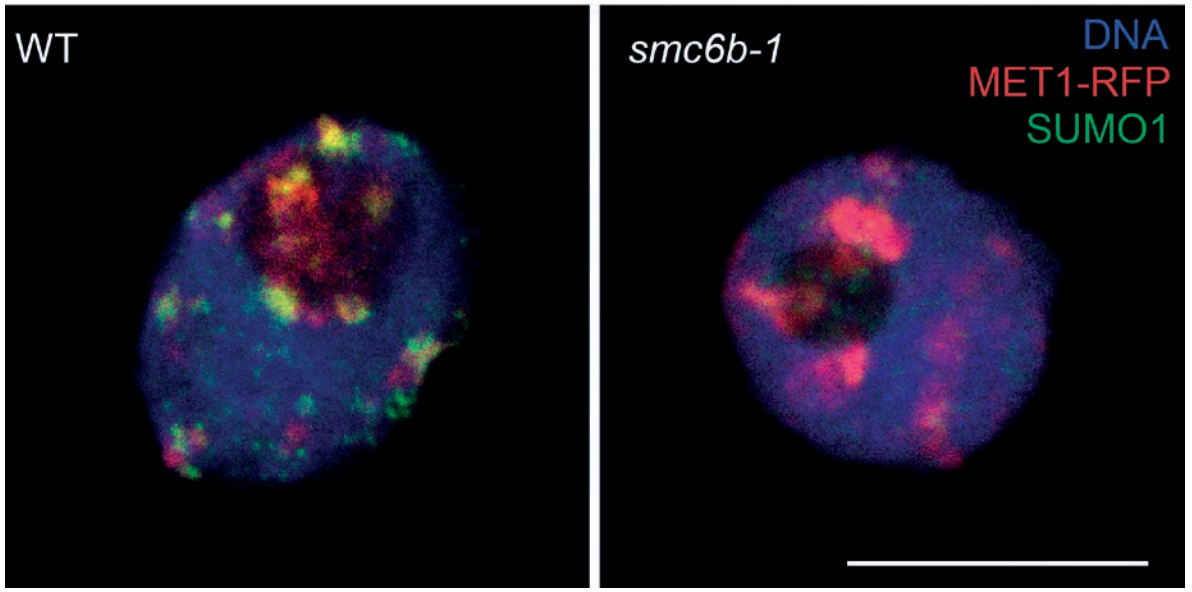

Figure 6. Nuclei from Arabidopsis wild type (WT) and *smc6b-1* mutant plants treated with zebularine. The DNA protein crosslinks are apparent by the accumulation of MET1-RFP (violet), and their SMC5/6 complex-dependent SUMOylation was visualised by SUMO1 (green/yellow) immunolabeling. Scale bar, 10 µm. Adopted from Dvořák Tomaštíková *et al.* (2023).

TO ZEBULARINE (HZE) and identified >70 candidate genes. Our analysis revealed HZE1, corresponding to the core subunit of the SMC5/6 complex SMC6B, as a key factor in DPC repair independent of the known repair pathways (Dvořák Tomaštíková *et al.* 2023). This new pathway depends on the SUMOylation activity of the SMC5/6 complex and thus links plant genome stability with SUMO modification (**Fig. 6**). Further characterisation of the SMC5/6 complex revealed its novel role in meiotic chromosome number reduction (Yang *et al.* 2021). This helps to discover new gene functions and mechanistically understand the role of SUMO in the maintenance of plant genome stability.

Application Laboratory for Agricultural Research

The Application Laboratory for Agricultural Research at the CSFG was the first unit at the Czech Academy of Sciences to link scientists with plant breeders and farmers. The main objective is to mediate the cutting-edge basic research findings and methodologies to the breeders and agricultural practice. Our team of specialists offers a broad theoretical and practical expertise in plant genomics that is shared with public and private organisations and breeders via consultations, workshops, seminars, and practical courses. We provide sample analysis using various advanced, state-of-the-art genomic and molecular biology instruments and methods, including ploidy level determination by flow cytometry, cytogenetic analyses, genotyping using different types of DNA markers, next-generation sequencing, and gene editing using new genomic techniques. The joint projects with partners from plant breeding institutes were supported by the Technology Agency of the Czech Republic and the Ministry of Agriculture of the Czech Republic. This allowed genome-wide association studies on agriculturally important traits, for example in cherries (Holušová *et al.* 2022).

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Research projects: 1, 3, 8, 11, 17, 22, 31–33, 38, 41–43, 47, 55–56, 61–67, 71, 81–83, 85, 94, 101–102, 104, 106, 109–110, 116

Imaging Facility

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The Imaging Facility of the institute (IFIEB) provides high-end instrumentation and expertise for demanding tasks in the field of non-invasive *in vivo* advanced fluorescence microscopy in high spatio-temporal resolution. In 2021-2023, the facility served on a number of projects performed in laboratories of the IEB CAS, including studies of membrane trafficking, cytoskeleton dynamics, hormonal regulations, the involvement of membrane lipids in signalling pathways, plant reproduction, the reaction of plants to pathogen attacks, and the dynamics of cell wall biosynthesis. In addition, it also offered microscopy services for external users from the Czech Republic and abroad as a part of the Czech-BioImaging and Euro-BioImaging infrastructure.

The Imaging Facility of IEB CAS (IFIEB; www.ueb.cas.cz/if) is a well-established facility that provides high-end instrumentation and expertise for demanding tasks in the field of fluorescence microscopy mainly in plant research. In 2021–2023, the instrumentation of the Imaging Facility was used by around seventy independent users (researchers, masters and Ph.D. students) each year, from both IEB CAS laboratories and external locations, including foreign research institutions. Their results using IFIEB instrumentation and support led to thirty original contributions being published in high impact journals (e.g. Martinek *et al.* 2023, Kashkan *et al.* 2022, Ortmannová *et al.* 2022; see also **Fig. 1**). IFIEB is located on premises that meet all the environmental requirements for high-end light microscopy instrumentation. In 2021, the light microscopy workplace expanded by one adjacent microscopy room. Since 2023, IFIEB has also supported a basic electron microscopy laboratory. The Imaging Facility runs ten microscopy-related systems, reservation software, a website/Twitter, and a data storage server. IFIEB is actively involved in a wide range of promotional and educational activities.

The Imaging Facility is a partner in the large research infrastructure project "National Infrastructure for Biological and Medical Imaging" (Czech-BioImaging, CzBI; www.czech-bioimaging.cz). CzBI was approved for funding by the Czech Ministry of Education, Youth and Sports for the periods 2020-2022 [project 88] and 2023 [project 89]. In 2020-2023 the IFIEB participated in European Regional Development Fund projects focused on the modernisation of the large research infrastructure Czech-BioImaging [project 12]. Through Czech-BioImaging, the IFIEB is integrated into the Prague node of the large European research infrastructure Euro-BioImaging (www.eurobioimaging.eu). Besides CzBI-related support, IFIEB is regularly successful in investment calls from the Czech Academy of Sciences and is supported by IEB. The combination of all these sources of funding enables the facility to continue to develop and provide high-end instrumentation and services to all users.

IFIEB is equipped with high-end microscopy systems serving a spectrum of techniques for the fast and sensitive detection of fluorescence signals. The IFIEB instrumentation portfolio was further optimised from 2021–2023. The bottleneck in confocal capacity has been solved by the purchase of a LSM 900 with Airyscan2 with Multiplex (**Fig. 2a**). The compact Airyscan2 system had a surprising level of sensitivity compared to

the LSM880 Airyscan, and the Multiplex setup became a very efficient and popular tool for fast imaging at a high resolution. The spinning disc microscope (**Fig. 2b**), our system for fast and gentle imaging, has been further enhanced (covered by the Czech Academy of Sciences). In addition to previous techniques, it enables simultaneous two-channel ultrafast imaging. IFIEB strengthened its image analysis service with an image analyst position, a powerful workstation, and Arivis image analysis software (IEB instrumentation calls). A dongle server has been installed to allow remote access to licenced software.

In addition to the commercial instrumentation, IFIEB runs a plant-optimised system that meets our vision of gentle imaging under near-natural conditions. Most microscopes place the sample horizontally, whereas plants grow vertically. Therefore, one of our confocal systems was previously turned by 90 degrees to optimise it for plant research. Vertical sample mounting now enables imaging of root growth in its natural (gravitational) orientation (**Fig. 2c**). Thanks to a collaboration with the Vienna IST Bioimaging Facility, root growth can be newly compensated for by macro-driven microscope stage movement. This means that the same region of the sample can be imaged over time in a single field of view. The integrated illumination of plant leaves has been added to preserve photosynthetic activity and facilitate photostimulation studies. To meet the needs of plant growth phenotyping, we are working on the production of self-made RaPiD-chambers. They allow non-invasive, long-term imaging (days to weeks) of plants grown on agar plates and subsequent phenotyping based on growth dynamics.

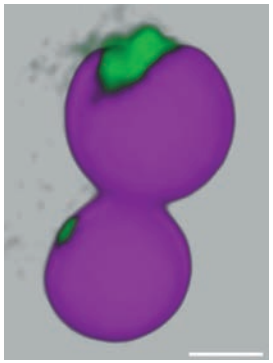

Figure 1. 3D view of ARP2/3 subunit NtARPC2 and peroxisome. Super-resolution Airyscan imaging of ARP2/3 subunit GFP-NtARPC2 (green) and peroxisome marker mCherry-mPTS1 (magenta). The two- channel Z-stack was displayed in Zen black 3D view and rendered in Transparent mode. Bar 1 µm. Adapted from Martinek *et al.* 2023.

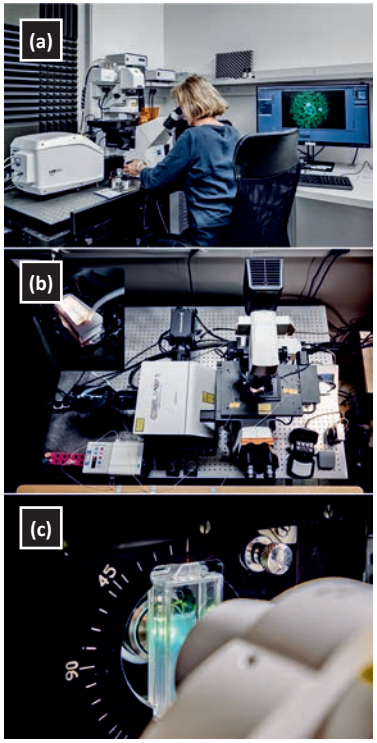

Figure 2. Microscopes for advanced fluorescence microscopy at IFIEB. (a) New Zeiss LSM 900 with Airyscan 2 with Multiplex. **(b)** The spinning disc confocal microscope after its upgrade in 2021 (top view of the system). The new Yokogava CSU-W1 spinning disc unit in a two-camera configuration enables ultra-fast simultaneous two-channel imaging. Detail of the home-made perfusion chamber system used for experiments requiring drug treatment. **(c)** Vertical stage microscopy – chambered coverglass with growing seedling.

IFIEB is actively involved in a wide range of promotional and educational activities. The facility organises a two-day course on Multimodal light microscopy imaging in plant research and regularly hosts company demonstrations of new technologies. IFIEB also provides instrumentation for courses organised by the external lecturers for students of Czech universities. In addition to supporting advanced microscopy techniques for basic research, IFIEB now serves several targeted research projects. Most of them are dedicated to crop plants, with barley as the main model plant. We would like to continue our mission of providing access to state-of-the-art instrumentation, sharing expertise in the challenging tasks of modern plant cell biology research, and helping to integrate students and young researchers into state-of-the-art imaging protocols.

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Publications by Facility members: 128, 133, 447
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Research projects: 9, 12, 88–89

Isotope Laboratory

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The major subject of interest of the Isotope Laboratory (IL) is the synthesis of chemical compounds labelled with stable or radioactive isotopes (e.g. deuterium, tritium, carbon ^{13}C and ^{14}C). These compounds are usually plant hormones and their derivatives, both of which are used in research at some laboratories of IEB and Palacký University. We also perform imaging techniques such as autoradiography and analytical chemistry of labelled compounds in animal and plant samples. Besides the chemistry of labelled compounds, our research is focused on 1) the development of biostimulants for agricultural use, 2) the investigation of pharmacologically important plant products, and 3) phytoremediation and other techniques for the cleaning of wastewater.

Radiolabeling

Long-term research in synthesising radiolabeled cytokinins resulted in joint scientific publications (Pokorná *et al.* 2021, Raspor *et al.* 2021), usually elucidating the role of plant hormones and their metabolites in plant development. In our most recent research in this field, we focused on the synthesis of polyamine standards (Bělíček *et al.* 2023), a precursor of ethylene, the ^{3}H-ACC or ^{14}C-labeled auxin-amino acid conjugates.

Biostimulants for agriculture

We continue to research the mechanism of action of the biostimulant MTU, which was developed at IEB (Nisler *et al.* 2018, 2023) and which, thanks to the company IntraCrop (UK), is already being used commercially in Great Britain, and in several EU countries including the Czech Republic. We have found that MTU delays both the age- and stress-induced senescence of wheat plants (*Triticum aestivum* L.) by enhancing the abundance of PSI supercomplex with LHCa antennae (PSI-LHCa) and promoting the cyclic electron flow (CEF) around Photosystem I. MTU appears to be the only chemical reported to date to have this activity (Nisler *et al.* 2023). Field trials also revealed that MTU enhances nutrient use efficiency, particularly the use of nitrogen.

The second line of our focus on biostimulants relates to the development of inhibitors of cytokinin degradation (CKX inhibitors). These compounds inhibit the function of cytokinin oxidase/dehydrogenase (CKX), thus enhancing the levels of cytokinins in plants. These compounds have a lot of potential in plant tissue culture techniques, as well as in agriculture (Nisler *et al.* 2021, 2022). Interestingly, some CKX inhibitors exhibited an extremely positive effect on root growth (**Fig. 1**), which is in contradiction with current knowledge. This regulation is being investigated.

Natural phytochemicals as potential nano-drug candidates

The current interest in this area is focused on the investigation of novel derivatives of triterpenoids and other plant products with potential supramolecular characteristics, cytotoxicity, antimicrobial activity, anti-HIV, anti-HSV, and other types of pharmaceutical activity. Cytotoxicity was tested on several important cancer cell lines, and additional studies of apoptosis were performed wherever possible. Selected physico-chemical parameters and supramolecular characteristics were measured to support experimentally obtained results. The physico-chemical characteristics of the prepared compounds were studied due to the investigation of the supramolecular characteristics of potential biological systems, and they contribute to the newly emerging area of supramolecular chemical biology and nano-drug application. A number of the prepared compounds, derived from natural triterpenoids, display improved bioavailability and enhanced pharmacological effects, despite the low bioavailability of the parent natural triterpenoids. The relationship between supramolecular characteristics and pharmacological activity has been described (Bildziukevich *et al.* 2023, Yang *et al.* 2022), resulting in the discovery of novel application patterns in supramolecular systems that, due to their nature, are capable of being used not only as drug carriers, but also as nano-drugs (**Fig. 2**).

In the picture (from left to right):
Front row: Mgr. Sándor Forczek, Ph.D. / researcher, Martina Wimmerová / technician, Doc. Ing. Libor Havlíček, CSc. / researcher, Mgr. Jaroslav Nisler, Ph.D. *et* Ph.D. / head of the laboratory.
Second row: Mgr. Uladzimir Bildziukevich, Ph.D. / researcher, Mgr. Mahfam Chalaki / Ph.D. student, Mgr. Meysam Aryafard, Ph.D. / researcher.
Not pictured:
Prof. Ing. Zdeněk Wimmer, DrSc. / researcher.

Figure 1. Some CKX inhibitors stimulate root growth in winter wheat. *Left:* control plant, *right:* plant treated by compound 82.

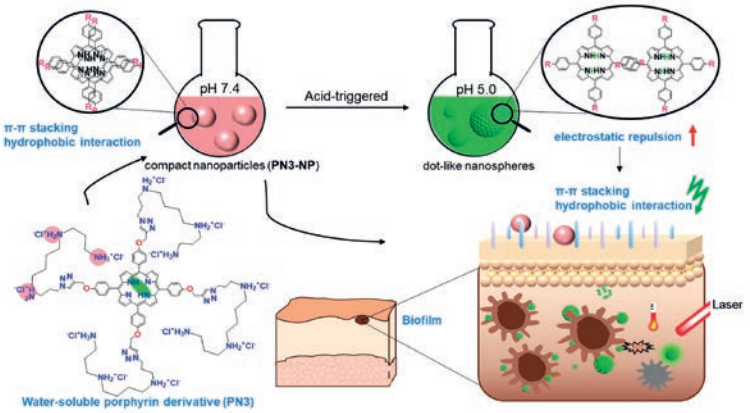

Figure 2. Schematic illustration of acid-activatable nanoporphyrin evolution based on water-soluble porphyrin derivative (PN3). PN3 can self-assemble to form compact nanoparticles (PN3-NP) with good stability under physiological environments and can be transformed into dot-like nanospheres-integrated fluorescence monitoring systems, and synergistic photodynamic and photothermal therapy under the activation of acid, which can be applied for the diagnosis/monitoring and treatment of biofilms.

Wastewater treatment and phytoremediation

We are modelling wastewater treatment (WWT) in constructed wetlands, where plants are used as root filters. During WWT, pollutants are partly degraded by microorganisms, which plants can utilise as nitrogen- and phosphorus-containing substances. Thus, N and P are removed, preventing the eutrophication of surface waters. In addition to nutrients, we study the uptake of micropollutants of pharmaceutical origin, which are potentially harmful to humans and nature. The uptake rates by plants or biochar, degradation rates, and degradation products are studied using analytical and radioanalytical methods. Our research is a collaborative project of the Pardubice region and the Czech Academy of Sciences.

Best results

The results, which have already been mentioned in the text, relate to the mode of action of MTU published in *Frontiers in Plant Science* (Nisler *et al.* 2023) and to the development of new CKX inhibitors published (Nisler *et al.* 2021, 2022) or submitted (Nisler *et al.* 2024) in/to the *Journal of Experimental Botany*. Our remarkable work concerning acid-responsive nanoporphyrin evolution for near-infrared fluorescence-guided photo-ablation of biofilm has been published in *Advanced Healthcare Materials* (Yang *et al.* 2022).

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Publications: 15, 91, 111, 136, 142–144, 151, 154, 163, 212, 230, 275, 283, 307, 312–315, 368, 373, 390, 394–395, 409, 432, 459, 465–466, 475, 518

Research projects: 20, 117

Laboratory of Biologically Active Compounds

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Second row: Mgr. Kateřina Eliášová, Ph.D. / head of the laboratory, RNDr. Lucie Fischerová, Ph.D. / researcher, RNDr. Zuzana Vondráková, CSc. / researcher.

The laboratory focuses on research into the role of secondary metabolites such as plant hormones, polyamines, carotenoids, and phenolic compounds in plant growth and development and the defence response of plants to abiotic stress factors. In recent years, the main interest of the laboratory has been the course and regulation of somatic embryogenesis in conifers, particularly Norway spruce. Our most recent publications focused primarily on the latter phases of somatic embryogenesis and explained the changes in somatic embryos essential for successful germination. In the future, we want to focus on improving the induction yield and the quality of embryogenic cultures. We plan to test novel cytokinin derivatives and to influence the redox status of proliferating embryogenic cultures. Our aim is to link the acquired techniques with forestry practices and to propagate elite genotypes for reforestation programmes.
In addition, in recent years we have been working on the *in vitro* propagation and transformation of *Cannabis sativa*. We have successfully derived transformed calluses of both medicinal and fibre-type genotypes. We have focused on the regeneration of this very recalcitrant plant by manipulating the levels of growth regulators.
To answer scientific questions, we employ different methodological approaches, including molecular biology, biochemistry, morphology and anatomy using histochemical microtechniques and immunolabelling in combination with advanced confocal fluorescence microscopy.

Somatic embryogenesis – effect of desiccation

The somatic embryogenesis of conifers proceeds in several steps controlled by phytohormones. These include the induction of embryogenic cultures from zygotic embryos, their multiplication in the presence of auxin and cytokinins, the maturation of somatic embryos in the presence of abscisic acid, and the desiccation of cotyledonary embryos under conditions of mostly high relative humidity (RH), which is usually a vital prerequisite for successful embryo germination and further plantlet growth. Recently, we have devoted ourselves to the comprehensive analysis of the processes associated with the transition of somatic embryos to germination during the desiccation phase.

We have demonstrated the positive effect of a high RH during desiccation as opposed to the effect of a reduced RH, which leads to reduced germination. The germination capacity of embryos desiccated at 100% RH was about three times higher than that of non-desiccated embryos. In comparison, reducing RH to 95% and 90% impaired subsequent embryo development (Fischerová *et al.* 2022). We have also shown that the processes occurring in Norway spruce somatic embryos during the desiccation phase are not only triggered by the ageing of the embryo. Embryos left on the maturation medium for an extended period of time corresponding to the usual desiccation time showed no reduction in abscisic acid content or total soluble carbohydrate and starch content, and no increase in raffinose family oligosaccharide content compared to embryos exposed to the desiccation treatment. A comparison of proteomic data from somatic embryos after a standard maturation period and after desiccation with data from zygotic embryos collected in summer (*i.e.* physiologically immature) showed similarities between zygotic embryos and somatic embryos exposed to the desiccation treatment. The physiological

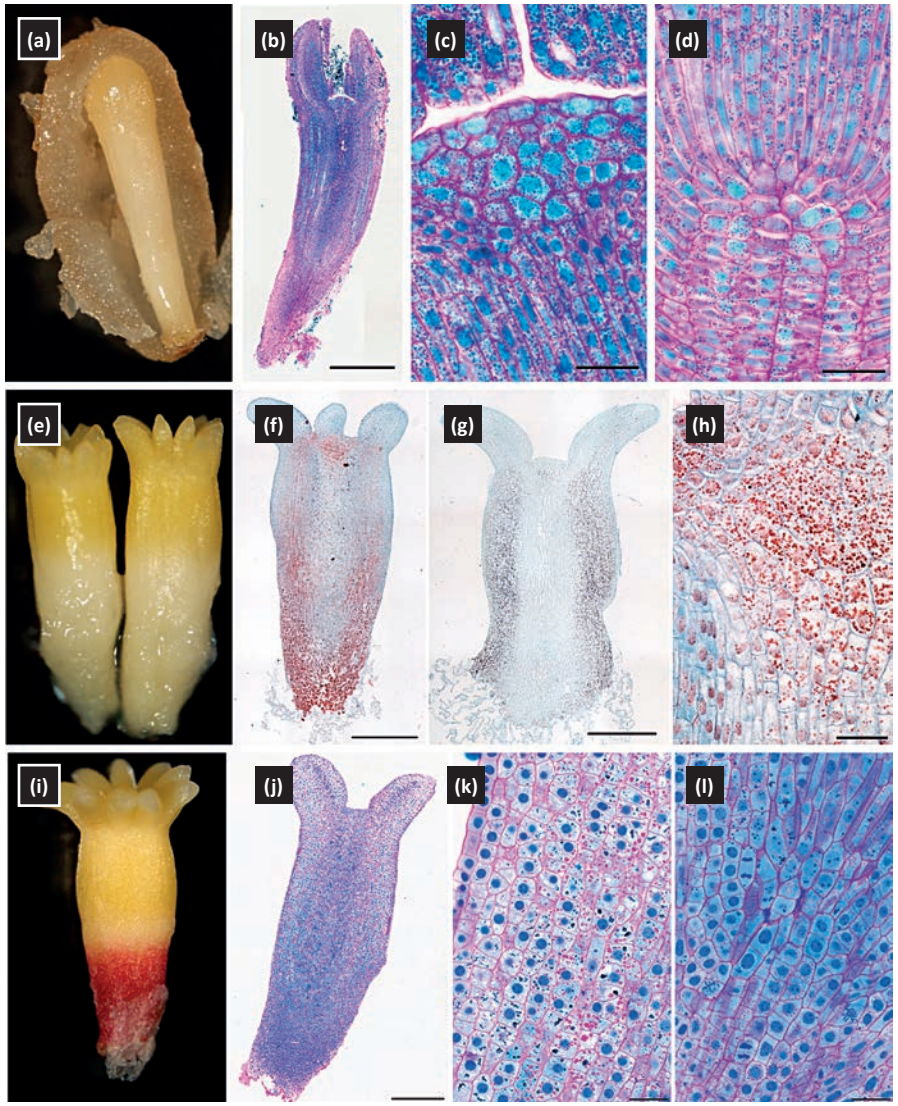

Figure 1. Cotyledonary zygotic and somatic embryos of Norway spruce. (a–d) zygotic embryos, **(e–h)** somatic embryos after 5 weeks of maturation, **(i–l)** somatic embryos after 3 weeks of desiccation at a high relative humidity. Fresh embryos **(a, e, i)** were fixed and embedded into paraffin **(b–d, f–h)** or resin **(j–l),** cut into sections, and stained to detect starch grains and storage proteins; **(b, f, g, j)** longitudinal section throughout the whole embryos, **(c)** detail of shoot apical meristem, **(d, l)** details of root apical meristems, **(h)** accumulation of protein storage vacuoles (red dots) below shoot apical meristem, **(k)** detail of the cells of the hypocotyl. Polysaccharides (*i.e.* cell walls and starch) in zygotic and desiccated somatic embryos **(b–d, j–l)** were stained with periodic acid and Schiff reagent (pink colour); storage proteins localised in protein storage vacuoles were stained there with Amido black (small blue dots; big blue particles are nuclei). Somatic embryos after maturation were stained with Ponceau xylidine to detect storage proteins (red dots in **f** and **h**) or with Lugol solution to detect starch grains (dark dots in **g**). Cell walls in these sections were counterstained with Azur (blue colour). Scale bars in **(b, f, g, j)** are 200 µm, and in **(c, d, h, k, l)** 50 µm.

changes revealed could be responsible for reorienting Norway spruce somatic embryos towards germination (Eliášová *et al.* 2022). The stress response was reflected in the activities of polyamine (PA) biosynthetic enzymes, which steadily increased in the embryos during desiccation at high RH, and decreased at lower RH. The total free PA content in embryos decreased gradually throughout desiccation at high RH, in contrast to an increase in free putrescine (Put) and insoluble Put conjugates at low RH. These changes were accompanied to some extent by the transcription of the genes for the PA biosynthetic enzymes. Desiccation at high RH also increased the activities of the cell wall-modifying enzymes β-1,3-glucanases and the transcription of β-1,3-glucanase and class IV chitinase genes, both of which were suppressed at low RH. Desiccation at lower RH appears to impede the processes leading to physiological maturity and the subsequent ability of embryos to develop into plantlets (Fischerová *et al.* 2022).

Collaborations

The laboratory has enjoyed a long-standing collaboration with the research group of Marie-Anne Lelu-Walter and Caroline Teyssier from BioForA INRA, Orléans, France. Recently, we have studied the impact of different desiccation conditions on the development of hybrid larch somatic embryos. The results will be published soon. We collaborated with Dr Ildiko Matušíková (University of Ss. Cyril and Methodius, Trnava, Slovakia) on the study of β-1,3-glucanase and chitinase activities. Together with Dr Teresa Hazubska-Przybyl (Institute of Dendrology, Polish Academy of Sciences, Kórnik, Poland) we plan to study the redox status in conifer embryogenic cultures. We continue to collaborate with teams from the Forestry and Game Management Research Institute, Strnady, and the Faculty of Tropical AgriSciences, Czech University of Life Sciences, Prague, Czechia. Our main partners in IEB are the Laboratories of Hormonal Regulations in Plants, Plant Growth Regulators, Plant Virology and Plant Reproduction. Our collaboration with the last one resulted in three articles (Abeyawardana *et al.* 2023, Gutierrez-Larruscain *et al.* 2022a, 2022b).

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Publications: 190, 250–251, 261–262, 379
Research projects: 8

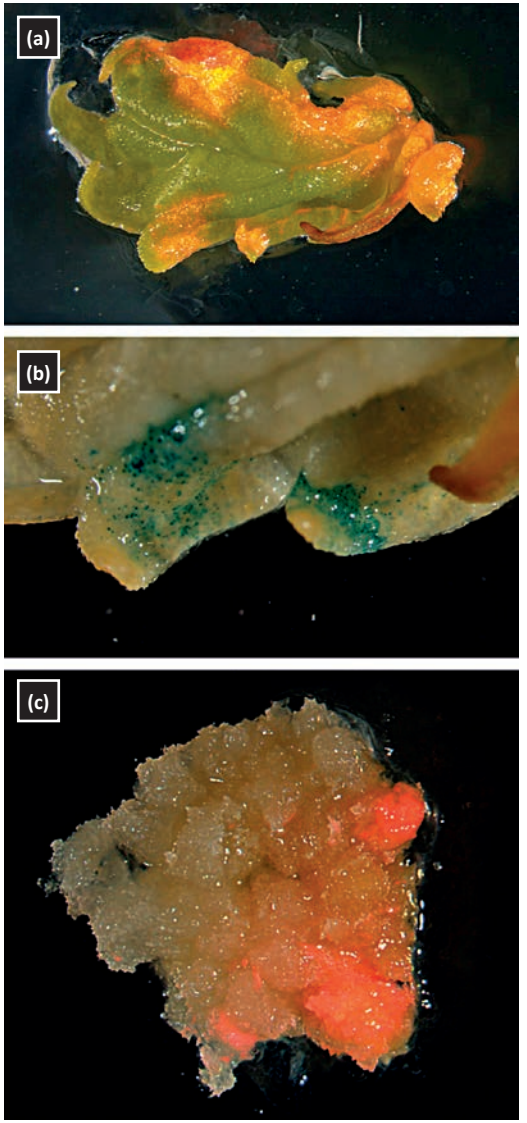

Figure 2. Transformation of the leaf segment of cannabis. Reporter genes RFP (red in **a**) and GUS (blue in **b**), and the derived callus **(c)**.

Laboratory of Cell Biology

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Our primary mission is to identify and understand the molecular modules that regulate plant cell polarity and morphogenesis, focusing on those operating at the intersection of the secretory pathway, the plasma membrane lipids, and the actin cytoskeleton. We divide our attention between two main processes that give plant cells their shape: oriented cell division and differential cell growth, and we focus on intracellular molecular mechanisms driving cellular morphogenesis, such as exocytosis.

The orchestration of cellular processes in eukaryotes hinges on transporting the extracellular matrix, membrane lipids, and proteins to the cell surface, predominantly achieved through exocytotic vesicles. The culmination of the secretory pathway is exocytosis, a finely tuned process involving the tethering, docking, and fusion of secretory vesicles with the plasma membrane, ultimately culminating in cargo release.

At the core of our investigations lies the exocyst, a conserved octameric protein complex that plays a central role in tethering secretory vesicles to the plasma membrane, thus serving as a pivotal exocytosis regulator. Our research efforts are centred around dissecting the functional intricacies of the exocyst across a diverse spectrum of plant species, ranging from angiosperms *Arabidopsis thaliana* and *Nicotiana tabacum* to bryophytes *Physcomitrium* patens and *Marchantia polymorpha*, as well as the streptophyte alga *Klebsormidium nitens*. An important aspect of our research is exploring the role of vesicular traffic,

In the picture (from left to right):
Bc. Jana Šťovíčková / technician, Edita Janková-Drdová, Ph.D. / researcher, Laiju Kalathodi, MSc. / Ph.D. student, Lukáš Synek, Ph.D. / researcher, Mgr. Matěj Drs / Ph.D. student, Viktor Žárský, Ph.D. / senior researcher, Přemysl Pejchar, Ph.D. / researcher, Martin Potocký, Ph.D. / head of the laboratory, Mgr. Samuel Haluška / Ph.D. student, Lucie Brejšková, Ph.D. / researcher, Ankush Saddhe, Ph.D. / postdoc, Mgr. Eliška Škrabálková / Ph.D. student, Andrea Potocká, Ph.D. / research specialist, Jitka Ormannová, Ph.D. / postdoc, Hana Soukupová, Ph.D. / research specialist.

Not pictured:
Tamara Pečenková, Ph.D., Michal Hála, Ph.D. / researchers, Anna Bartáková / BSc. student, Vladyslav Nikolov / MSc. student.

specifically the exocyst, in plant reactions to abiotic stresses and in plant-microbe interactions. Concurrently, our research extends into deciphering the roles of negatively charged phospholipids in establishing and maintaining plant cell polarity. Although quantitatively minor, these anionic lipids are pivotal in governing membrane characteristics, charge, curvature, signalling, and protein recruitment. The collaborative regulatory role of these phospholipids in orchestrating vesicular traffic at the plasma membrane is of particular interest. In this context, we also examine the lipid kinases and phospholipases that catalyse the production of anionic lipid phosphatidic acid at the plasma membrane.

Molecular insight into the function and evolution of the plant exocyst complex

We have uncovered the modular structure of the exocyst complex, pinpointing the role of the EXO70A1 subunit in tethering the plant exocyst to the lateral plasma membrane in roots. This binding is mediated through interactions with an array of anionic phospho-

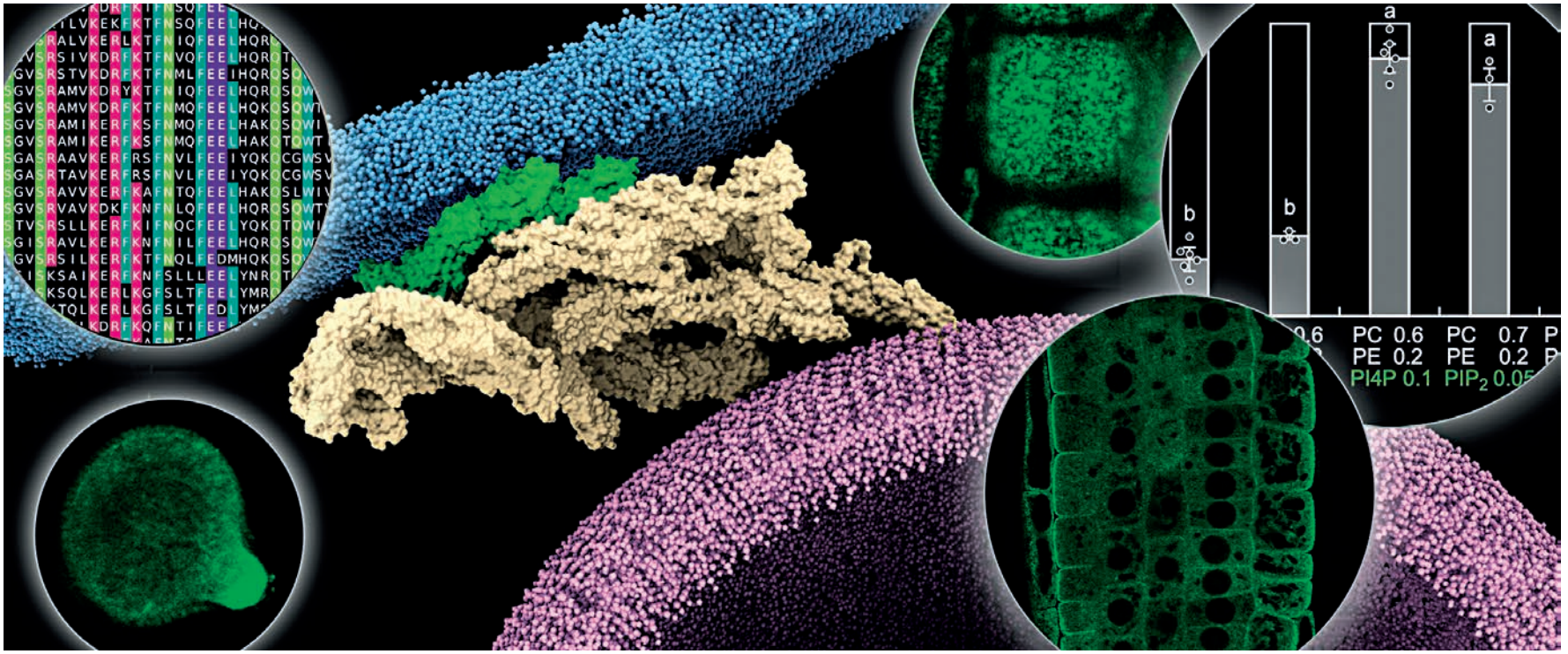

Figure 1. Model suggesting how Arabidopsis exocyst complex tethers a secretory vesicle to the plasma membrane via the isoform EXO70A1 and multiple anionic phospholipids. The insets showcase the multifaceted workflow of our lab, which combines state-of-the-art microscopical, computational, biochemical, and *in planta* approaches. Inspired by the results published in Synek, Pleskot, Sekereš *et al.* (2021).

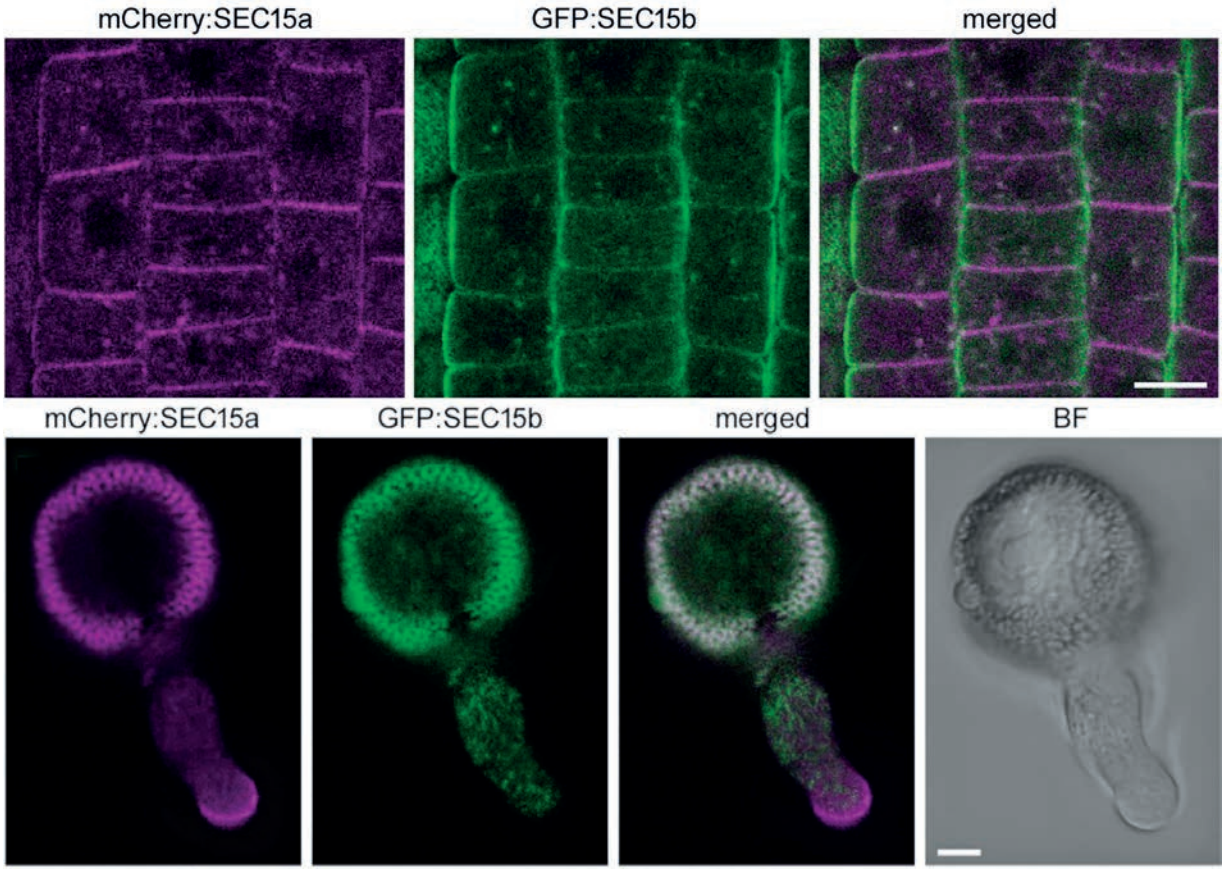

Figure 2. Different localisation of fluorescently-tagged exocyst subunits SEC15A and SEC15B in Arabidopsis roots and pollen tubes, suggesting the co-existence of functionally distinct exocyst complexes within one cell in different cell types. Scale bar, 10 µm. Adapted from Batystová *et al.* (2022).

lipids. Through a multifaceted approach encompassing biochemical, genetic, microscopic, and computational techniques, we have shown the role of phosphatidylinositol 4-phosphate and phosphatidic acid in controlling exocyst binding to the plant plasma membrane (**Fig. 1**). This study also emphasises the pivotal role of membrane charge in governing interactions between peripheral proteins and membranes (Synek, Pleskot, Sekereš *et al.* 2021).

We elucidated the evolutionary history of the land plant exocyst complex, revealing a multistep pattern of evolution of distinct exocyst subunits. We have dissected the evolution of the EXO70 subunit, revealing its division into three well-established subfamilies, each exhibiting unique functional attributes. Through cross-complementation assays, we have highlighted the deep conservation of the canonical EXO70.1 subfamily and the independent evolution of the non-canonical EXO70.2 and EXO70.3 subfamilies (Haluška, Janková-Drdová *et al.*, submitted). Additionally, our investigations into the SEC15a and SEC15b exocyst subunit isoforms have illuminated their preferred roles in the male gametophyte and sporophyte, respectively, and revealed a surprising acquisition of novel function for SEC15a in the sporophyte (**Fig. 2**; Batystová *et al.* 2022). Our functional study of the exocyst in the moss *Physcomitrium patens* has provided insight into the significance of the SEC6 exocyst subunit in cell division and in the organisation of cell assemblies. Our observations document the role of the exocyst complex in the transition from simple filamentous structures to complex morphological arrangements in plant organs (Brejšková *et al.* 2021).

Role of vesicular traffic in plant-microbe interactions

In the reaction to pathogenic fungi, plants employ intensive exocytosis to deposit cell wall reinforcements (papillae or encasements), which block the spread of infection throughout the plant. We identified a vital role of the exocyst complex containing subunit EXO70B2 in the papillae membrane domains important for callose deposition and encasement formation. Our results have highlighted the importance of the EXO70B2-containing exocyst in delivering the key SNARE protein SYP121 to the papillae membrane domains, offering new insights into mechanisms of penetration resistance. (Ortmannová *et al.* 2022). We found that the closely-related exocyst subunit EXO70B1 plays a vital role in responses to various abiotic stresses, operating at both endomembranes and the plasma membrane in a complex cooperation/competition mode with EXO70B2 (Drs *et al.*, in preparation).

Our research shed new light on the enigmatic cellular processing, trafficking, localisation, secretion, and function of the pathogenesis-related 1 (PR1) protein. By generating a spectrum of tagged Arabidopsis PR1 variants, we have provided insights into the dynamic modulation of defence responses and immune pathways through PR1 protein processing. This intricate interplay is dependent on cellular localisation and plant age, highlighting the multifaceted nature of this crucial plant defence protein (Pečenková *et al.* 2022).

To unravel the interplay between cell morphogenesis and biotic stress responses, we began to investigate how the immunity elicitor chitosan impacts the polar growth of plant cells. We found that chitosan triggers root hair callose deposition and growth inhibition (**Fig. 3**). Our findings revealed the deep conservation of this strategy in analogous root hair-like structures in lycopods and bryophytes, underpinning the significance of this response in mild biotic stress situations. (Drs *et al.*, submitted).

Lipid signaling at the plant plasma membrane

Pollen tubes require a tightly regulated pectin secretion mechanism to maintain the cell wall plasticity required for polar tip growth. Phosphoinositides and phosphatidic acid participate in this regulation at the apical plasma membrane, but the processes regulating their production remain unclear. In collaboration with Till Ischebeck's group at the University of Göttingen, Germany, we have shown that diacylglycerol kinase 5 plays a significant role in the regulation of pectin secretion in pollen tubes growing at the apex (**Fig. 4**; Scholz, Pejchar *et al.* 2022).

In two extensive collaborative studies led by Daniel

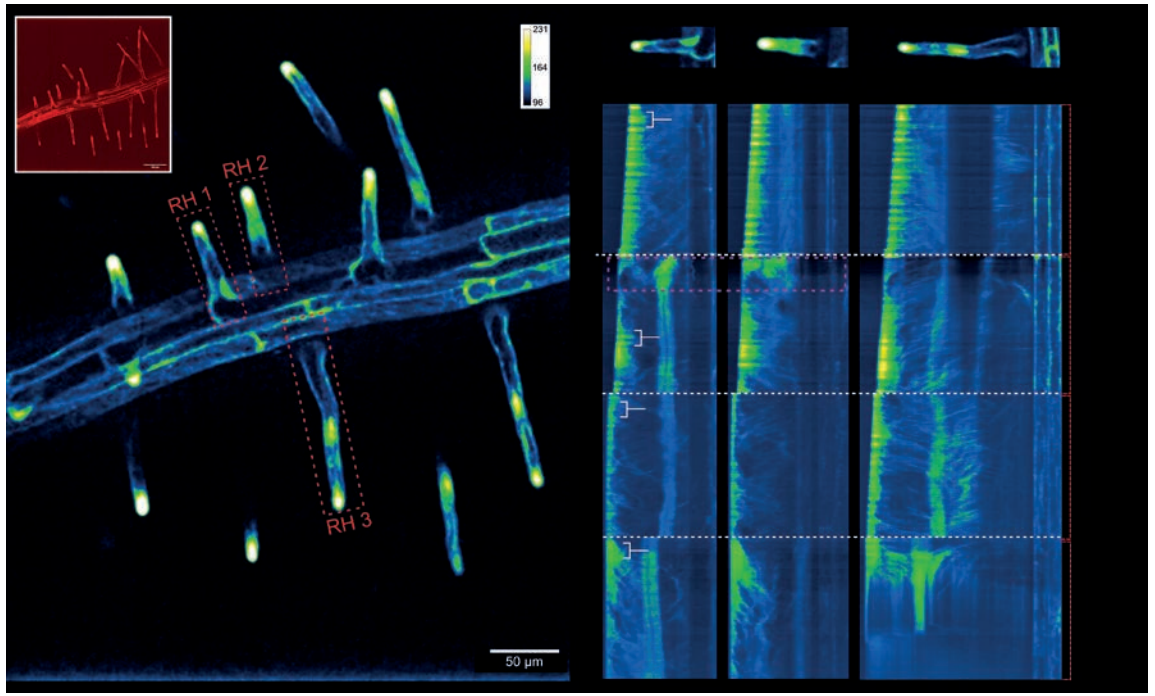

Figure 3. Intracellular calcium dynamics in Arabidopsis root hairs treated with low (0.001%, LCC) or high (0.01%, HCC) chitosan concentration. Calcium is visualised by genetically-encoded marker R-GECO. Kymographs show the dynamics of R-GECO fluorescence within selected root hairs. Scale bar, 50 µm. Adapted from Drs et al. (submitted).

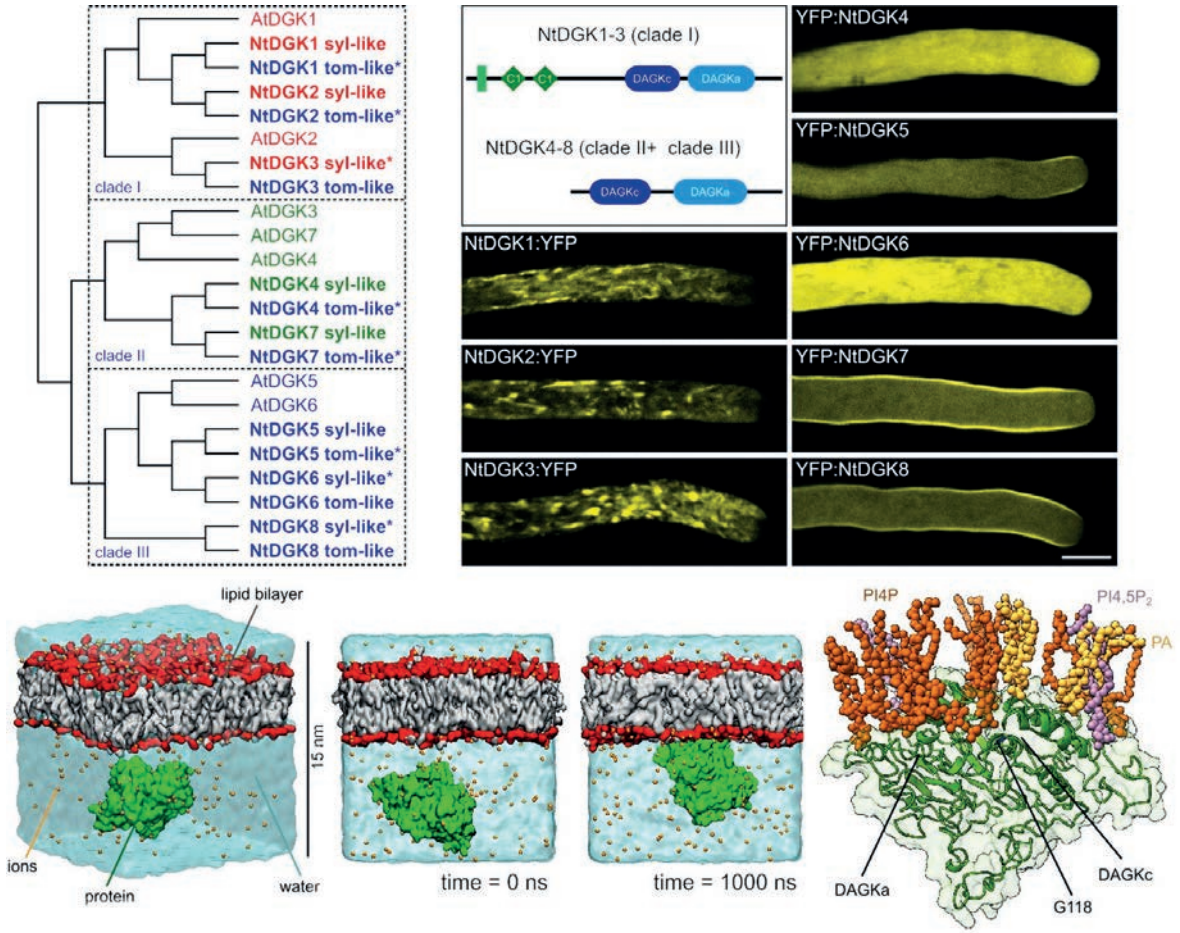

Figure 4. Tobacco diacylglycerol kinase isoforms show distinct localisation patterns in pollen tubes and bind to the plasma membrane via phosphoinositides. Left, phylogeny of Arabidopsis and *Nicotiana tabacum* diacylglycerol kinase (DGK) isoforms and their distribution into three clades. Right, domain distribution and localisation of YFP-tagged NtDGK1-8 in actively growing pollen tubes. The lower panel illustrates the molecular dynamics simulations showing mechanistic details of the NtDGK5-membrane interaction. Scale bar, 10 µm. Adapted from Scholz, Pejchar *et al.* (2022).

Van Damme (PSB VIB Ghent, Belgium) and Roman Pleskot (Laboratory of Integrative Structural Biology, IEB), our group joined forces to unravel the role of anionic lipids in the regulation of plant clathrin-mediated endocytosis, namely the structure and regulation of the TPLATE complex (TPC). We focused on the lipid-binding properties of several TPC subunits, showing the distinct but complementary roles of anionic lipids in the recruitment of TPC to the plasma membrane (Yperman *et al.* 2021a, 2021b). Recently, we revealed that anionic lipids play a role in the biogenesis of biomolecular condensates of specific TPC subunits (Dragwidge *et al.*, submitted).

As membrane lipids are important players in plant responses to various stresses, we investigated the involvement of lipid signalling in flagellin perception, which is a keystone of pattern-triggered immunity in plants. In collaboration with the Laboratory of Pathological Plant Physiology and the group of Eric Ruelland (CNRS, Compiègne, France), we found that flagellin-derived peptide flg22 caused rapid and transient changes in Arabidopsis lipid dynamics. We identified diacylglycerol kinase 5 (DGK5), the enzyme producing phosphatidic acid, as the responsible gene. Importantly, *dgk5.1* mutant plants produced less phosphatidic acid in response to flg22 and showed impaired resistance. The enzymatic activity of plasma membrane-localised DGK5 is thus vital for flagellin signalling and early immune responses in plant-microbe interactions (Kalachova, Škrabálková *et al.* 2022).

National and international collaboration

Within the country, we benefit from a long-standing collaboration with the Laboratory of Cell Morphogenesis (Charles University, Prague, Czech Republic), led by Viktor Žárský and Fatima Cvrčková. In the past two years, we have enjoyed an extremely fruitful collabora-

Figure 5. Collection of cover pages published between 2021–2023 highlighting the outcomes of our work (Batystová *et al.* 2022, Synek *et al.* 2022, Marković *et al.* 2021).

tion with the Laboratory of Integrative Structural Biology at the IEB, led by Roman Pleskot, a former member of our team. On an international level, between 2021-2023 we published joint papers with laboratories led by Daniël Van Damme (PSB VIB, Ghent, Belgium), Till Ischebeck (now at the University of Münster, Germany), Fabien Nogué (IJPB INRAE Versailles, France), Eric Ruelland (CNRS, Compiègne, France), and Claus Schwechheimer (TUM, Freising, Germany).

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Research projects: 5, 8, 10, 25, 44, 48, 52, 58, 70, 73, 77

Laboratory of Growth Regulators

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The Laboratory of Growth Regulators (LGR) was established in 1996 as a joint facility of the Institute of Experimental Botany of the Czech Academy of Sciences and the Faculty of Science of Palacký University. The purpose of the LGR is to pursue research in the field of molecular and physiological mechanisms of the action of growth regulators in living organisms and to develop the necessary technology. The LGR focuses on scientific research and teaching in the field of Experimental Biology, especially in the preparation of new growth regulators with potent biological activities, in the development of relevant analytical methods, and in the study of functions and effects on the growth and developmental process in cells, tissues, and whole organisms, including the development of drugs derived from plant hormones. Research on genes, mechanisms that regulate their expression, and the development of mutant organisms with controlled gene expression are also included in the LGR's scientific profile. Researchers at the LGR work mainly with cytokinins (CKs), but they have recently also worked with other groups of plant growth regulators. One globally renowned contribution of the LGR in this field is the expansion of a number of CKs, especially the aromatic CK **topolins** and their anti-tumour derivatives (**olomoucine**, **bohemine**, **roscovitine**, **olomoucine II**, etc.), which are effective inhibitors of cyclin-dependent kinases – key enzymes of the cell division cycle. Roscovitine was licenced by Cyclacel Pharmaceuticals, Inc. and under its commercial name, **Seliciclib®**, is undergoing phase II clinical trials for the treatment of Cushing disease in the USA. Another product, under the trade name **Pyratine®**, is also derived from CKs. This not only treats skin roughness, wrinkles, and pigmentation, but is also effective for treating various forms of acne. The LGR has also been successful in agricultural research. For example, we discovered how to increase the amount of endogenous CKs using an inhibitor of cytokinin oxidase/dehydrogenase called **INCYDE**. Applying this compound, we were able to increase the yield of a number of agricultural crops and improve plant stress resistance. Recently, LGR was also successful with the concept of using derivatives of the plant growth regulator **MTU (Status®)**, which helps create longer growth periods, protection from stress, larger plants, and potentially less nutrient loss per unit of fertiliser applied.

In the picture (from left to right):
Mgr. Ota Blahoušek / research assistant, Mgr. Kateřina Perničková, Ph.D. / research assistant, Bc. Jitka Hansgutová, DiS. / secretary, Mgr. Jana Oklešťková, Ph.D. / researcher, Prof. Mgr. Ondřej Novák, Ph.D. / head of the laboratory, Ing. Věra Doleželová / project manager, Mgr. Aleš Pěnčík, Ph.D. / researcher, Mgr. Ivan Petřík / Ph.D. student, Miroslava Špičáková / technician, Mgr. Michaela Mrvková / research assistant, Mgr. Tereza Miksteinová / Ph.D. student, Prof. Ing. Miroslav Strnad, CSc., DSc. / full professor, Mgr. Jitka Kopková / project manager, Prof. RNDr. Martin Fellner, Ph.D. / full professor, Mgr. Tomáš Vlčko, Ph.D. / researcher, Mgr. Terezie Urbanová, Ph.D. / researcher, Mgr. Karel Doležal, Dr., DSc. / researcher, Ing. Ludmila Ohnoutková, Ph.D. / researcher, Mgr. Pavel Jaworek, Ph.D. / researcher.

Not pictured:
Doc. Mgr. Lucie Plíhalová, Ph.D., Doc. RNDr. Jiří Pospíšil, Ph.D. / associate professors, Mgr. Jakub Hajný, Ph.D., Mgr. Jakub Hrdlička, Ph.D., RNDr. Miroslav Kvasnica, Ph.D., Mgr. Marie Kvasnicová Ph.D., Lutfun Nahar, Ph.D., Mgr. Lenka Plačková, Ph.D., Mgr. Tomáš Pospíšil, Ph.D., Mgr. Lucie Rárová, Ph.D., Pharm.Dr. Jitka Široká, Ph.D., Mgr. Danuše Tarkowská, Ph.D., Mgr. Veronika Turečková, Ph.D. / researchers, Mgr. Petra Bublavá, Mgr. Martin Hudeček, Mgr. Veronika Kábrtová, Mgr. Natálie Závorková / Ph.D. students, Ing. Libor Hájek / technology scout, Lenka Gajdošíková, Xénie Hanáková, Mgr. Tomáš Jirsa, Pavel Sedláček / technicians.

Research activity and characterisation of the main scientific results

During the period 2021-2023, the research programs were divided into four main parts reflecting the structure of the LGR. The original results achieved over the last three years demonstrate the high scientific quality of the teams, both in a national as well as in an international context (see also List of publications and patents).

New phytohormones, biostimulants, and biomolecules (leader: Karel Doležal)

Purine and anti-senescence compounds

The solubility of growth regulators is essential for their use in agriculture. Therefore, four new CK mesylate salts have been prepared. The mesylates were several orders of magnitude more water-soluble than the original CKs and also highly biologically active, so these salts can find some applications in agriculture (Klos *et al.* 2022). Furthermore, a new group of isoprenoid 2′-deoxyribosides and 2′,3′-dideoxyribosides was prepared by various synthetic approaches and their CK activity was determined. The prepared compounds were found to be non-toxic to human cells, and the majority of assays exhibited the highest activity of free bases, while 2′,3′-dideoxyribosides had very weak or no activity (Matušková *et al.* 2023). Highly targeted drug micro applications can be used in plant research for the regulation of physiological processes on tissue and cellular levels. Here, for the first time, an organic electronic ion pump (OEIP) was reported that can transport an isoprenoid-type CK, N^6-isopentenyladenine (iP) to intact plants. The results from the application of the high-resolution OIEP treatment method confirm previously published findings showing that the influence of CKs may vary at different stages of lateral root development (Pařízková *et al.* 2022).

Steroid chemistry

Based on molecular docking experiments, two groups of brassinosteroid (BR) analogues with short and long side chains were prepared to study the impact of side chain length on plants. A total of 25 new BR analogues were prepared and tested in an Arabidopsis root sensitivity bioassay and cytotoxicity screening. The synthesised substances showed no significant inhibitory activity compared to natural 24-epibrassinolide. In contrast, at low concentration, several BR-based compounds showed interesting growth-promoting activity. The cytotoxicity assay showed no toxicity of the prepared compounds on cancer and normal cell lines (Diachkov *et al.* 2021). Using novel BR analogues, we demonstrated that plasmodesmata (PD) mediate BR passage between adjacent cells (**Figure 1**). Moreover, intracellular BR content is in turn able to modulate PD permeability to optimise its own mobility, thereby manipulating BR biosynthesis and signalling (Wang *et al.* 2023).

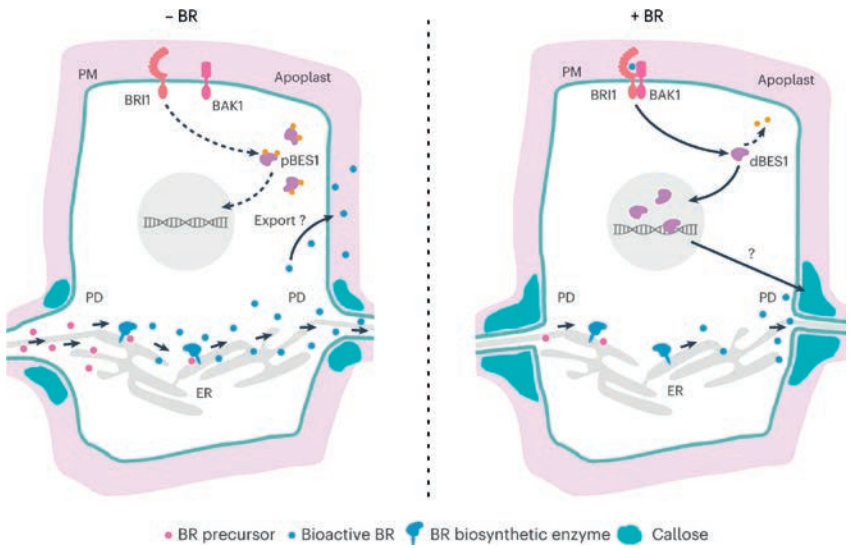

Figure 1. Plasmodesmata-mediated transport and homeostasis of brassinosteroid (BR) biosynthesis and signalling. BR biosynthetic enzymes are expressed in neighbouring cells, requiring the exchange of BR intermediates through PD to produce bioactive BRs. Once synthesised, BRs exit the cell via an unknown mechanism and reach the apoplast (*left panel*). Once in the extracellular matrix, bioactive BRs bind to the BRI1 receptor and co-receptor BAK1 and initiate a signalling cascade that leads to dephosphorylation of the BES1 transcription factor. BES1 can then enter the nucleus and initiate transcriptional responses. High levels of BRs increase callose deposition and decrease PD permeability, possibly via BR signalling-initiated transcriptional regulation. Restricted BR precursor movements reduce hormone production and contribute to optimal BR signalling level maintenance (*right panel*). BR, brassinosteroid; PM, plasma membrane; PD, plasmodesmata; ER, endoplasmic reticulum. (Wang *et al.* 2023).

Indole chemistry

The introduction of a fluorophore to an auxin molecule represents a sensitive and non-invasive method of directly visualising auxin localisation with high spatiotemporal resolution. Despite partial metabolisation *in vivo*, the fluorescent auxins display

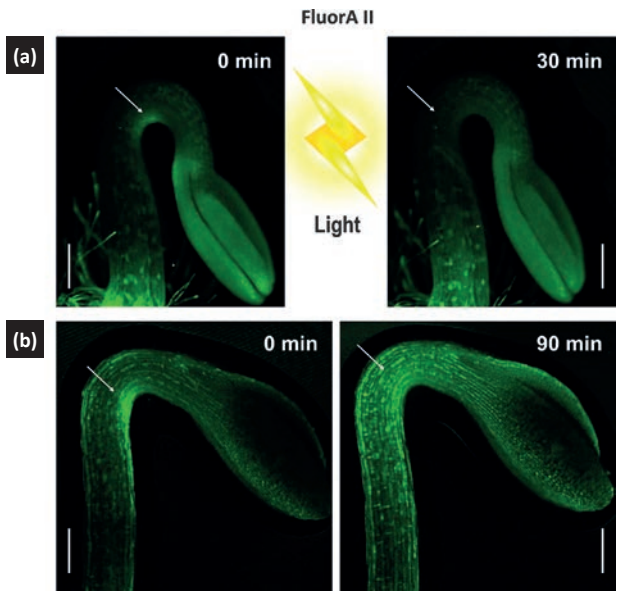

Figure 2. Tissue-specific distribution of fluorescent auxin analogue (FluorA II) in response to light in apical hook. Three-day-old dark-grown seedlings of Arabidopsis Col-0 WT treated with 2 µM FluorA II for 15 min. The accumulation of FluorA II in the apical hook was evaluated before (0 min) and 30 min after a light stimulus **(a)** or without a light stimulus **(b)**. Scale bars represent 1 mm. The white arrows indicate the redistribution of FluorA II signal in the apical hook after light stimulation. (Pařízková *et al.* 2021).

an uneven and dynamic distribution, leading to the formation of fluorescence maxima in tissues known to concentrate natural auxin, such as the concave side of the apical hook (**Figure 2**). Moreover, we characterised the subcellular localisation of the fluorescent auxin analogues as being present in the endoplasmic reticulum and endosomes (Pařízková *et al.* 2021).

Bioanalytical chemistry of phytohormones and its application (Leader: Ondřej Novák)

Plant hormonomics

In order to better understand the network regulation of hormone action, it is necessary to measure multiple hormone concentrations simultaneously, i.e. characterise the 'hormone-metabolome'. Therefore, a high-throughput sample preparation method for liquid chromatography-tandem mass spectrometry determination of 25 acidic phytohormones classed as auxins, jasmonates, abscisates, and salicylic acid was optimised. The method developed enables high-throughput profiling of acidic phytohormones with minute amounts of plant material, and it is suitable for large-scale interspecies studies (Široká *et al.* 2022). We also developed a sensitive method for the determination of two highly biologically active karrikins (KAR1 and KAR2) in minute amounts of plant material (<20 mg fresh weight). The developed protocol combines the optimised extraction and efficient single-step sample purification with ultra-high performance liquid chromatography-tandem mass spectrometry (Hrdlička *et al.* 2021). Recently, we have introduced an approach for the synthesis of *cis*-12-oxophytodienoic acid (*cis*-OPDA) conjugates with amino acids and a highly sensitive method based on liquid chromatography-tandem mass spectrometry that enables the identification and accurate quantification of these compounds in minute amounts of plant tissues (Mik *et al.* 2023).

(Sub)cellular phytohormone profiling

Single-cell suspensions can be obtained through digestion of the cells walls and the release of the so-called protoplasts (plants without their cell walls). We have published best practices for protoplast preparation, and for analysis by flow cytometry and cell sorting. Finally, the numerous downstream applications involving sorted protoplasts have been discussed (Antoniadi *et al.* 2021). We also presented an optimised protocol for endoplasmic reticulum (ER) isolation from *Arabidopsis thaliana* seedlings and subsequent mass spectrometric determination of ER-specific auxin metabolite profiles. Analysis of auxin metabolites revealed highly elevated levels of free indole-3-acetic acid (IAA) within the ER compared to whole plants (Včelařová *et al.* 2021). Moreover, we tested several published isolation protocols based on differential centrifugation or flow cytometry to present the first complex report on the auxinome of isolated nuclei from cell cultures of Arabidopsis and tobacco. We also concluded that the methodological procedure combining flow cytometry and mass spectrometry offers new horizons for the study of phytohormone homeostasis at the subcellular level (Skalický *et al.* 2021). This confirmed the introduction of a breakthrough technique, the so-called Fluorescence-Activated multi-Organelle Sorting (FAmOS), for the simultaneous fractionation of up to four organelle populations (nuclei, chloroplasts, mitochondria, and ER) from a single sample of plant cell cultures. The unique combination of our developed FAmOS tool with bioanalytical methods enables a high-resolution mapping of plant hormones in isolated organelles (**Figure 3**). Our data showed different subcellular distribution of auxin and CKs, revealing the formation of phytohormone gradients that have been suggested by the subcellular localisation of auxin and CK transporters, receptors, and metabolic enzymes (Skalický *et al.* 2023).

Mass spectrometry imaging of phytohormones

We also introduced a tool using desorption electrospray ionisation mass spectrometry imaging (DESI-MSI) for high-throughput visualisation and evaluation of wound-induced phytohormones inside *Arabidopsis*

thaliana leaves (Zhang *et al.* 2021). The results showed higher levels of jasmonates, salicylic acid, abscisic acid (ABA) and IAA in their ion intensity maps established from wounded leaves compared to control leaves (**Figure 4**).

Applications of novel methodological approaches

Our new technologies have led to many international collaborations and important discoveries. For example, we found that shade combined with a warm temperature produces a synergistic hypocotyl growth response that is dependent on PHYTOCHROME-INTERACTING FACTOR 7 (PIF7) and auxin (Burko *et al.* 2022). Our data also provided new insights into the roles of group II members of the GRETCHEN HAGEN 3 (GH3) gene family in IAA metabolism and hormone-regulated plant development (Casanova-Sáez *et al.* 2022). Peptide RAPID ALKALINIZATION FACTORS 1 (RALF1) and IAA are key growth regulators with characteristic rapid and long-term effects. Our study revealed that those two signals trigger rapid growth inhibition (<1 min) by rapidly alkalinising the apoplast, a result of rapid net H^+ influx across the plasma membrane; nonetheless, their signalling mechanisms are independent (Li *et al.* 2022). Root meristem organisation in Arabidopsis is maintained by an interplay between the interacting hormones BRs and auxin. Their interaction maintains the meristem to the outer epidermis and lateral root cap tissues and demonstrates the essentiality of BR signalling in these tissues for meristem response to BRs (Ackerman-Lavert *et al.* 2021). We also demonstrated that BR biosynthesis is largely restricted to the root elongation zone, where it overlaps with BR signalling maxima (Vukašinović *et al.* 2021). Furthermore, we published findings that ethylene regulates these distinct root growth responses using different downstream signals, auxin, and ABA (Huang *et al.* 2022). In the cytokinin field, we showed that within the root meristem, xylem cells act as a local organiser of vascular development by non-autonomously regulating CK levels in neighbouring procambium cells via sequential induction and repression modules (Yang *et al.* 2021). Despite numerous studies showing the central role of CKs in nodulation, the importance of CK transport in the symbiosis is unknown. We studied the role of a full-size ATP-binding cassette (ABC) transporter in the early stages of the nodulation. Our data indicate that ABCG-mediated CK transport is important for the proper establishment of N-fixing nodules (Jarzyniak *et al.* 2021).

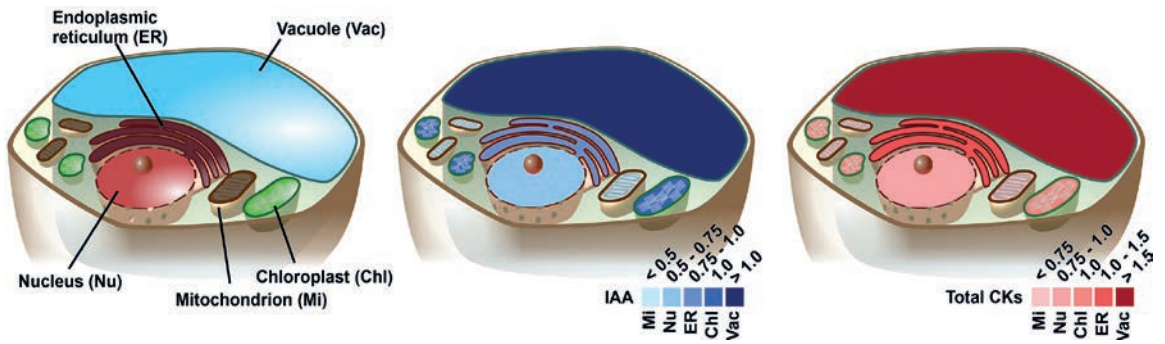

Figure 3. Subcellular map of endogenous phytohormones within the Arabidopsis cell. Scheme of a plant cell (*left*), relative distribution of auxin (*middle, in blue*), and total cytokinin metabolites (*right, in red*) within the plant cell. The results represent 5 biological replicates. The metabolites were quantified in pmol/µg of proteins, and the ratios of the respective organelle to chloroplast were determined. (Skalický *et al.* 2023).

Medicinal chemistry (Leader: Vladimír Kryštof)

3,5,7-Trisubstituted pyrazolo[4,3-d]pyrimidines have been identified as potent inhibitors of cyclin-dependent kinases (CDKs), which are established drug targets. We described their further structural modifications leading to novel nanomolar inhibitors with strong antiproliferative activity (Jorda *et al.* 2022). Moreover, we reported a protocol that allows the direct oxidative coupling of heteroaryl thiols and primary amines, readily available and inexpensive commodity chemicals. The transformation proceeds under mild reaction conditions and yields the desired N-alkylated sulfonamides in good yields. These are important antimicrobial drugs (Iakovenko *et al.* 2022). Novel triterpene derivatives were further prepared and evaluated in salsolinol (SAL)- and glutamate (Glu)-induced models of neurodegeneration in neuron-like SH-SY5Y cells. Among the tested compounds, betulin triazole bearing a tetraacetyl-β-d-glucose substituent showed a highly potent neuroprotective effect (Gonzalez *et al.* 2021).

Plant molecular physiology (Leader: Martin Fellner)

Seed germination

The physiological and biophysical mechanisms underpinning cold-induced secondary dormancy include the chilling-induced accumulation of ABA in the seeds, a

reduction in the embryo growth potential, and a block in the weakening of the endosperm covering the embryonic root. Here, we proposed a model integrating the hormonal signalling and master regulator expression with the temperature-control of seed dormancy and maturation programmes (Hourston *et al.* 2022). The dihydrochalcone and putative allelochemical myrigalone A (MyA) inhibits seed germination and seedling growth. A comparative analysis of MyA and other phytotoxins revealed differences in the specific regulatory mechanisms and auxin transporter genes targeted to interfere with auxin homeostasis. We conclude that MyA exerts its phytotoxic activity by multiple auxin-dependent and independent molecular mechanisms (Nakabayashi *et al.* 2022).

Stress physiology

The redistribution of growth between shoots and roots is a common response to drought, promoting plant survival, but reducing yield. Therefore, we studied the effect of progressive drought on the content of gibberellins (GAs) and other hormones. In contrast to GAs, the other hormones analysed responded to drought similarly in the leaf and roots, indicating organ-specific differential regulation of GA metabolism in response to drought (Ptošková *et al.* 2022). Compaction is intuitively thought to reduce root growth by limiting the ability of roots to penetrate harder soils. We reported that root growth in compacted soil is instead actively suppressed by the volatile hormone ethylene, which acts as an early warning signal for roots to avoid compacted soils (Pandey *et al.* 2021). Recently, we analysed the natural variation in types of strigolactones exuded from maize roots. Maize genotypes that produced mainly zealactol suffered less Striga infection than those that produced mainly zealactone. A single cytochrome P450 catalyses several of the oxidative steps in strigolactone biosynthesis, including conversion of precursors to either zealactol or zealactone (Li *et al.* 2023).

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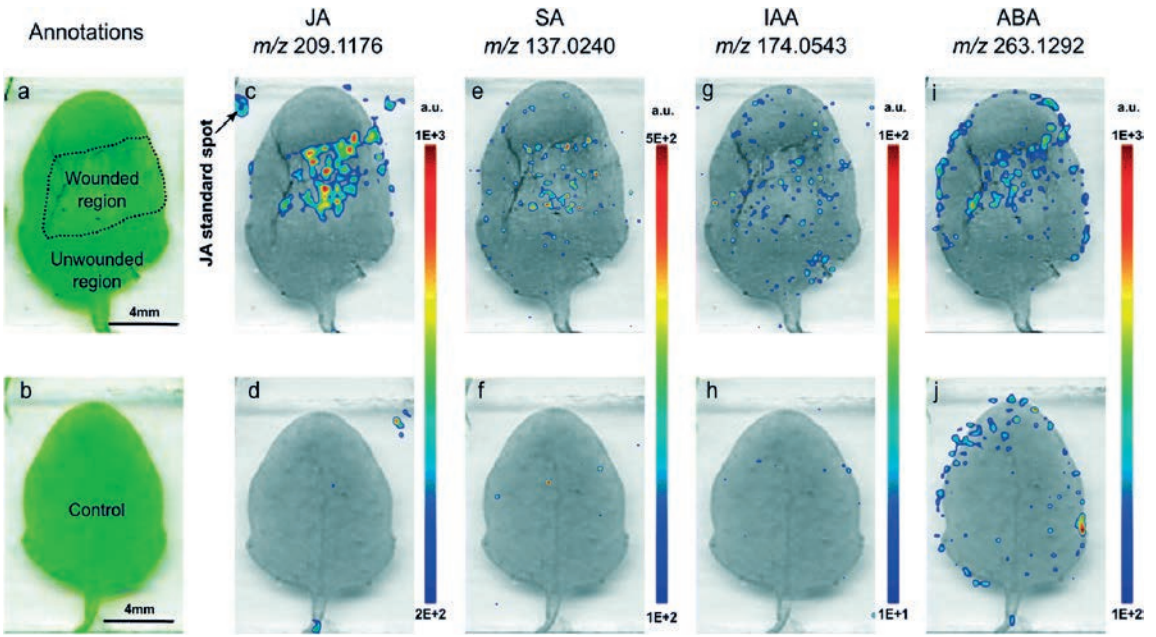

Figure 4. Direct DESI-MSI images describing the localisation of phytohormones (JA, SA, IAA, and ABA) in a pair of sample leaves. Leaf morphology patterns are shown in the **(a)** annotated image of the wounded and unwounded regions delimited inside the stressed leaf and **(b)** image of a control leaf. Distribution of JA in the wounded **(c)** and control **(d)** leaves; distribution of SA in the wounded **(e)** and control **(f)** leaves; distribution of IAA in the wounded **(g)** and control **(h)** leaves; and distribution of ABA in the wounded **(i)** and control **(j)** leaves. The peak intensity levels are displayed in the adjacent scale bar. (Zhang *et al.* 2022).

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Research projects: 8, 10–11, 13, 30, 34, 50–51, 54, 57, 68, 72, 74, 76, 84, 117

Laboratory of Hormonal Regulations in Plants

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The Laboratory of Hormonal Regulations in Plants continued its efforts to understand the role of phytohormones in coordinating plant development and responses to the environment. From 2021–2023, we published original contributions and reviews focused on auxin metabolism and transport, cytokinin metabolism, the role of RNA processing in plant hormonal pathways, profiling of phytohormone levels in an evolutionary framework, and novel players in the cytokinin-mediated abiotic stress response of plants. Our long-lasting objective is to comprehend the multifaceted role that plant hormones play. To achieve this goal, we combine several approaches, such as molecular genetics, profiling of transcriptomes and proteomes, plant phenotyping, biochemistry, analysing phytohormones and their metabolites, advanced fluorescence confocal microscopy, and mathematical modelling.

In the picture (from left to right):
Upper row: Jan Petrášek, Ph.D. / head of the laboratory, Milada Čovanová, Ph.D. / researcher, Petre Dobrev, CSc. / researcher, Nikoleta Dupľáková, Ph.D. / researcher, Klára Hoyerová, Ph.D. / researcher, Jana Jarošová, Ph.D. / researcher, Adriana Jelínková, Ph.D. / researcher, Petr Klíma, Ph.D. / researcher, Kateřina Malínská, Ph.D. / researcher, Václav Motyka, CSc. / researcher, Karel Müller, Ph.D. / researcher, Kamil Růžička, Dr. rer. nat. / researcher, Assoc. Prof. Radomíra Vaňková, CSc., DSc. / researcher, Prof. Eva Zažímalová, CSc. / researcher.
Middle row: Tomáš Hluska, Ph.D. / postdoc, Lucia Hlusková, Ph.D. / postdoc, Petr Hošek, Ph.D. / postdoc, Mike Karampelias, Ph.D. / postdoc, Ivan Kashkan, Ph.D. / postdoc, Eva Pokorná, Ph.D. / postdoc, Sylva Přerostová, Ph.D. / postdoc, Katarzyna Retzer, Ph.D. / postdoc, Roman Skokan, Ph.D. / postdoc, Martina Knirsch, Ph.D. / research assistant, Mgr. Roberta Filepová / research specialist, Mgr. Alena Gaudinová / research specialist, Mgr. Zuzana Vondráková / research specialist, Mgr. Nikola Drážná / technician.
Bottom row: Bc. Vojtěch Knirsch / technician, Eva Kobzová / technician, Bc. Marie Korecká / technician, Bc. Tereza Košťálová / technician, Ing. Alena Trávníčková / research specialist , Nayyer Abdollahi Sisi, Ph.D. / Ph.D. student, Judith Garcia Gonzales / Ph.D. student, Mgr. Lenka Helusová / Ph.D. student, Mgr. Jozef Lacek / Ph.D. student, Mgr. Daniel Nedvěd / Ph.D. student, Mgr. Vojtěch Schmidt / Ph.D. student, Ksenia Timofeyenko, MSc. / Ph.D. student, Elena Zemlyanskaya, MSc. / Ph.D. student, Anastasiia Holoborodko, BSc. / master's student.

Auxin metabolism and homeostasis

Together with auxin transport, auxin metabolism is a key determinant of auxin signalling output by plant cells. Enzymatic machinery involved in auxin metabolism is subject to regulation based on numerous inputs, including the concentration of auxin itself. Our lab utilises a well-established model of tobacco cell line BY-2, which allows for the detailed characterisation of altered auxin availability and subsequent changes in auxin metabolism, thus elucidating the function and regulatory role of individual elements in the auxin metabolic machinery. In collaboration with the Laboratory of Growth Regulators IEB CAS, utilising a complex transcriptomic, proteomic, and analytical approach, we discovered that the concentration of the oxidised form of IAA aspartate (oxIAA-Asp), the most abundant auxin metabolite produced in control BY-2 cells (**Fig. 1**), significantly decreases in auxin-depleted cells (Müller *et al.* 2021). This decrease was accompanied by a significant downregulation of all tobacco homologs of Arabidopsis DIOXYGENASE FOR AUXIN OXIDATION 1 (DAO1), at both the transcript and protein levels. Profiling of auxin metabolism in mutants of BY-2 (siRNA and CRISPR-Cas9 in NtDAO1) and Arabidopsis revealed lower levels of oxIAA. We also confirmed the existence of this novel enzymatic activity in a bacterial system.

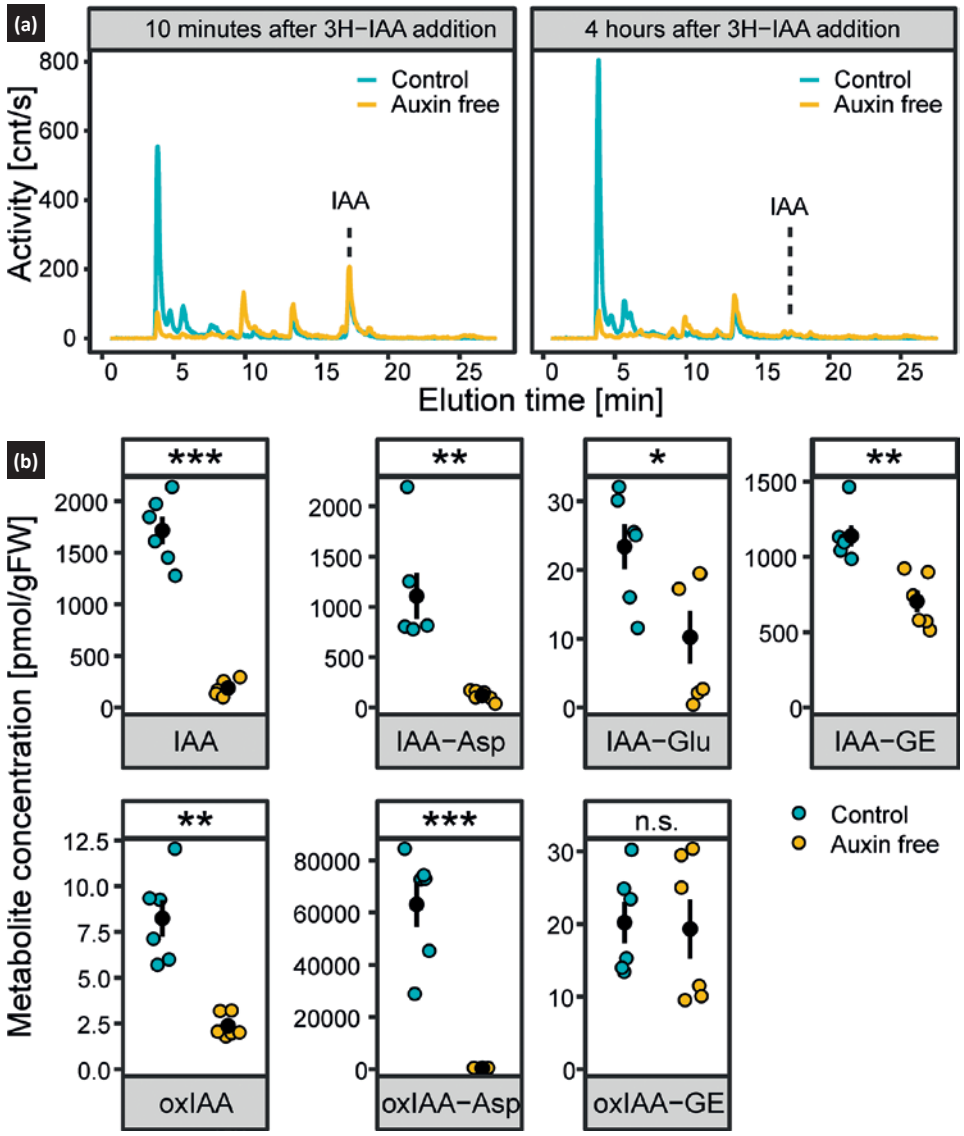

Figure 1: Auxin metabolism in 2,4-D-supplemented and auxin-starved BY-2 cells. **(a)** Auxin metabolite profiling of control and auxin-starved cells performed by HPLC chromatography 10 min and 4 h after application of 3H-IAA into the media. **(b)** Mass-spectrometry-based quantification of free IAA and its metabolites in control and auxin-starved cells 2 h after application of 1 mM IAA into the media. Data points and error bars indicating mean 6 SE are shown. B, Student's t test P-values: *P < 0.05, **P < 0.01, ***P < 0.001. Modified from Müller *et al.* 2021.

Our results thus represent direct evidence of DAO1 activity on IAA amino acid conjugates.

In order to identify a complete spectrum of IAA metabolites, we further performed a comprehensive analysis of IAA metabolism in tobacco cells and several plant species (Dobrev *et al.* 2023). A combination of labelled/unlabelled substrate feeding, global untargeted mass spectrometric (MS) scanning, and selective MS filtering allowed for the detection of 17 auxin metabolites, 15 of which were identified. Subsequent study of intermediate metabolism and dynamics revealed eight major pathways: three amino acid conjugation pathways with aspartate, glutamate, and glutamine, followed by their 2-oxidation with the help of the DAO enzyme; side-chain glucosyl ester formation; direct 2-oxidation; two decarboxylation pathways; and a pathway producing an unidentified metabolite. Our finding that the IAA decarboxylation pathway occurs *in planta*, and the previous reports of auxin activity of some metabolites of this pathway, suggest that at least some of the biological effects of IAA may be explained by its conversion to decarboxylative metabolites.

The continuation of our collaboration with PSB VIB, Ghent, Belgium led to the discovery of the piperonylic acid (PA) interference with the conjugation of IAA (El Houari *et al.* 2023). PA is an inhibitor of CINNAMATE-4-HYDROXYLASE (C4H), often used to inhibit lignin biosynthesis. However, we found that PA is recognised as a substrate by GRETCHEN HAGEN 3.6 (GH3.6), an amido synthetase involved in the formation of the indole-3-acetic acid (IAA) conjugate IAA-Asp. Our experiments using BY-2 cells showed a clear decrease in the relative levels of IAA over time in control cells, but not in PA-treated cells (**Fig. 2**). By competing for the same enzyme, PA interferes with IAA conjugation, resulting in an increase in IAA concentrations in the plant. We concluded that the deregulation

of phytohormone homeostasis by the occupation of the conjugation machinery in the plant is likely a general phenomenon when using chemical inhibitors.

Cytokinin metabolism

Incorporating phylogenetic, physiological, biochemical, and molecular approaches, we have investigated numerous aspects of the *N*- and *O*-glucosyltransferase pathways thought to be involved in the irreversible or reversible inactivation of cytokinins (CKs). The irreplaceable role of these metabolic pathways leading to the production of the corresponding CK *N*- and *O*-glycoconjugates has been demonstrated. Widespread distribution of CK *N*-glucosides biosynthesised by specific glucosyltransferases encoded by the UGT76C1 and UGT76C2 genes was demonstrated throughout the land plants, with increasing abundance from evolutionarily older to evolutionarily younger species. Based on data documenting the biological activity and degradation of CK N-glucosides in some model systems, the deactivating role of the N-glucosylation pathway has been revised and the putative irreversibility of its products questioned (Pokorná *et al.* 2021). Contradicting the long-held and generally accepted hypothesis that N-glucosylation irreversibly inactivates CKs, we confirmed our previous results on CK *N*-glucoside cleavage in *Arabidopsis thaliana* and showed that CK N-glucosides can undergo metabolic transformations that differ between *N7*- and *N9*-glucosides in oats (Pokorná *et al.* 2021). These results were also confronted with current knowledge on the metabolism of CKs in a review article (Hluska *et al.* 2021).

As part of the study of the evolutionary history of CK-specific glucosyltransferases, a phylogenetic reconstruction of the zeatin O-glucosyltransferase (ZOG) genes that spread during the transition from algae to vascular plants was performed. Phylogenetic analyses revealed that the *cis*ZOG gene (encoding *cis*-zeatin-O-glucosyltransferase) is present only in angiosperms, but not in *Arabidopsis thaliana* and other members of the Brassicaceae family (Záveská Drábková *et al.* 2021a).

In collaboration with the Laboratory of Growth Regulators IEB CAS and PSB VIB Ghent, Belgium, we also participated in the discovery of a novel transcriptional regulation of cytokinin levels during xylem development in Arabidopsis root. Extensive analysis revealed the role of enzymes involved in cytokinin metabolism. We participated in the functional analysis of the cytokinin hydrolase BGLU44, which contributes to the production of active cytokinins by cleaving cytokinin O-glucosides and O-glucoside ribosides (Yang *et al.* 2021).

Phytohormone profiling during plant development and on the evolutionary scale

Thanks to the development of sensitive methods of extraction, purification, and analytical determination of phytohormones, we are gradually expanding the spectrum that can be determined in a single sample. This allows us to analyse complex hormonomes in various contexts.

Firstly, we performed a comprehensive analysis of multiple phytohormones systematically across streptophyte algae, and in several land plants and chlorophyte algae for comparison (Schmidt and Skokan *et al.* 2023). We showed that the biosynthesis of many compounds recognised as active phytohormones preceded the evolution of their receptors. Auxin, tRNA-derived cytokinins, and salicylic acid were found ubiquitously,

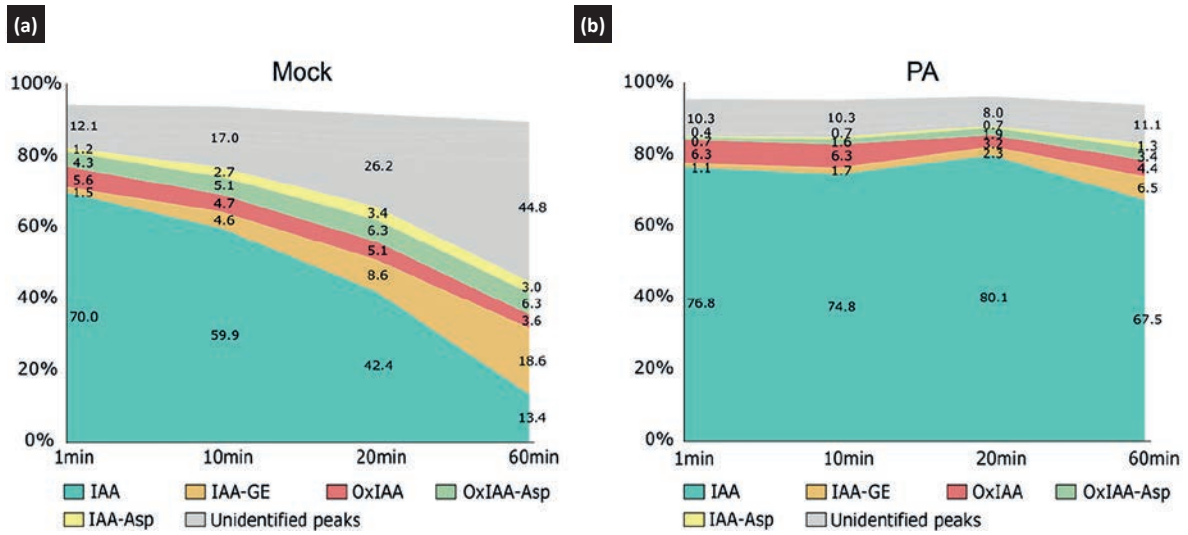

Figure 2: Conjugation of Piperonylic acid (PA) impacts on phytohormone homeostasis. Quantification of IAA and IAA conjugates in BY-2 cells over time upon addition of 3H-IAA and treatment without **(a)** or with **(b)** PA (n = 4). Modified from El Houari *et al.* 2023.

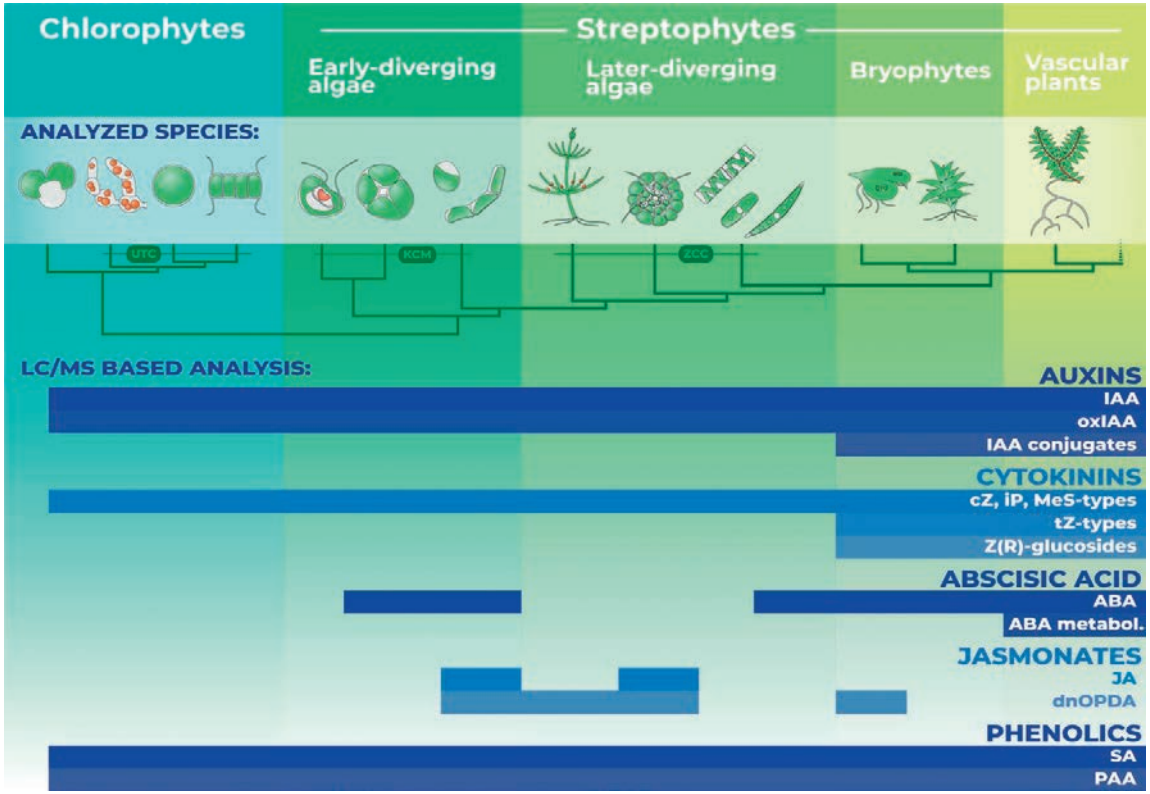

Figure 3: LC/MS-based analysis of plant hormones in the green lineage. Schematic depiction of individual representatives of chlorophyte and streptophyte algae and land plants with indicated presence of individual plant hormones as shown in Schmidt, Skokan *et al.* 2023.

whereas abscisic acid and jasmonates only occasionally. In collaboration with Ghent University, Belgium, we also showed that gaseous ethylene was released by most green algae in trace amounts, but in significantly higher quantities by all land plants. Land plants were likewise unique in their signs of auxin and cytokinin homeostasis and in the consistent detection of abscisic acid, indicating that these compounds only acquired phytohormone identity in ancestral land plants (**Fig. 3**). We discussed our results in the context of current plant hormonomics and phylogenomics.

Secondly, in collaboration with the Laboratory of Pollen Biology IEB CAS, we analysed microgametogenesis in four different *Nicotiana* species (*N. tabacum, N. alata, N. langsdorffii,* and *N. mutabilis*) and showed that they could even be species-specific (Záveská Drábková *et al.* 2021b). Thirdly, in collaboration with the Laboratory of Biologically Active Compounds IEB CAS, we determined a wide range of phytohormones during somatic embryogenesis of Norway spruce (*Picea abies*). Here, we identified a desiccation-specific composition of phytohormones that are responsible for the germination competence priming in both zygotic and somatic embryos (Eliášová *et al.* 2022). Finally, in a joint project with the Institute of Botany CAS, the role of auxins and cytokinins in determining the root-sprouting ability of plants was investigated. We showed that root sprouting ability is determined by phytohormonal regulation and that a low auxin/CK ratio is a critical factor leading to adventitious bud formation on roots and root sprouting ability (Martínková *et al.* 2023a, b).

RNA processing in plant hormonal pathways

RNA processing represents an important, highly intricate, and underexplored layer of gene expression in plants. In contrast, plant hormonal pathways have been relatively well elucidated. In the RNA Processing Group, we utilise the excellent lab expertise on auxin and abscisic acid-dependent processes to explore the relevance of key steps of the processing of mRNA, particularly alternative splicing (AS) and m^6A mRNA methylation. Overall, the physiological role and the actual impact of AS on gene expression are highly discussed. Along this line, we have demonstrated how AS impacts auxin transport by modifying a short internal amino-acid motif in the auxin carrier PIN7 (Kashkan *et al.* 2022a). This work brought a characterisation of one of the few well-evidenced plant AS events (Kashkan *et*

al. 2022b). This study also shed new light on the mechanistic understanding of PIN-mediated transport. We revealed that PIN7 carriers homo- and heterodimerise and proposed that the native constraints of their mobility within the plasma membrane are a prerequisite for auxin transport activity (Kashkan *et al.* 2022a).

The AS events that are evolutionarily conserved are likely physiologically relevant. For estimating the role of AS in hormonal pathways, we developed an algorithm that determines the evolutional conservation of given alternative proteins. As our machine learning-based pipeline also functions well on eukaryotic alternative proteins different from plants, we expanded our searches. Strikingly, we found that plant but not animal AS events show a high rate of evolutional parallelism in producing new (analogous) alternative proteins. As no such user-friendly tool has been released for both plant and animal communities, a web interface is available at https://catsnap.cesnet.cz/ to help researchers select physiologically relevant AS event for further characterisation (Timofeyenko *et al.* 2023).

m^{6}A is the most common covalent modification of mRNA. We have previously isolated mutants with dramatically reduced m^{6}A levels. Recently, we found that they display remarkable auxin-related defects and a strong resistance to exogenously applied auxin. As the phenotypes of these mutants are largely pleiotropic, we further point out the multilevel impact of m^{6}A on auxin-dependent processes. Moreover, we also propose the role of m^{6}A in the ground tissue formation in the primary root, likely orchestrated by the auxin signaling activity in the ground tissue founder cells (Zemlyanskaya *et al.* 2023).

Phytohormones in heat and cold stress signalling

We continued in our effort to decipher how cytokinins act in the signal transduction during plant reactions to heat and cold stress and which factors that interfere with their action during plant acclimation to stress conditions. In our approach, we correlated analytical phytohormone profiling with transcriptomics and several biochemical methods, mainly focused on the determination of photosynthetic parameters, membrane damage, and analysis of volatiles. In collaboration with the Laboratory of Plant Biotechnologies IEB CAS, we showed for the first time that cytokinins are supported in their beneficial effects on rice thermotolerance by volatile organic compounds (Přerostová *et al.* 2023). Exogenous cytokinin stimulated the emission of volatile organic compounds (VOC), especially 2,3-butanediol. The strongest thermo-protective effect of cytokinins was observed when their application was combined with heat acclimation, and this also led to the strongest stimulation of volatiles. Our work also pointed to inter-organ communication during the response of rice plants to heat stress conditions. The selective application of stress to either roots or shoots, the analysis of hormonome, the expression of selected heat shock response proteins and alternative oxidases, and the ascorbate peroxidase activity all collectively suggested that the highest protection could be seen in crowns and roots. In contrast, there was a weaker protection of leaves, which probably reflects the growth strategy of rice (Přerostová *et al.* 2022). We also significantly participated in a review article focusing on the diverse structure, subcellular localisation, and interactions of histidin receptor kinases, as well as their signalling functions during development and environmental responses across different plant species (Hoang *et al.* 2021).

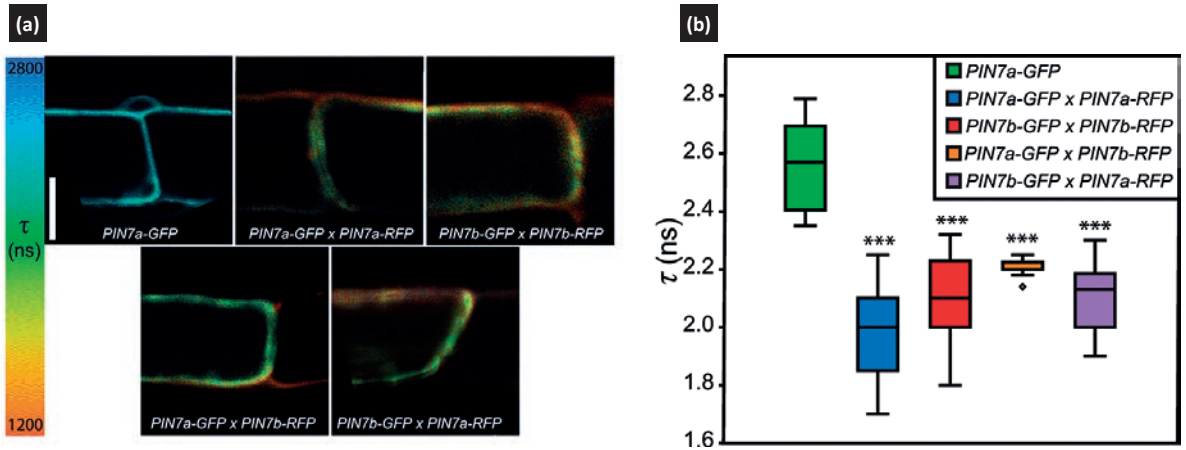

Figure 4: Lifetime imaging detection of the interaction between individual splicing variants of PIN7. PIN7a and PIN7b can form homo- and heteromers on PM *in vivo*. GFP fluorescence lifetime (s) was determined from the tagged PIN7a and PIN7b proteins and compared to that of PIN7a-GFP alone, presented as a representative image heat map **(a)** and quantified **(b)**. Proteins were expressed under control of the G10-90::XVE promoter in Arabidopsis root tips and induced with 5 µM b-estradiol. On the box plots, the middle line corresponds to the median, the box corresponds to the 25% and 75% quantiles, and the whiskers represent the minima and maxima. In (b), asterisks indicate a significant difference between the respective protein pair and the PIN7a-GFP control (*, P < 0.05; ***, P < 0.001 by one-way ANOVA). For each line, n ≥ 12. Bars: 10 µm. Modified from Kashkan *et al.* 2022a.

The response of plants to cold stress is strongly influenced by light, both its intensity and its quality. Plants have been found to adapt very dynamically to low light intensity, being able to effectively eliminate damage. However, low levels of photosynthesis will not allow them to increase the hardiness associated with intense dehydrin synthesis (Přerostová *et al.* 2021a). Both cryptochromes and phytochromes are involved in the cold response, with cryptochromes playing the most prominent role at optimal intensity and phytochrome A at low intensity. We have shown that cytokinins have a positive effect on plant cold tolerance and that this effect depends on light. In the dark, the effect of cytokinins is not apparent (Přerostová *et al.* 2021b).

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Research projects: 2, 4, 8, 10, 15, 18, 39, 40, 45, 49, 53, 78, 79, 86, 91, 97, 103, 113, 117

Laboratory of Integrative Structural Biology

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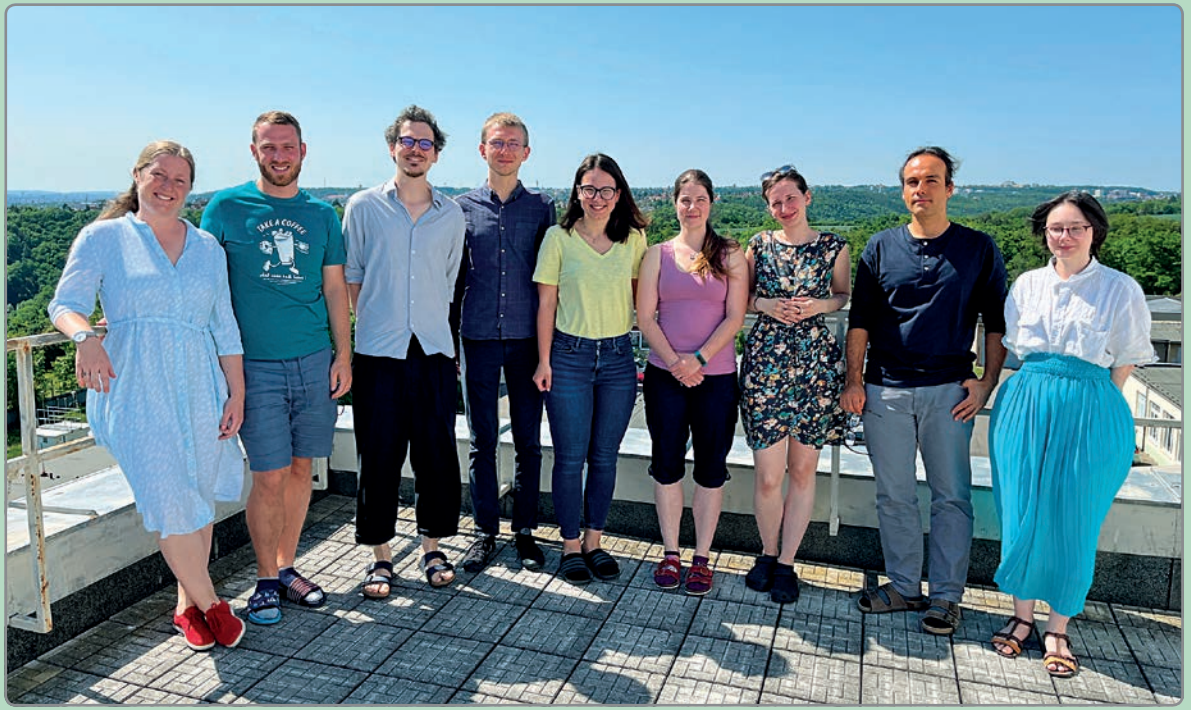

In the picture (from left to right):
Mgr. Petra Cifrová / research assistant, Mgr. Roman Hudeček / lab manager, research assistant, Roman Pleskot, Ph.D. / head of the laboratory, Mgr. David Ušák / Ph.D. student, Ing. Michaela Neubergerová / Ph.D. student, Ing. Šárka Mattauchová / Ph.D. student, Ing. Tereza Korec Podmanická / research assistant, Michal Daněk, Ph.D. / postdoc, MSc. Maria Voloshina / Ph.D. student.

Not pictured:
Daniela Kocourková, Ph.D. / research assistant.

The laboratory aims to understand how **the wiring of different biomolecules** drives the fundamental processes of the plant cell, such as cytokinesis, cell-wall material secretion, and nutrient uptake. We employ a **multidisciplinary strategy** combining experimental and computational approaches to achieve this goal. We utilise methods of molecular biology, biochemistry, and state-of-the-art microscopy. By combining the experimental techniques with the computational ones (structural bioinformatics, molecular dynamics) in an **integrative fashion**, we can obtain unprecedented details of a given process, leading to **novel biological implications**.

Delineation of the spatial and temporal arrangements of biological systems is essential in formulating hypotheses about their function and evolution. In integrative structural approaches, diverse information at different levels of description is synthesised to yield a common view of a biological system. The integrative approach builds a system representation by simultaneously combining information from various sources, both experimental (such as chemical cross-linking with mass spectrometry, protein co-immunoprecipitation, or yeast two-hybrid assays) and theoretical (physical theories, statistical analysis, or evolutionary analysis). When all available information about the modelled system is used, the resulting model's accuracy, precision, and completeness are maximised, thus significantly surpassing any of the single structural methods. Moreover, the integrative approaches provide unprecedented insight into the conformational dynamics of large biological assemblies.

Cell division

Without cell division, there would be no life on the planet Earth. Cell division is finalised by cytokinesis, which divides two daughter nuclei. The key element of plant cytokinesis is the cell plate, a transient, membranous compartment that grows between two daughter cells and develops into a new cell wall and plasma membrane. The cell plate, which is composed of lipids, proteins, and polysaccharides, predominantly callose,

is formed by the fusion of transport vesicles at the cell equator. We aim to take advantage of highly coordinated cell plate development and use it as a time axis by which to study the assembly of different protein complexes and their dynamic interplay with lipids and polysaccharides. This research interest is supported by Junior Star Project nr. 22-35680M from the Czech Science Foundation.

Endocytosis

Eukaryotic cells rely on endocytosis to regulate their plasma membrane proteome and lipidome. Apart from fungi and animals, most eukaryotic groups have retained the evolutionary ancient TSET complex as an endocytic regulator. In close collaboration with the Daniel Van Damme laboratory (PSB/UGent, Belgium), we revealed the molecular architecture of plant TSET [TPLATE complex (TPC)] using an integrative structural approach. We identified crucial roles for specific TPC subunits in complex assembly, and together with the Laboratory of Cell Biology, led by Martin Potocký (IEB), we described TPC-membrane interactions (Yperman *et al.* 2021a, 2021b). We identified how TPC recognises and interacts with the cargo proteins (Yperman *et al.* 2021b, Grones *et al.* 2022, Wang *et al.* 2023). Our work provided new implications for the evolution of membrane trafficking in eukaryotes. Furthermore, the knowledge of the TPC structure enabled the generation of a new conditional tool to interfere with endocytosis in plant cells (Wang *et al.* 2021).

Callose synthase

Polar callose deposition into the extracellular matrix is tightly controlled in time and space. Its presence in the cell wall modifies the properties of the surrounding area, which is fundamental for the correct execution of numerous processes such as cell division, male gametophyte development, intercellular transport, and responses to biotic and abiotic stresses. The extracellular deposition of callose in plants depends on callose synthase (CalS), a large 200-kDa integral membrane protein. We provided a robust phylogenetic analysis of CalS across the plant kingdom, which implies a 3-subfamily distribution of CalS and has implications for the function of individual CalS homologs (Ušák *et al.* 2023).

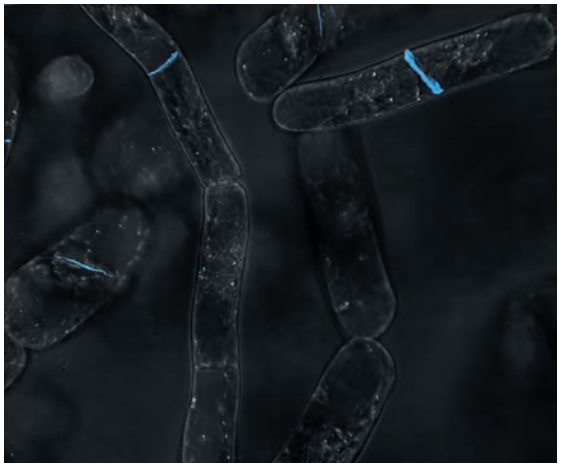

Figure 2. Dividing tobacco cells. Cell plates (cyan) are labelled by callose staining using aniline blue dye.

Protein-membrane interfaces

Anionic phospholipids (phosphatidic acid, phosphatidylserine, phosphatidylinositol, and its phosphorylated derivatives, phosphoinositides) are essential regulators of many cellular processes in plants, including signal-

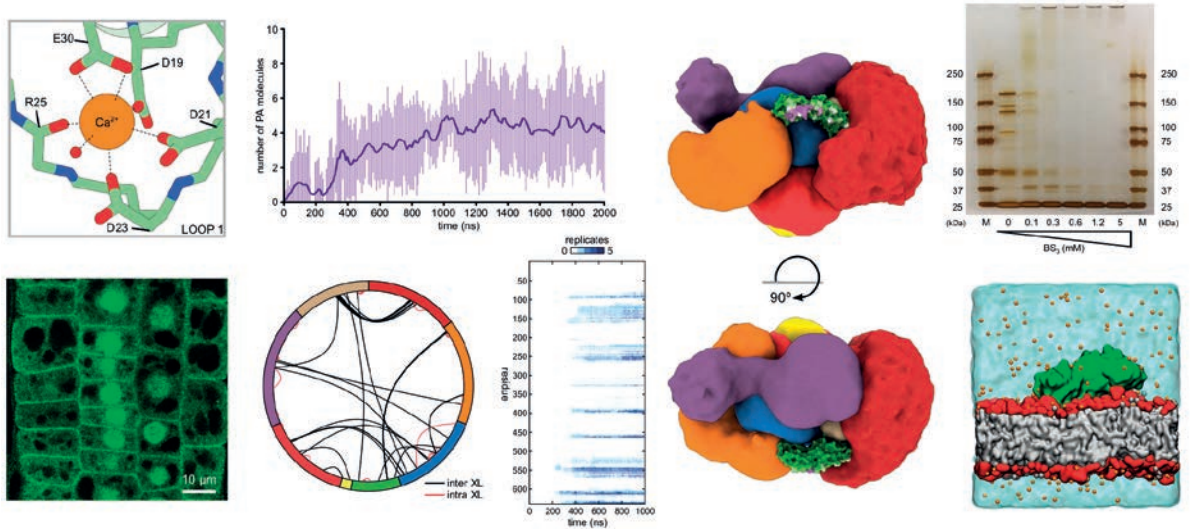

Figure 1. Graphical illustration of the research summary.

ling, vesicle trafficking, and cell growth and division. They can modulate the physical properties of membranes, establish cell polarity, act as signalling molecules, and mediate interactions with peripheral membrane proteins. By combining methods of molecular biology, state-of-the-art microscopy with structural modelling, and molecular dynamics simulations, our laboratory, together with colleagues from various research institutions (Martin Potocký – IEB, Daniel Van Damme – PSB/UGent, Yvon Jaillais – CNRS, Till Ischebeck – University of Münster), has described the membrane recruitment of different proteins and protein complexes, such as diacylglycerol kinase, LIPID DROPLET PLASMA MEMBRANE ADAPTOR protein, subunits of the TPLATE complex, the PI4 kinase complex, and the EXOCYST complex (Yperman *et al.* 2021b, Yperman *et al.* 2021a, Synek *et al.* 2021, Noack *et al.* 2022, Krawczyk *et al.* 2022, Scholz *et al.* 2022). Collectively, these results have uncovered mechanistic details of the specific protein-membrane interactions, leading to a better understanding of protein-membrane interfaces in general.

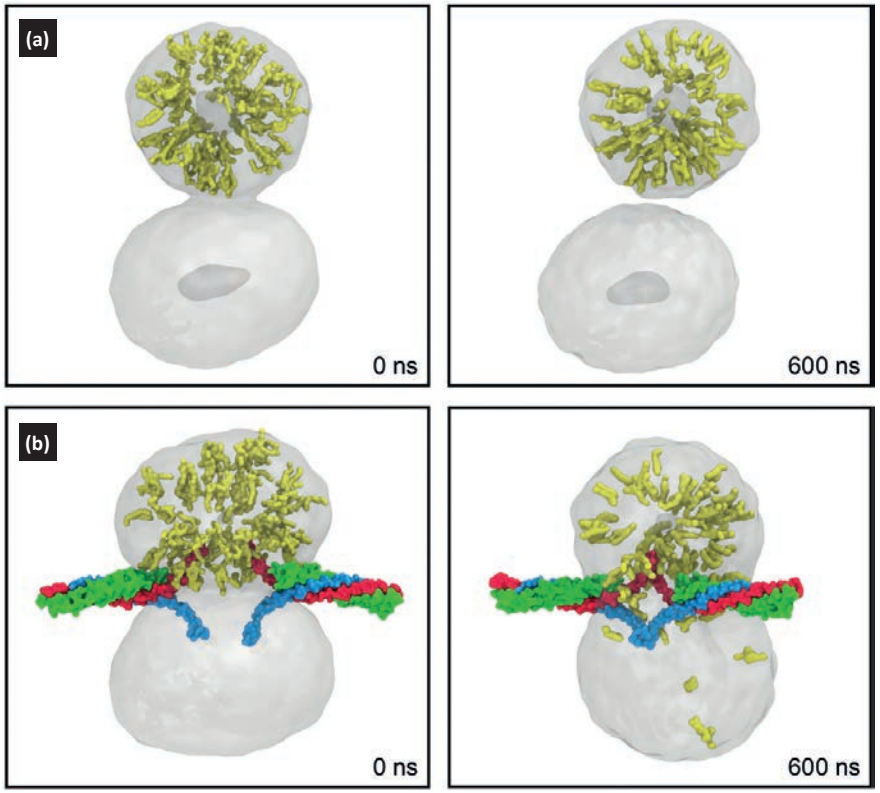

Figure 3. The plant SNARE complex-mediated vesicle fusion studied by molecular dynamics simulations. (**a**) Lipid vesicles do not fuse in the control simulations (no SNARE complex). (**b**) The plant SNARE complex mediates vesicle fusion marked by the movement of phosphatidylserine, depicted in yellow.

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Publications: 259, 276, 292, 308, 341, 360, 463, 508, 515
Research projects: 8, 87

Laboratory of Pathological Plant Physiology

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Our laboratory focuses on important aspects of plant-microbe interactions underlying host resistance to pathogens. The main interest of the laboratory is hormonal and phospholipid signalling pathways implicated in plant defence responses. In addition to plant pathogens, the studied pathosystems have been extended to herbivores and molecular mechanisms involved in tri-trophic interactions.
Our recent research has been devoted to: (1) The role of phospholipid signalling pathways in plant immunity and hormone balance regulation; (2) alternative methods of plant protection: the search for novel bio-based resistance inducers, the application of bacteriophages to treat plant bacterioses, exploring the role of soil microbiome in plant defences; (3) the fungal phytopathogen *Leptosphaeria maculans* – mechanisms of pathogenicity and the mapping of avirulence genes distribution; and (4) three-way interactions between plant, pathogen, and herbivores.

Molecular basis of plant immunity

We are particularly interested in the role of phospholipid signalling pathways in plant defence and hormone balance regulation. Salicylic acid (SA) is a phytohormone regulating many physiological processes and is one of the most important molecules regulating a plant's reaction to infection. We study the connection between the SA biosynthesis/signalling and other plant signalling systems, as well as the impact of abiotic factors on immunity (cold stress, elevated temperature, and salinity).

In exploring the cross-talk between stress-associated signalling pathways, we found that the action of stress-related phytohormones (SA, ABA or brassinosteroids, or microbial elicitors, e.g. chitin) results in the production of lipid-derived secondary messengers, like phosphatidic acid (Kretinin *et al.* 2021). We discovered that in the case of the elicitor flagellin, this is a result of the activity of diacylglycerolkinase 5 (DGK5). Moreover, functional DGK5 is necessary for the activation of a particular defence-related gene cluster and the establishment of PAMP-triggered immunity and overall resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (**Fig. 1**, Kalachova *et al.* 2022b).

In the picture (from left to right):
Ing. Barbora Jindřichová, Ph.D. / researcher, Mag. Ing. Agr. Nikoleta Rubil / Ph.D. student, MSc. Anzhela Antonova / technician, Doc. Ing. Lenka Burketová, CSc. / head of the laboratory, Mgr. Hana Leontovyčová, Ph.D. / postdoctoral fellow, MSc. Tetiana Kalachova, Ph.D. / researcher, Mgr. Romana Pospíchalová / technician.

Not pictured:
MSc. Marzieh Mohri / Ph.D. student, Ing. Vladimír Šašek, Ph.D. / researcher (currently farmer), Bc. David Řejha, Bc. Veronika Brožková, Bc. Oleksandra Bondarenko / MSc. students, Anastasiia Zhivaeva, Jakub Jančík, Bc. Gabriela Růžičková, Berenika Vojtěchová, Yuliia Omelchenko / BSc. students.

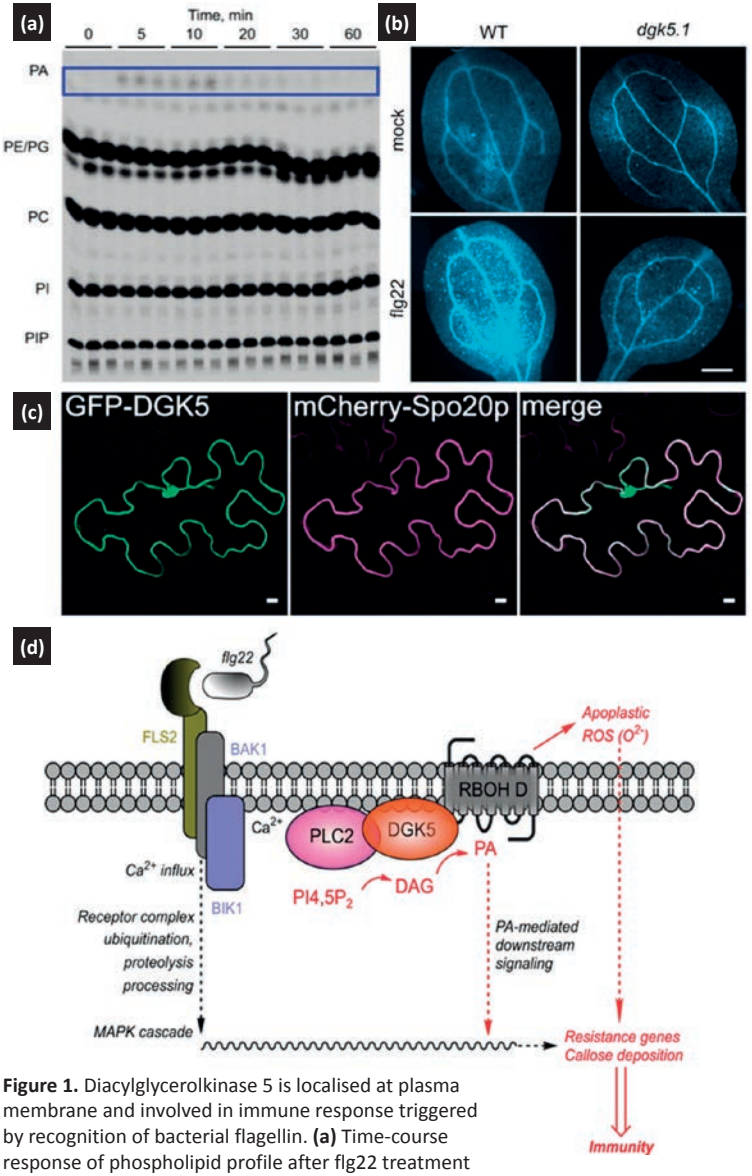

Figure 1. Diacylglycerolkinase 5 is localised at plasma membrane and involved in immune response triggered by recognition of bacterial flagellin. **(a)** Time-course response of phospholipid profile after flg22 treatment of *Arabidopsis thaliana* suspension cells, 33P radioactivity associated with phosphatidic acid (PA, blue line), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylinositol (PI) and its phosphate (PIP). Accumulation of PA occurs at 5 min and peaks at 10 min. **(b)** Deposition of defence-related polysaccharide callose in *A. thaliana* cotyledons after flg22 treatment is impaired in *dgk5.1* mutant. **(c)** Plasma membrane co-localisation of GFP-tagged DGK5 and its product, PA (visualised by fluorescent PA sensor mCherry-Spo20p) in *Nicotiana benthamiana* epidermis cells. **(d)** Updated model of signalling pathway triggered by bacterial flagellin including lipid signalling and DGK5.

Our previous research showed that some of the key players in lipid signalling and endomembrane trafficking, phosphatidylinositol-4-kinases β1 and β2 (PI4Kβ1/β2) enzymes, are negative regulators of SA biosynthesis. We then went deeper, characterising the particular transcriptomic and proteomic changes in *pi4kβ1β2* mutant plants (Junková *et al.* 2021). Interestingly, PI4Kβ1/β2 enzymes have distinct roles in the morphogenesis of shoots and roots, and the effect in roots is independent of SA, but relies on the altered metabolism of auxins (Starodubtseva *et al.* 2022).

Alternative methods of plant protection

With the European programs directed to the reduction of pesticide use, we are focused on the possibility of relying on the natural environment in mitigating plant-pathogen interactions.

Novel resistance inducers from natural sources

Our research has turned to the practical side of this topic, as we explore biologically active compounds derived from natural sources, such as plant, bacterial, yeast, and fungal extracts. We have recently found efficient compounds among the extracts from the algae *Ulva lactuca* (Přerovská *et al.* 2022) and suggested their mode of action based on the activation of distinct phytohormonal pathways (**Fig. 2**).

Exploring the potential of bacteriophages

Bacteriophages, viruses of bacteria, are an effective alternative to antibiotics that are just finding their way into agriculture (Korniienko *et al.* 2022b). We isolated and fully characterised two novel bacteriophages from the natural environment, Pseudomonas phage Eir4 and Eisa9, which are efficient against bacterial infections caused by *Pseudomonas and Xanthomonas* spp. in plants (Korniienko *et al.* 2022a).

Improving resistance via interactions with soil microbiome

We performed a model experiment with iterative selection of soil microbiome upon exposure to the *Arabidopsis-Pseudomonas* pathosystem. After ten iterations (each lasting for a month of plant cultivation and a week of infection), we succeeded in establishing a microbial community that helps to protect the next cultivated plants from bacterial infection (Kalachova *et al.* 2022a).

Noble metal nanoparticles as environmental contaminants

Nanoparticles of noble metals have recently become widely used. Despite their undisputed advantages in the possibility of engineering their novel properties, safety

questions arise concerning their wide exploitation. Nanoparticles interact with living organisms, which can interfere with essential life processes (Burketová *et al.* 2022). We explore the impact of noble nanoparticles and their ions on plants, plant-pathogen interactions, and soil microbiome (Macůrková *et al.* 2021, Maryška *et al.* 2023).

Three-way interactions among plant, pathogen, and insect

Research on the interconnection between plant defence systems activated during a concurrent attack by pathogens and herbivores was introduced in the laboratory relatively recently. Signalling pathways and defence mechanisms are investigated using the model plant *Arabidopsis thaliana* and the economically important crop oilseed rape, infected by the bacteria *Pseudomonas syringae* and the fungus *L. maculans*, respectively, and infested with the chewing herbivore *Plutella xylostella* (**Fig. 3**) and the sucking non-specialist *Myzus persicae* or specialist *Brevicoryne brassicae* (**Fig. 4**). To precisely map the activation of distinct branches of phytohormonal signalling and defence responses, we use genetically encoded fluorescent biosensors, histochemistry, and qRT-PCR. We have found a rapid induction of salicylic and jasmonic acid signalling markers in cells surrounding a stylet puncture, co-localising with callose deposition. Our research brings detailed insight into the spatiotemporal complexity of plant defence activation during a specialist aphid attack. (Rubil *et al.* 2022).

Collaboration with other institutions and applied research

The laboratory collaborates with several institutions within the Czech Republic. Phospholipid signalling in biotic stress is investigated within a well-established collaboration with Prof. Valentová's laboratory at the University of Chemistry and Technology, Prague. Students of this university, as well as of the Faculty of Science of Charles University, work in our laboratory on their bachelor's, master's, and Ph.D. theses. A new collaboration has been initiated with the University of South Bohemia in České Budějovice (laboratory of Dr. Martin Janda) and Masaryk University in Brno (laboratory of Dr. Markéta Šámalová). Collaboration with institutions involved in applied research concerns the project funded by the Ministry of Agriculture aimed at monitoring avirulence genes in *L. maculans* populations in Czech oilseed rape cultivation areas. *L. maculans* isolates are collected and identified at the Institute of Oilseed Crops (OSEVA Pro. Ltd.), OSEVA Development and Research Ltd., and the University of Life Sciences, Prague.

International collaboration

International collaboration is of great importance

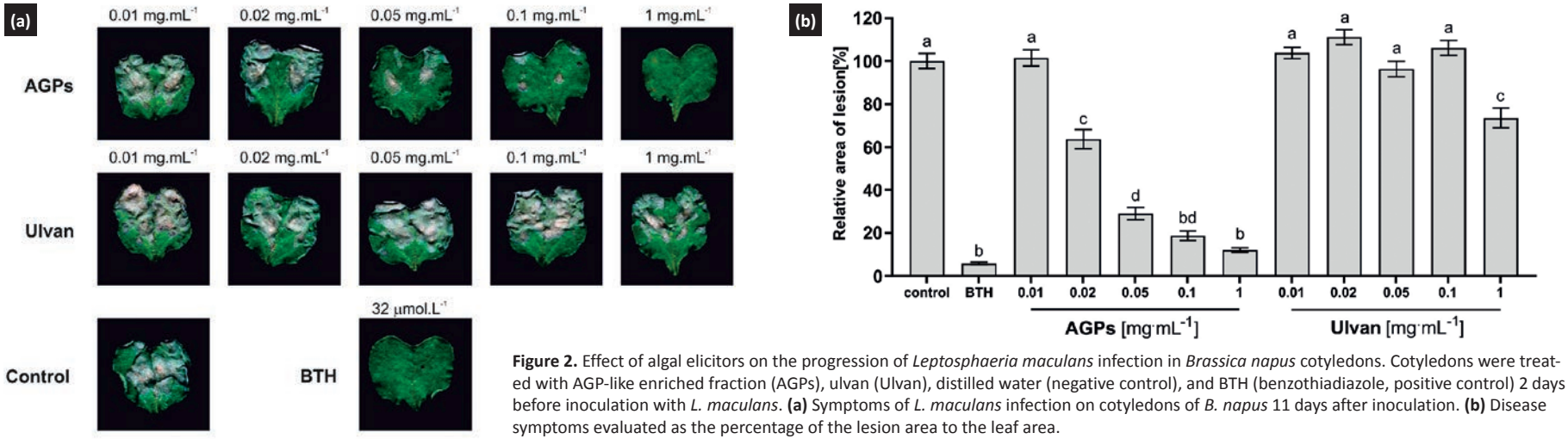

Figure 2. Effect of algal elicitors on the progression of *Leptosphaeria maculans* infection in *Brassica napus* cotyledons. Cotyledons were treated with AGP-like enriched fraction (AGPs), ulvan (Ulvan), distilled water (negative control), and BTH (benzothiadiazole, positive control) 2 days before inoculation with *L. maculans*. **(a)** Symptoms of *L. maculans* infection on cotyledons of *B. napus* 11 days after inoculation. **(b)** Disease symptoms evaluated as the percentage of the lesion area to the leaf area.

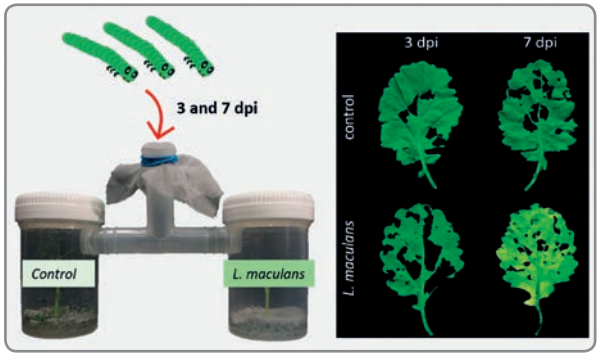

Figure 3. Feeding choice test performed in the vial system with one non-inoculated leaf inside the left vial and one *Leptosphaeria maculans*-inoculated leaf inside the right vial; three *Plutella xylostella* caterpillars are placed in the T-tube with a fine brush 3 and 7 days post inoculation (dpi) and are given the opportunity to feed on both leaves; leaves are then scanned after 48 h of caterpillar feeding and the percentage of leaf damage is evaluated.

for the laboratory. Research on phospholipid signalling has been proceeding in close collaboration with Prof. Eric Ruelland (Université de Technologie de Compiègne, France) and Dr. Jean-Luc Cacas (Institute of Jean Pierre Bourgin, INRAE, France). The role of intercellular communication in induced resistance is being investigated in a newly established collaboration with Dr. Christine Faulkner (John Innes Centre, UK). Novel resistance inducers are being studied in collaboration with Dr. Eric Nguema-Ona (Agro Innovation International TIMAC AGRO, France). Microbiome studies are ongoing through cooperation with Dr. Ruben Puga-Freitas (Université Paris Est Creteil, France). Collaboration on the study of herbivores is underway with Prof. Thure Hauser (University of Copenhagen, Denmark). Research on bacteriophages is being performed together with Dr. Alla Kharina and Prof. Iryna Budzanivska (Taras Schevchenko National University of Kyiv, Ukraine) and Nikita Zrelovs (Latvian Centre of Biomedical Research). The work involving *L. maculans* is being carried out in collaboration with the laboratory of Prof. Thierry Rouxel (INRAE, Centre de recherché de Versailles-Grignon, France), namely with Dr. Isabelle Fudal.

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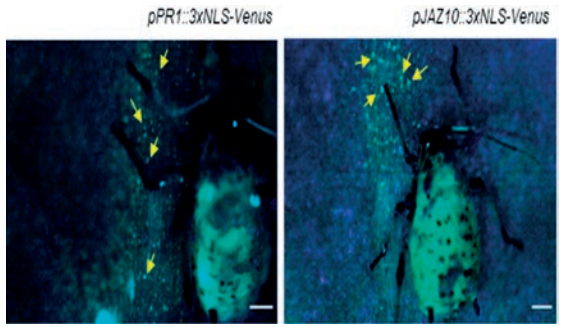

Figure 4. Local activation of *PR1* and *JAZ10* transcription in *Arabidopsis thaliana* veins upon infestation around the feeding sites of *Brevicoryne brassicae*, 48 hours post inoculation (hpi). Nuclei with induced gene expression are marked with yellow arrows.

Publications: 81, 96, 109, 161, 183, 234, 276, 290–291, 329, 336, 350, 427, 450, 458, 509

Research projects: 6–8, 10, 21, 105, 117

Laboratory of Plant Biotechnologies

Head of the laboratory:

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From 2020 to 2023, our laboratory focused on a research program centred on plant-xenobiotic interactions, the identification of secondary metabolites in plants, and the synthesis of their analogues. Throughout this period, we published 28 papers in international journals with significant impact factors and achieved practical results. The research was financially supported by 11 grant projects from various grant agencies, and it encompassed the following key areas:

1. Fate of engineered nanoparticles in plants: We investigated the fate of engineered nanoparticles in plants and their impact on plant metabolism.
2. Synthesis of analogues of biologically active plant metabolites: Special emphasis was given to strigolactones and anti-inflammatory compounds.
3. Volatile research: Plants release volatile organic compounds (VOCs) as a means of warning other plants of impending danger. We explored the communication and defense mechanisms triggered by VOCs.
4. Lipidomics: The study of the role of lipids in plant cell membranes, energy storage, and defence reactions, particularly during stress responses.
5. Phytoremediation for environmental protection: Our extensive knowledge of plant use in environmental protection was applied from the laboratory scale to semi-real and real conditions, resolving environmental contamination issues.

In the picture (from left to right):
Upper row: RNDr. Mgr. Petr Soudek, Ph.D. / head of the laboratory, RNDr. Mgr. Tomáš Vaněk, Ph.D. / researcher, Mgr. Marcela Dvořáková, Ph.D. / researcher, Mgr. Daniel Haisel, Ph.D. / researcher, Ing. Přemysl Landa, Ph.D. / researcher, Ing. Lenka Langhansová, Ph.D. / researcher, Mgr. Petr Maršík, Ph.D. / researcher.
Lower row: Ing. Kateřina Moťková / technician, Antonio Pavičić / Ph.D. student, Ing. Šárka Petrová, Ph.D. / researcher, Mgr. Radka Podlipná, Ph.D. / researcher, Ing. Jan Rezek, Ph.D. / researcher, Prof. Tetiana Satarova, D.Sc. / researcher, Ing. Miroslav Šíša, Ph.D. / researcher.

Selected insights on plants and the environment

Our research delved deeply into evaluating the environmental risks posed by emerging contaminants, encompassing pharmaceuticals, veterinary drugs, and engineered nanoparticles. By unraveling the complex fate of these contaminants within plants, we contributed to the understanding of their ecotoxicological consequences, especially in the context of their potential incorporation into the human food chain. The study of the effects of stress on plant lipids and volatile signaling was supported within the frame of the European Regional Development Fund-Project "Centre for Experimental Plant Biology."

Pharmaceuticals

Our investigations focused on monitoring the pathways of xenobiotics in plants, especially the risk of pharmaceuticals entering the environment and infiltrating the food chain. Notably, we conducted experiments involving clover grown in soil enriched with anthelmintics, to characterise their absorption, metabolism, and translocation. We also explored the uptake of anthelmintic fenbendazole in soybean, uncovering potential ramifications for plant yield and food safety (Langhansová *et al.* 2021, Podlipná *et al.* 2021, Sochacki *et al.* 2021).

Pesticides and fungicides

Our research highlighted the negative role of pesticides in agriculture and emphasised the need for riparian buffer zones in order to mitigate water body contamination. We investigated the effects of specific pesticides on poplar and hybrid aspen, revealing intriguing insights into their upward and downward transfer within plants. We also studied the fate of azole fungicides in constructed wetlands with plants (Hanková *et al.* 2023, Maršík *et al.* 2021).

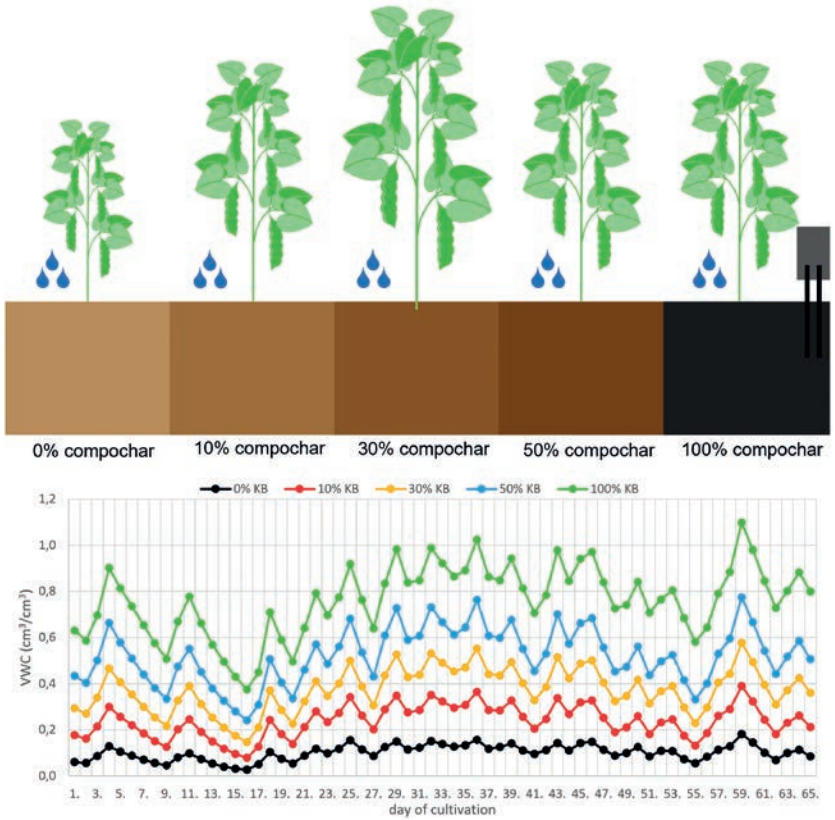

Figure 1. Soil moisture changes when common beans grow with different additions of compochar.

Plant toxicity stress

The possible toxic impact of borate additives was investigated. Zinc borate was found to be toxic to plants, affecting frond growth and causing damage to the photosynthetic apparatus. In another study, we analysed thorium interactions with phosphorus and the impact on *Arabidopsis* plants with RNA-seq profiling. Thorium reduced phosphorus availability, leading to a phytotoxic effect, while nano-hydroxyapatite reduced Th toxicity but caused a deficiency in essential metals. Our research also focused on bio-indicator studies. Our inquiry into wood/chip wastewater unveiled its potential toxicity to aquatic organisms, contingent upon the wood species (Petrová and Soudek 2022, Sackey *et al.* 2021, Landa *et al.* 2024).

Volatile plant signaling

The dynamics of stress signaling in rice plants through volatile organic compounds (VOCs) emerged as a captivating field of study. We elucidated how cytokinins contribute to enhanced thermotolerance in rice plants under acclimation and heat stress conditions, potentially revolutionising strategies for bolstering plant resilience to high temperatures (Přerostová *et al.* 2023).

Nanoparticles

Our international collaborations extended to unraveling the uptake and fate of gold nanoparticles within potatoes and vegetables. We found that plants are able to modify the nanoparticles they take up by changing their size. We also investigated the potential benefits of nanoparticles for plant growth, particularly root elongation under cadmium stress (Landa *et al.* 2023, Malejko *et al.* 2021).

Plant metabolites and analogues

Our research focused on the identification of new anti-inflammatory agents, specifically COX-1 and COX-2 inhibitors. We tested the effectiveness of fractions isolated from different *Pleurotus* spp. against the activity of COXs. Furthermore, we designed, synthesised, and tested compounds with potential selectivity for the COX-1 isoform. The activity of COX-1 plays a role in some types of cancer and in cardiovascular events. Our quinazoline derivatives, as well as mimetics of known COX-1 inhibitors prepared in our laboratory, showed excellent inhibitory activity and COX-1 selectivity.

N-alkylaminoferrocene-bile acid conjugates-based prodrugs displayed strong anticancer bioactivity with high specificity. These prodrugs are accumulated in the endoplasmic reticulum, causing the oxidative stress leading to cancer cell death

(Šíša *et al.* 2023, Šťastný *et al.* 2022, Twilley *et al.* 2022, Dvořáková *et al.* 2021, Xu *et al.* 2021).

Plant breeding

Molecular genetic passports were developed for perspective maize inbreds to determine their affinity and heterotic potential, allowing for reference control in registration, certification, and intellectual property defence. Inbreds of the Iodent and BSSS germplasms showed unique allelic states of specific SNP-markers and high heterotic potential in the Iodent×BSSS breeding model (Satarova *et al.* 2023).

Research for practice

Our laboratory extended its international cooperation and participated in two COST Actions, collaborating with scientists from other European countries. Additionally, we engaged in projects related to rainwater purification, agrochemical cleaning in constructed wetlands, and the identification of surviving individuals of forest tree species in calamity areas.

Potential fern utilisation

Our research on European ferns shows that ferns are a rich source of antioxidants, essential fatty acids, and bioactive compounds that can enrich the human diet. Although some species contain toxic compounds, most are considered safe for consumption; conversely, our research has demonstrated a potential hepatoprotective effect. Furthermore, our investigation of anthelmintic activity has shown the potential of fern extracts in combating parasitic nematodes (even drug-resistant strains of nematodes), offering a sustainable solution for livestock health (Dvořáková *et al.* 2021, Langhansová *et al.* 2021, Pavičić *et al.* 2023).

Figure 2. Pilot scale of constructed wetland for veterinary drug removal.

Biochar for drought prevention

Our profound inquiry into biochar's impact on soil properties and crop yield under severe drought stress revealed its potential to mitigate adverse effects and enhance yields, particularly with specific compochar amendments. Additionally, our foray into using biochar for soil remediation exhibited promising possibilities for tackling metal contamination (Lebrun *et al.* 2022, Soudek *et al.* 2024, Mocová *et al.* 2022).

International collaboration

Our laboratory continued international cooperation with researchers from Israel, the USA, China, South Africa, Ukraine, and other countries. We participated in two COST Actions, providing opportunities for collaboration with scientists from various European countries. Our team also actively contributed to research organisations and served on the editorial boards of several scientific journals.

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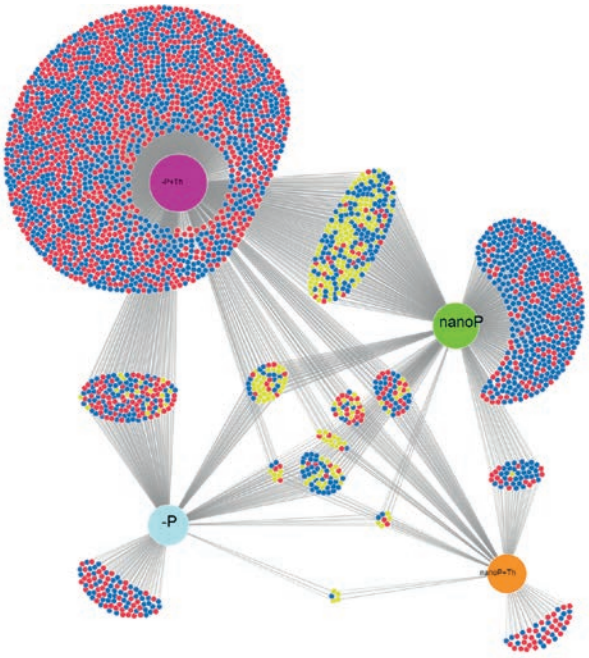

Figure 3. Up- and down-regulated genes in N8508 *Arabidopsis thaliana* mutant plants. Overlaps between the treatments.

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Publications: 29–30, 101–103, 114, 118, 122, 127, 150, 168, 181, 203, 245, 296, 301, 322, 351, 359, 391, 419, 436, 474, 480, 490, 492, 501, 509, 512
Research projects: 19, 28–29, 96, 107, 111–112, 117

Laboratory of Plant Reproduction

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The Laboratory of Plant Reproduction, established in 2007, investigates two main research topics: the regulation of flowering in the Chenopodiaceae family and mitochondrial-nuclear interactions, namely cytoplasmic male sterility (CMS) in *Silene* (campion). It also conducts collaborative investigations in the field of mycorrhiza. The lab uses advanced methods like RNAseq, PacBio, and ONT sequencing in combination with classical Mendelian genetics (controlled crosses).
The main focus of the past three years was the analysis of floral induction and development in *Chenopodium ficifolium*, a close relative of the traditional crop of the Andes – *Chenopodium quinoa*. Because of the acceleration of global warming and aridification, *C. quinoa* is becoming a promising crop owing to its tolerance to salinity and drought. To move the cultivation of *C. quinoa* to higher latitudes, new cultivars responding appropriately to seasonal changes will need to be bred. *C. quinoa* is a tetraploid species with many genes participating in the regulation of flowering. There are 11 genes homologous to the essential integrator of flowering *FLOWERING LOCUS T* (*FT;* Štorchová 2021). The study of flowering is therefore much easier in the diploid *C. ficifolium* because it contains only half of the genes.

FTL genes in *Chenopodium*

The comprehensive transcriptomic and hormonomic study of the floral induction in the seedlings of the short-day ecotype *C. ficifolium* 459 revealed the high elevation of the contents of abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) under long days, caused by long photoperiods (18 h). However, unlike some other species (e.g., Lemnaceae), the high concentrations of the stress-related phytohormones did not promote flowering (Gutierrez-Larruscain *et al.* 2022a, 2022b). The floral induction was accompanied by the dramatic upregulation of the floral promoter

In the picture (from left to right):
Ing. Oushadee A. J. Abeywardana, Ph.D. / postdoc, RNDr. Helena Štorchová, CSc., Dsc. / head of the laboratory, Manuela Krüger, Ph.D. / postdoc.

Not pictured:
MSc. Niluka Wickramasinghe / research assistant.

FLOWERING LOCUS T LIKE 1 (FTL1). As *C. ficifolium* is recalcitrant to transformation, we provided evidence of the *FTL1* function by way of the heterologous transformation of *Arabidopsis thaliana* with the *FTL* genes of *C. ficifolium*. The overexpression of *FTL1* promoted flowering in *A. thaliana* ft- mutants (Abeyawardana *et al.* 2023), similar to the effect of other angiosperm *FT* genes.

C. ficifolium contains another *FT* homolog, *FTL2-1*. Its transcription was very low in the course of the floral induction in *C. ficifolium*. However, the transformation of *A. thaliana* with *FTL2-1* from *C. ficifolium* and *C. quinoa* led to a surprising result – the *35S::FTL2-1* expression was lethal. When the *FTL2-1* gene was inserted in the inducible cassette and expressed early in plant development, it caused immediate flowering at the cotyledon stage (Abeyawardana *et al.* 2023); see **Fig. 1.** The *FTL2-1* genes of *C. ficifolium* and *C. quinoa* behave as extremely powerful floral activators, which opens up new possibilities in the research of flowering.

To overcome the constraints given by the absence of transformation protocols in *C. ficifolium* and *C. rubrum*, the Virus-induced gene silencing (VIGS) method was

optimized. The marker gene *PHYTOENE DESATURASE (PDS)* was silenced (**Fig. 2**), which enabled to conduct similar experiments with flowering-related genes and to confirm their function.

The genomics of *Silene vulgaris* and its relatives

The mitochondrial genome (mitogenome) of *Silene vulgaris* is extremely variable at the species level, owing to the repeats mediating frequent intramolecular recombinations, which rearrange the genome and generate chimeric genes. Some chimeric genes interfere with mitochondrial functions, which leads to pollen abortion and CMS. We developed a bioinformatic pipeline to assemble the highly complex plant mitogenomes of close relatives of *S. vulgaris*. The hermaphroditic species *Silene fabaria* has a shorter mitogenome with few repeats, unlike gynodioecious *Silene uniflora*, which suggests a correlation between the mode of reproduction and the mitogenome structure. The mitogenomes are shared between *S. uniflora* and *S. vulgaris*, with some *S. uniflora* populations carrying a nearly identical mitogenome (but with a distinct CMS gene) to *S. vulgaris*. We are now finalising the complete, high-quality nuclear genomic sequence of *S. vulgaris* (in collaboration with Hana Šimková of IEB CAS and Peter Fields and Douglas E. Taylor of the University of Virginia, USA; see Moraga *et al.* 2023) to enable the identification of fertility restorer genes for the specific CMS types by means of Genome-Wide Association Studies (GWAS).

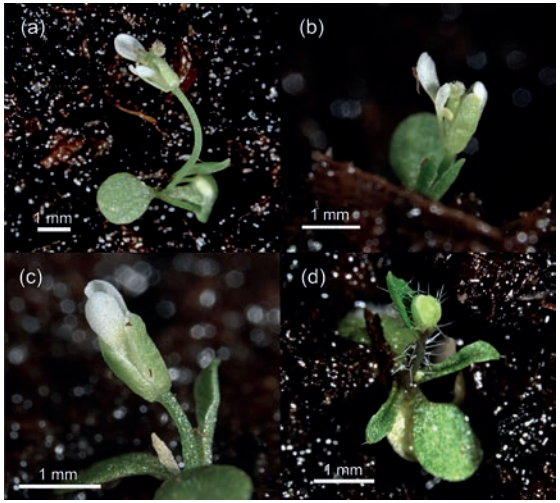

Figure 1. *Arabidopsis thaliana* transformed with the *CfFTL2-1* gene flowers immediately after germination (Abeyawardana *et al.* 2023).

Genomics of fungi

The bioinformatic approaches used while working on the *Silene* and *Chenopodium* projects were also applied to the genomics of mycorrhizal fungi, namely *Geosiphon pyriformis*, and to their identification.

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Figure 2. *Chenopodium quinoa* infected by the modified Apple Latent Spherical Virus ALSV carrying a fragment of the *PDS* gene in antisense orientation. *PDS* silencing decreases chlorophyll synthesis. Yellow chlorotic spots are spread along the veins in leaves.

Publications: 52, 108, 113, 124, 261–262, 379, 485, 509
Research projects: 8, 10, 35, 92

Laboratory of Pollen Biology

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The laboratory has continuously dedicated its activities to the research of plant reproductive development, sexual reproduction in plants, and genome stability. We have traditionally employed integrated omics to study regulatory levels of gene expression active during reproductive development. 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).
Our results also contributed to the establishment of the current paradigm in male gametophyte research with regard to asking and answering specific gene-oriented questions. Moreover, novel strategies for manipulating the gametophyte development and function are of current interest in agriculture and breeding in a rapidly ever-changing climate. This is related to the introduction of new model species like *Physcomitrium, Amborella, Eschscholzia*, and tomato. Finally, we are continuously extending our activities and our research towards various aspects of pollen communication with female tissues, male-female crosstalk, and embryo development.

In the picture (from left to right):
Standing front row: Vinod Kumar, MSc. / Ph.D. student, Ing. Jana Kůrková / research assistant, RNDr. Lenka Steinbachová, Ph.D. / researcher, Elnura Torutaeva, MSc. / Ph.D. student, prof. RNDr. David Honys, Ph.D. / head of the laboratory, Said Hafidh, Ph.D. / researcher, deputy head of the laboratory, Mgr. Alena Náprstková / Ph.D. student, Mgr. Anna Popelářová / Ph.D. student, Palash Chandra Mondol, Ph.D. / postdoctoral fellow, Mgr. Karel Raabe / Ph.D. student, Mariana Limones Mendez, Ph.D. / postdoctoral fellow.
Standing back row: Janto Pieters, MSc. / Ph.D. student, RNDr. Jan Fíla, Ph.D. / postdoctoral fellow.
Kneeling: Zahra Aghcheh Kahrízí, MSc. / Ph.D. student, Mgr. Božena Klodová / Ph.D. student.

Not pictured:
RNDr. Karel J. Angelis, CSc., RNDr. Lenka Záveská Drábková, Ph.D. / researchers, Mgr. Marcela Holá, Ph.D., Daniela Impe, Ph.D., Christos Michailidis, Ph.D., Anna J. Wiese, Ph.D. / postdoctoral fellows, Ing. Radka Vágnerová / Ph.D. student, Ing. Klára Čermáková, Ing. Jana Feciková, RNDr. Zuzana Gadiou, Ph.D., Ing. Iveta Jelínková, Petra Rožnovská / research assistants, Bc. Peter Darivčak, Marek Földi, Bc. Veronika Jirásková, Mgr. Helena Kočová, Mgr. Katarína Kulichová, Mgr. Oliver Pitoňak, Bc. Petr Šesták / students.

Reproductive development under standard/ normal and stress conditions

Regulation of reproductive development

Male gametophyte development leading to the formation of a mature pollen is precisely controlled at various levels, including transcriptional, post-transcriptional, and post-translational. Environmental conditions play an important role in the modulation of reproductive fitness. As members of a large international consortium, we generated the first gene expression atlases for various reproductive organs and gametes from ten plant species (Julca *et al.* 2021) comprising model plants and important agricultural crops (**Fig. 1**). A comparative analysis of the atlases identified hundreds of organ- and gamete-specific orthogroups and revealed that most of the specific transcriptomes

are significantly conserved. Interestingly, our results suggested that the co-option of existing genes is the predominant mechanism for evolving new organs. In contrast to female gametes, male gametes showed a high number and conservation of specific genes, which indicates that male reproduction is highly specialised (Julca *et al.* 2021).

We used the *Arabidopsis thaliana* datasets to extend, refine, and substantially update our previous studies (Honys and Twell 2003, 2004) of global gene expression dynamics in developing pollen for two *Arabidopsis* accessions (Klodová *et al.* 2023). Despite the superiority of RNA-seq over microarray-based platforms, we demonstrated high reproducibility and comparability. We identified thousands of long non-coding RNAs as potential regulators of pollen development, as well as hundreds of changes in alternative splicing, and we provided insight into the mRNA translation rate and storage in developing pollen. We also studied specific processes of pollen maturation, in particular pollen dehydration and cell wall biogenesis. We were interested in the molecular changes underlying pollen dehydration; we identified an unrecognised cluster of transcripts accumulated between the 'early'- and 'late'-pollen-expressed genes, suggesting that dehydration is initiated after bicellular pollen is formed. Our analyses revealed many new transcripts and proteins that accompany dehydration in developing pollen, pointing to multiple biological processes during pollen development, including the protection of developing pollen from hyperosmotic stress, the remodelling of membranes and walls, and the stabilisation of pre-synthesised mRNA and proteins in condensates in dry pollen (Sze *et al.* 2023). These results helped us to identify and analyse the INAPERTURATE POLLEN1 (INP1) ortholog from the basal eudicot *Eschscholzia californica* (EcINP1) and study its role in aperture formation. We found that EcINP1 expression peaks at the tetrad stage of pollen development, consistent with its role in aperture formation, and we showed, via gene silencing, that the role of INP1 as an important aperture factor extends to basal eudicots. Using germination assays, we demonstrated that, in *Eschscholzia*, apertures are dispensable for pollen germination (Mazuecos-Aguilera *et al.* 2021).

The results of our studies of plant reproductive development and stress response were summarised and put in context in a few high-profile reviews. In *Annual Review of Plant Biology* (Hafidh and Honys 2021), we reviewed the evolutionary origins of the male gametophyte among land plants and, in particular, its ontogenesis in flowering plants. We described two phases of pollen ontogenesis: a developmental phase leading to the differentiation of the male germline and the formation of a mature pollen grain, and a functional phase representing pollen tube growth, beginning with the landing of the pollen grain on the stigma and ending with double fertilisation. We highlighted recent advances in the complex regulatory mechanisms involved, including post-transcriptional regulation and

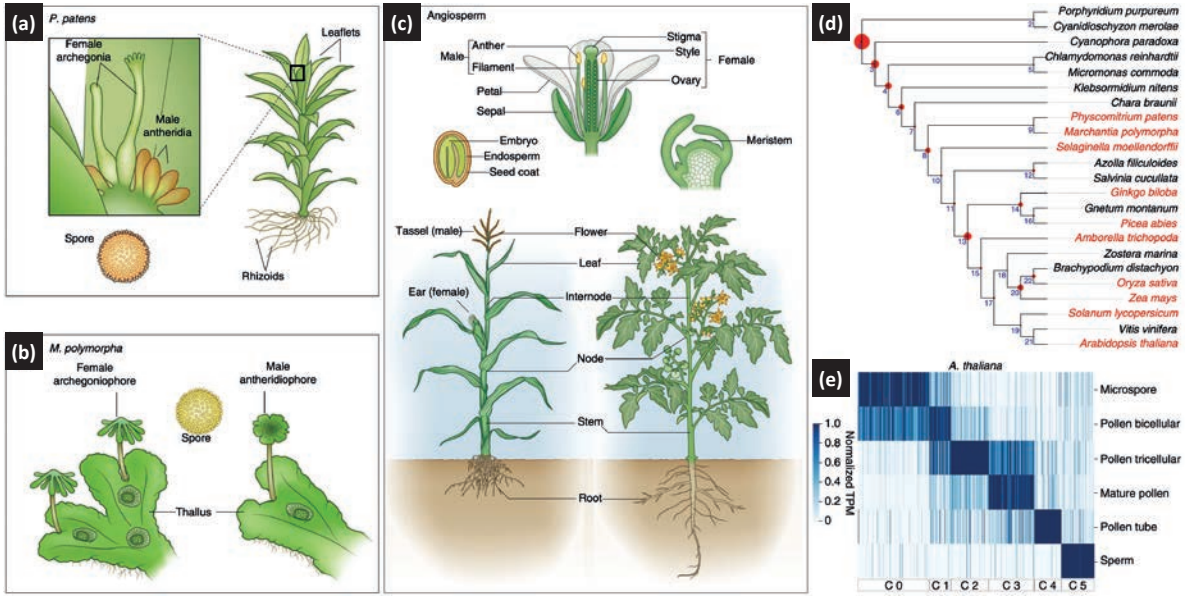

Figure 1. Expression atlases for selected land plant species (Julca et al. 2021). **(a–c)** Depiction of the different organs, tissues, and cells collected for **(a)** *Physcomitrium patens*, **(b)** *Marchantia polymorpha*, and **(c)** angiosperms. **(d)** The phylogenetic relationship of the analysed plant species; species in red are the ones with transcriptomic data available. **(e)** Heatmaps showing the expression of male sample genes for *Arabidopsis thaliana*. Bars to the bottom indicate the k-means clusters.

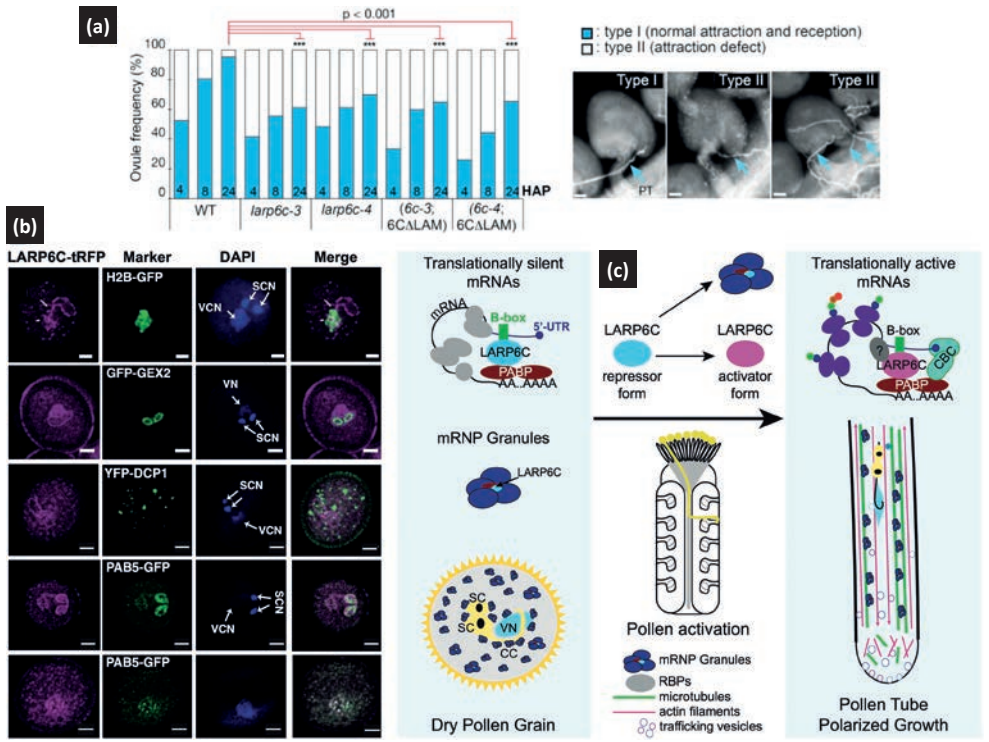

Figure 2. (a) LARP6C is required for pollen tube guidance. The WT pistils were pollinated in planta with pollen from various homozygous genotypes and the behaviour of pollen tubes monitored and scored over a time course. Type I (blue bars): targeting and reception are normal, type II (white bars): attraction (targeting) is defective. **(b)** Co-localisation of LARP6C-tRFP and several fusion proteins – H2B-GFP, GFP-GEX2, YFP-DCP1, and PAB5-GFP – in mature pollen. The arrows indicate the position of the nucleolus (No), generative cell nucleus (GCN), vegetative nucleus (VCN), sperm cell nucleus (SCN), and cytoplasmic connection (CC). **(c)** Model of the molecular functions of LARP6C in male fertilisation. In mature dry pollen grain, LARP6C is part of mRNP granules and acts as a repressor of the translation of its target transcripts. During pollen hydration and in the progamic phase, LARP6C shifts from a repressor to an activator of translation in association with PABP protein (Billey *et al.* 2021).

transcript storage (Hafidh and Honys 2021). We also summarised the ways in which pollen was affected by heat stress and the molecular mechanisms employed during the stress period, as revealed by classical and omics studies (Chaturvedi *et al.* 2021).

Pollen as a model for hierarchical regulatory levels of gene expression

Functional analyses of male gametophytic transcription factors

Within the frame of "transcriptional" regulation, we have focused on the identification and functional characterisation of the *Arabidopsis* pollen-expressed transcription factors (TF) involved in the regulation of pollen development, with a specific interest on dimeric basic leucine zipper (bZIP) TF family members. Developing *Arabidopsis* pollen expresses a versatile module of bZIP TFs from Groups E (bZIP34, bZIP61) and I (bZIP18, bZIP52, bZIP 59, and bZIP69). We revealed the dimerisation preferences among individual bZIP TFs, with some (bZIP18) dimerising more broadly than others. We showed the involvement of bZIP18 and bZIP52 in the heat stress response in seedlings (Wiese *et al.* 2021). Under standard conditions, the localisation of both bZIP18-GFP and bZIP52-GFP are partitioned between the cytoplasm and nucleus. Following heat stress, they accumulate in nuclei due to phosphorylation/dephosphorylation and 14-3-3 binding. Following heat stress, specific serine residues become dephosphorylated, allowing the bZIP TFs to dissociate from 14-3-3 proteins and re-localise to nuclei. A similar mechanism in pollen is currently being investigated.

The role of post-transcriptional regulatory levels in pollen development and function

Recently, we initiated a completely new direction in our research, pollen translatomics. 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, the germination of many pollen species has been shown to be largely independent of transcription but vitally dependent on the translation of stored mRNAs. We demonstrated that non-translating monosomes were formed in immature pollen, where they contained translationally silent mRNAs, and then served as a long-term storage for mRNA transported along with the translational machinery to the tip region where the translation took place (Hafidh *et al.* 2018, Hafidh and Honys 2021). Such an organisation is extremely useful in a pollen tube with rapid tip growth. Moreover, the asymmetric mRNA distribution is the determinant of the protein gradient influencing cell polarity, cell fate, and overall patterning during development.

Increasing evidence suggests that post-transcriptional regulation is under the control of RNA-binding proteins (RBPs). We demonstrated that in *A. thaliana*, the evolutionarily conserved RNA-binding protein LARP6C formed cytoplasmic granules containing poly(A) binding protein. LARP6C is necessary for the transition from dry pollen to pollen tubes and their guided growth towards the ovule to promote male fertilisation in plants (Billey *et al.* 2021). LARP6C shifts from a repressor to an activator of translation when the pollen grain enters the progamic phase, thus orchestrating the timely post-transcriptional regulation of a subset of mRNAs during the transition to the active state and along the progamic phase, as we proposed in the respective model (**Fig. 2**). ALBA DNA/RNA-binding proteins are also associated with RNA metabolism, mRNA translatability, and stress response. The study of ALBA dynamics during reproductive development in *A. thaliana* demonstrated the co-localisation of ALBA4 and ALBA6 in mature pollen with poly(A) binding protein 3 (PABP3) and the engagement of ALBA proteins in male reproductive development and heat stress response (**Fig. 3**), highlighting their involvement in mRNA storage and/or translational control in pollen upon heat stress (Náprstková *et al.* 2021).

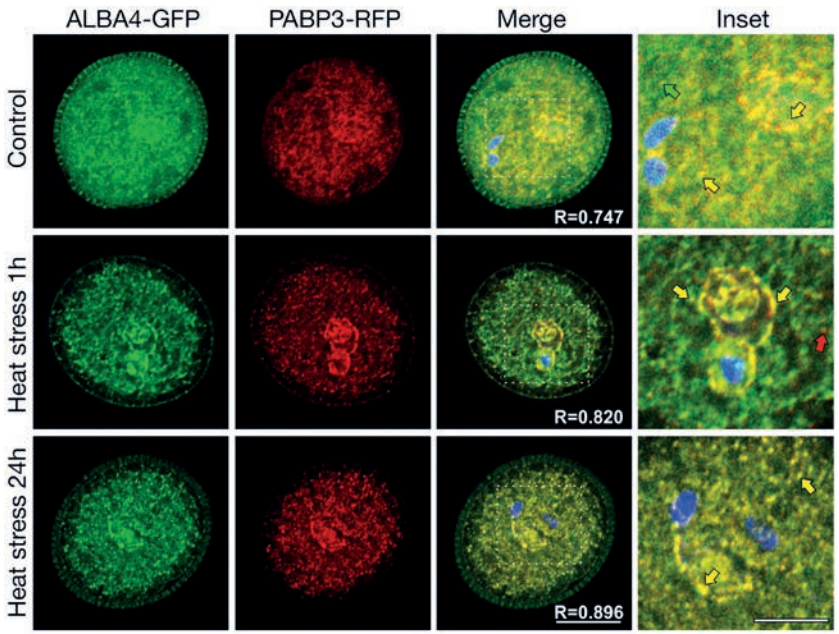

Figure 3. Co-localisation of ALBA4-GFP (green) and PABP3-RFP (red) in mature *Arabidopsis thaliana* pollen grain before and after heat stress (Náprstková *et al.* 2021). DAPI staining (blue) is included in the overlay image, and insets are indicated by dotted rectangles. The co-localisation of green and red channels appears as yellow. Arrows in the inset point to spots with GFP (green arrow), RFP (red arrow), and both fluorophores (yellow arrow).

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 a novel approach, we 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, University of Arizona, USA. As a joint effort with Z. Zdráhal's group (CEITEC MU, Brno), we performed gel-free LC-MS/MS for high throughput analysis of pollen-tube-secreted proteins (Hafidh and Honys 2020). The pollen tube secretome was comprised vastly of non-classical types of secreted proteins. Intriguingly, we discovered that TCTP1, a non-classically secreted protein, hijacked the classical secretory pathway and co-localised with the nanovesicle exosome marker Ole-e-1 (Hafidh *et al.* 2016). Our broader analyses of secretomes of pollen tubes grown *in vitro* from basal angiosperm *Amborella*, monocot maize, and dicot tobacco, performed in collaboration with T. Dresselhaus, University of Regensburg, also identified novel small secreted peptides (Flores-Tornero *et al.* 2021). The link between pollen tube translation and protein secretion is currently being evaluated.

Pollen tubes are not the only secreting tissues important for plant reproduction; flower stigma secretions are important in early stages of sexual reproduction. Previous chemical and proteomic characterization of these exudates provided insights into their biological function. Nevertheless, the presence of nucleic acids in the stigma exudates has not been previously reported. We showed that the stigma exudates of *Pyrus communis*, *P. pyrifolia*, and *P. syriaca* harbor extracellular RNAs of various sizes (Ambastha *et al.* 2023). RNA sequencing revealed, for the first time, the presence of known Rosaceae mature microRNAs (miRs), also abundant in the stigma source tissue. Several of their target genes are pollen transcribed, suggesting possible involvement of exudate miRs in transcriptional regulation of pollen. Moreover, extracellular miRs can potentially act across kingdoms and target genes of stigma interacting organisms/microorganisms, thus opening novel applicable avenues in horticulture.

Molecular insights into genome stability

Telomeres, repetitive nucleotide sequences associated with specialised proteins

at the ends of linear chromosomes, are crucial for genome stability and cell survival. Their maintenance is studied as an "alternative" mechanism not dependent on telomerase and its RNA template subunit (TR), but rather on employing a mechanism of recombination. The sequence diversity of TRs hinders their comprehensive characterisation. Nevertheless, by combining conserved sequence elements and minimal template regions, P. Fajkus (CEITEC, MU Brno) identified TR candidates across diverse organisms, including early diverging Viridiplantae and Diaphoretickes lineages. To validate the templating role of one of the candidates, we generated TR knock-out mutants in *Physcomitrium patens* via gene targeting (GT) by replacing the TR locus with a 35S:HygR cassette through homology-directed repair, using Cas9-induced DNA double-strand cleavage within the TR locus (Fajkus *et al.* 2021). Telomere repeat binding proteins (TRBs) belong to a family of proteins possessing a Myb-like domain, which binds to telomeric repeats. Three members of this family (TRB1, TRB2, TRB3) from *A. thaliana* are associated with terminal telomeric repeats (telomeres) or short interstitial telomeric repeats in gene promoters (telo-boxes). We characterised two novel TRB family members, TRB4 and TRB5. They share common TRB motifs while differing in several others and seem to have an earlier phylogenetic origin than TRB1-3. We determined the minimal recognition motif of all TRBs as one telo-box and showed that despite the distinct localisation patterns of TRB1-3 and TRB4-5 *in situ*, all members of the TRB family mutually interact and bind to telomerase/PRC2/PEAT complexes (Kusová *et al.* 2023).

RUVBL1 and RUVBL2, plant orthologues of human Pontin and Reptin, respectively, belong to the evolutionarily highly conserved AAA+ family of ATPases linked to a wide range of cellular processes, including

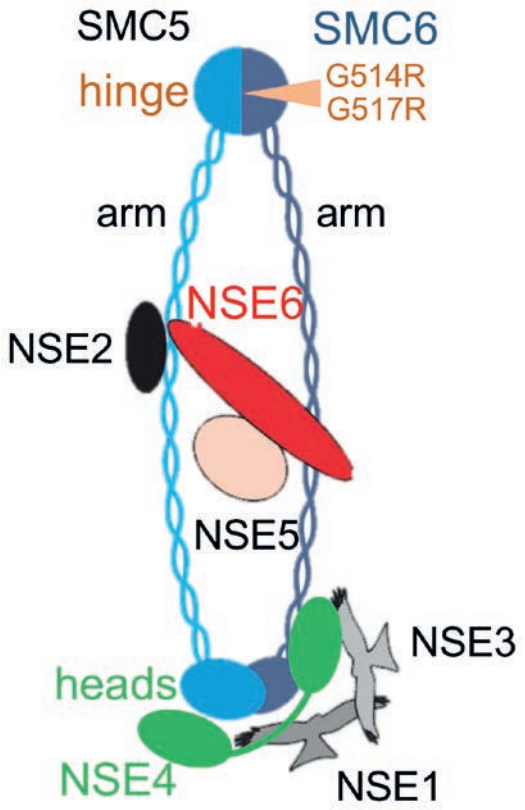

Figure 4. Model of the moss PpSMC5/6 ring complex with details of the PpNSE2 and PpNSE6 binding to the SMC5 arm (Lelkes *et al.* 2023).

the assembly of telomerase holoenzyme. We reported that RUVBL1 and RUVBL2A also play a role in *Arabidopsis* reproductive development, as they are essential for the proper development of both male and particularly female gametophytes (Dvořák Tomaštíková *et al.* 2023).

In cooperation with J. Paleček (MU Brno), we launched a broad study of the role of the essential complex of the structural maintenance of chromosomes SMC5/6 and its NSEx subunits. We addressed the circularisation of SMC5/6, which is thought to be crucial for its function, either by interfering with the expression of kleisin NSE4 (a member of the NSE1, 3 and 4 subcomplex), which holds together the heads of SMC5/6 (**Fig. 4**), or by targeted mutation disrupting the interaction in the hinge regions of SMC5 and 6. The ring structure of SMC5/6, mediated by NSE4, is crucial for DNA repair and cell renewal (Holá *et al.* 2021). We further studied the roles of NSE6 subunits and NSE5 subunits, which likely form the SMC5/6 subcomplex. The conserved CANIN domain in NSE6 regulates SMC5/6 dynamics, with a deficiency in *nse6* showing sensitivity to DNA damage and developmental abnormalities (Lelkes *et al.* 2023). These findings underscore the complex interplay of SMC5/6 complex components and their regulatory subunits in maintaining genome stability and orchestrating repair mechanisms.

We also contributed to the understanding of the DNA double-strand break (DSB) repair process, which is essential for genome maintenance, by describing the evolution and roles of the key homologous recombination (HR) gene RAD51 and its paralogs RAD51-1, RAD51-2, and RAD51B in *P. patens*. RAD51-1 and RAD51-2 knockout lines revealed their importance in the efficient execution of HR during DSB repair. An absence of RAD51 leads to shifts towards non-homologous end joining (NHEJ), which affects genome stability (Angelis *et al.* 2023).

National and international collaborators

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Publications: 16, 24, 32, 37, 53, 59, 80, 88, 120, 133, 151, 184, 201, 210–211, 233, 295, 340, 384a, 385, 430, 435, 437, 481, 504, 509

Research projects: 2, 4, 8, 10, 15, 18, 39–40, 45, 49, 53, 78–79, 91, 97, 103, 113

Laboratory of Virology

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The Laboratory of Virology conducts research in several interrelated areas, with research priorities shaped by the available funding sources. Our primary focus is on exploiting the unique capabilities of plants to produce valuable compounds for diagnostics, medicine, and industry. To this end, we use modified plant viruses, known as viral vectors, and perform transient or permanent plant transformations facilitated by the plant pathogen *Agrobacterium tumefaciens*. In addition to protein overexpression, viral vectors enable the study of gene function through virus-induced gene silencing (VIGS) and direct modification of plant genomes through virus-induced gene editing (VIGE).
Another important area of our research focuses on the detection and diagnosis of viral plant diseases, complemented by the in-house development of diagnostic enzymes with novel properties. Over time, our focus on creating and optimising diverse genetic constructs and circuits has led to an extensive collection of standardised DNA components and genetic modules that enrich collaborations with research teams inside and outside our institute.

Viral vectors

To transiently express valuable proteins, we created and remodelled infectious clones of several plant viruses. These include Bean Yellow Dwarf virus, Tobacco Mosaic virus (strains U1 and Cg8, tailored for the infection of *Arabidopsis thaliana*), Potato virus X (Plchová *et al.* 2022), Tobacco Rattle virus, and Apple Latent Spherical virus (ALSV). These viral vectors are adapted to conform to the GoldenBraid standard, which facilitates the exchange of genetic components between these viruses and provides access to a large collection of existing GoldenBraid/MoClo gene modules for use in basic plant virology, virus-induced gene silencing (VIGS), or virus-induced gene editing (VIGE). In combination with our extended suite of GoldenBraid vectors (Dušek *et al.* 2020), this allows us to construct increasingly complex genetic circuits for applications in metabolic engineering and gene editing.

In the picture (from left to right):
Top row: Mgr. Tomáš Moravec, Ph.D. / head of the laboratory, RNDr. Oldřich Navrátil, CSc. / researcher, Lenka Kolčabová / technician, Bc. Radek Vítek / Ph.D. student, doc. RNDr. Noemi Čeřovská, CSc. / researcher.
Front row: Jitka Svobodová / technician, Mgr. Jan Fousek, Ph.D. / technician, Ing. Jakub Dušek, Ph.D. / post-doctoral fellow, Mgr. Hana Hoffmeisterová, Ph.D. / researcher.

Not pictured:
Ing. Jiban Kumar, Ph.D., Dr. rer. nat. / researcher, Mgr. Kateřina Kratochvílová / Ph.D. student (maternity leave).

Quick diagnostics

In addition to our work in plant-based pharmaceutical protein expression, we also specialise in the diagnosis of viral diseases. Early detection of viral infections is essential in order to prevent significant economic losses. Current diagnostic techniques based on detection of the viral genome (RNA, DNA), while providing exceptional sensitivity and precision, are limited to centralised, certified laboratories that are often located far from the field, leaving a significant portion of suspect plant material unanalysed.

Recent advances in enzyme functionality have opened up the possibility of improving both the efficiency and cost-effectiveness of virus diagnostics. Using

RTX reverse transcriptase/DNA polymerase, we have developed a robust one-tube protocol for RNA plant virus detection that eliminates the need for RNA extraction, purification, and separate cDNA synthesis steps. All these functions are seamlessly integrated into the enzyme itself, reducing the time required for virus detection from one and a half days to only 3 hours (Hoffmeisterová *et al.* 2022). However, this method still requires a well-equipped laboratory with a PCR cycler and a gel electrophoresis instrument. We are currently developing isothermal amplification methods that eliminate the need for traditional laboratory equipment.

International cooperation

As a relatively small research group, we are looking for opportunities to expand our network of collaborators both domestically and internationally. Our ongoing collaboration with Prof. Ed Rybicki's lab at the University of Cape Town, South Africa focuses on the use of viral vectors for biopharmaceutical production. In partnership with Prof. Andreas Voloudakis at the University of Athens, we are exploring the use of VIGS and VIGE vectors to improve the quality of vegetable crops. A particularly promising collaboration has developed with Prof. Thanh Ha Duong at ITODYS, Université Paris Cité, where we are jointly investigating the use of modified plant viruses as biotemplates for advanced nanomaterials (Da Silva *et al.* 2023).

Figure 1. Viruses as tools for analysing gene function in plants. This figure illustrates the use of viruses in deciphering gene function in plants through gene silencing. The image shows a novel viral vector derived from the Apple Latent Spherical Virus carrying a small segment of tobacco's PDS gene, which is responsible for the biosynthesis of photosynthetic pigments.
As viral infection progresses, the plant's innate antiviral defence mechanisms target both the viral RNA and its own PDS mRNA, effectively halting photosynthetic pigment production. This is evident from the observed bleached phenotype. This mechanism can similarly be used to deactivate other plant genes.

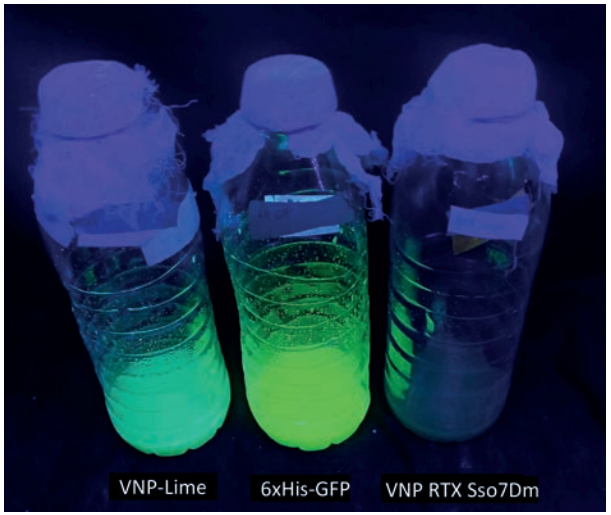

Figure 2. Expression of recombinant proteins in *Escherichia coli* bacteria. Two reporter proteins, amajLime from coral and GFP from jellyfish, and a modified thermostable polymerase originally from hot springs archea were expressed in the bacteria. The proteins are purified and used as standards for quantification (Lime and GFP) or as reagents for RT-PCR detection of plant viruses.

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Publications: 63–64, 128, 229, 266, 291, 319, 324, 379, 384, 405, 478, 501, 509
Research projects: 10, 100, 115, 117

Transcriptomics and Cloning Facility

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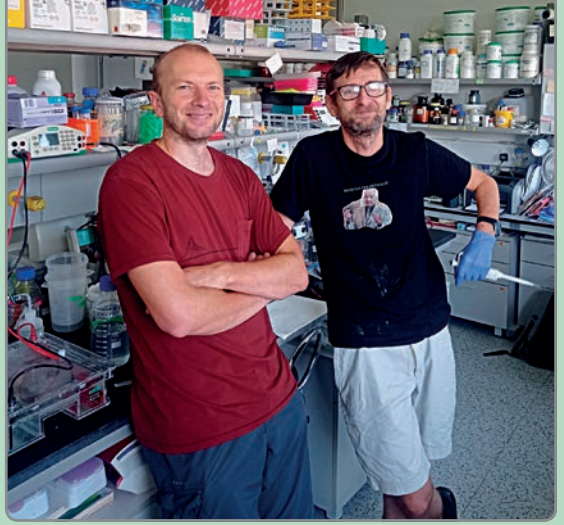
The main task of the service laboratory is to provide both assistance with the realisation of transcriptomic analysis using whole RNA sequencing and assistance with the design and preparation of plasmid constructs. The group was established in 2023 and was founded by two researchers with many years of practical experience.

Next generation sequencing (NGS) is an example of the effective tools in modern biology. While transcriptomic studies using NGS may appear to be a routine method complementary to most biological experiments, the actual implementation is still complicated for many research teams. For novices just starting out in the area of transcriptomics, several dilemmas arise: where to access the service, what exactly to ask for, what kind of information to extract from the experiment, how to process the data, and how to interpret it, for example. And last but not least: What happens afterwards? Transcriptomic experiments require not only knowledge of the biological background of the problem under investigation, but also laboratory experience (isolation and handling of RNA) and bioinformatic knowledge. The analysis of RNA-seq results is a typical example of Big Data processing and data mining. All these skills are difficult to acquire and maintain, especially for smaller research teams.

To maintain expertise and provide support for transcriptomic studies both within and outside of the IEB, the Transcriptomics and Cloning Facility was recently established. Our team provides support for all of the steps of transcriptomic analysis mentioned



In the picture (from left to right):
Ing. Karel Müller, Ph.D. / head of the facility,
Mgr. Tomáš Moravec, Ph.D. / researcher.
Not pictured:
Ing. Alena Trávníčková / graduated technical assistant,
Ing. Jitka Svobodová / graduated technical assistant.

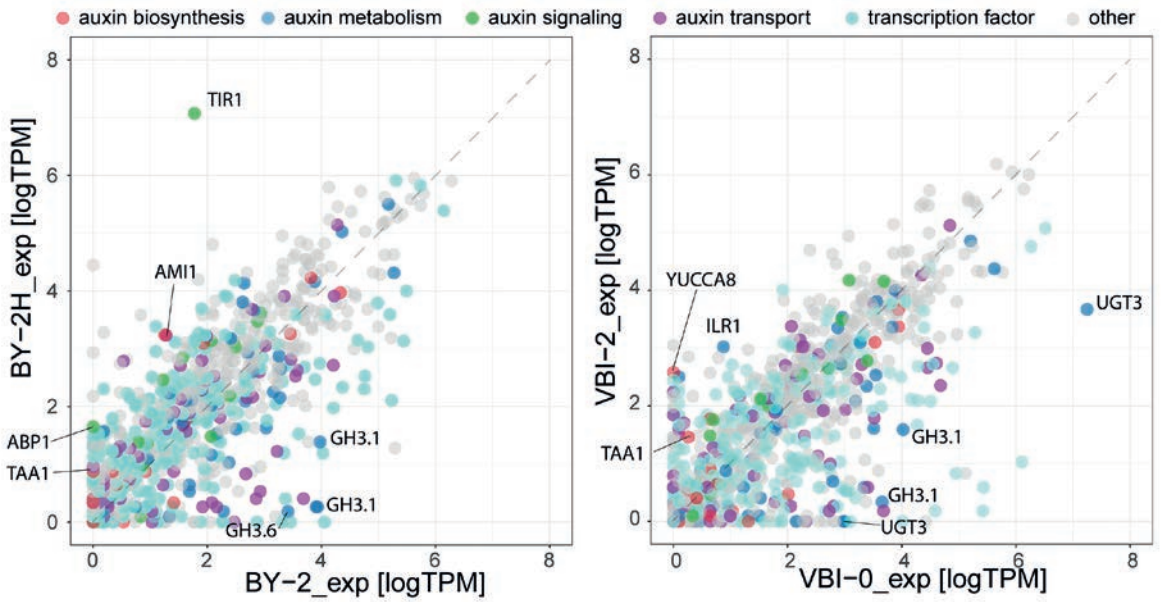


Figure 1. Comparison of transcription of auxin-related genes in auxin-autonomous and auxin-dependent cells of tobacco cell lines BY-2 and VBI.

above. We accept harvested and frozen samples or already isolated RNA and arrange the generation of the sequencing data. Sequencing data are processed using a high-performance computational cluster of the IEB using need-specific programmes (e.g. Star, Salmon, Trinity, etc.). Finally, a comprehensive table with the transcripts, their basic annotation, the normalised transcript abundances, and the statistical evaluation is provided. Of course, we also take care of the data deposition (e.g. GEO submission) and help with the preparation of manuscripts.

Transcriptome analysis should be followed regularly by experiments aimed at determining or validating the function of candidate genes selected from transcriptome comparison or at analysing in detail the mechanism of the biological event in question. Such experiments often depend on plasmid constructs with various functions (e.g. expression of proteins tagged with fluorescent labels, up- or down-regulation of specific proteins or their targeted mutation, promoter analysis, etc.). Similar to transcriptome workflow experts, not all research groups have "cloning masters" and all the necessary tools and instruments for synthetic biology. Therefore, our facility also offers cloning services. We maintain a large collection of cloned elements, including a variety of promoters, terminators, protein tags, antibiotic resistance cassettes, elements for CRISPR-Cas9-driven targeted mutagenesis, and much more. In general, we follow the protocols and strategies based on GoldenBraid cloning. We offer preparation of whole plasmid constructs-of-interest or support and training in this technique for our colleagues, especially students, so that they can use the methodology for their future careers.

Research projects: 118

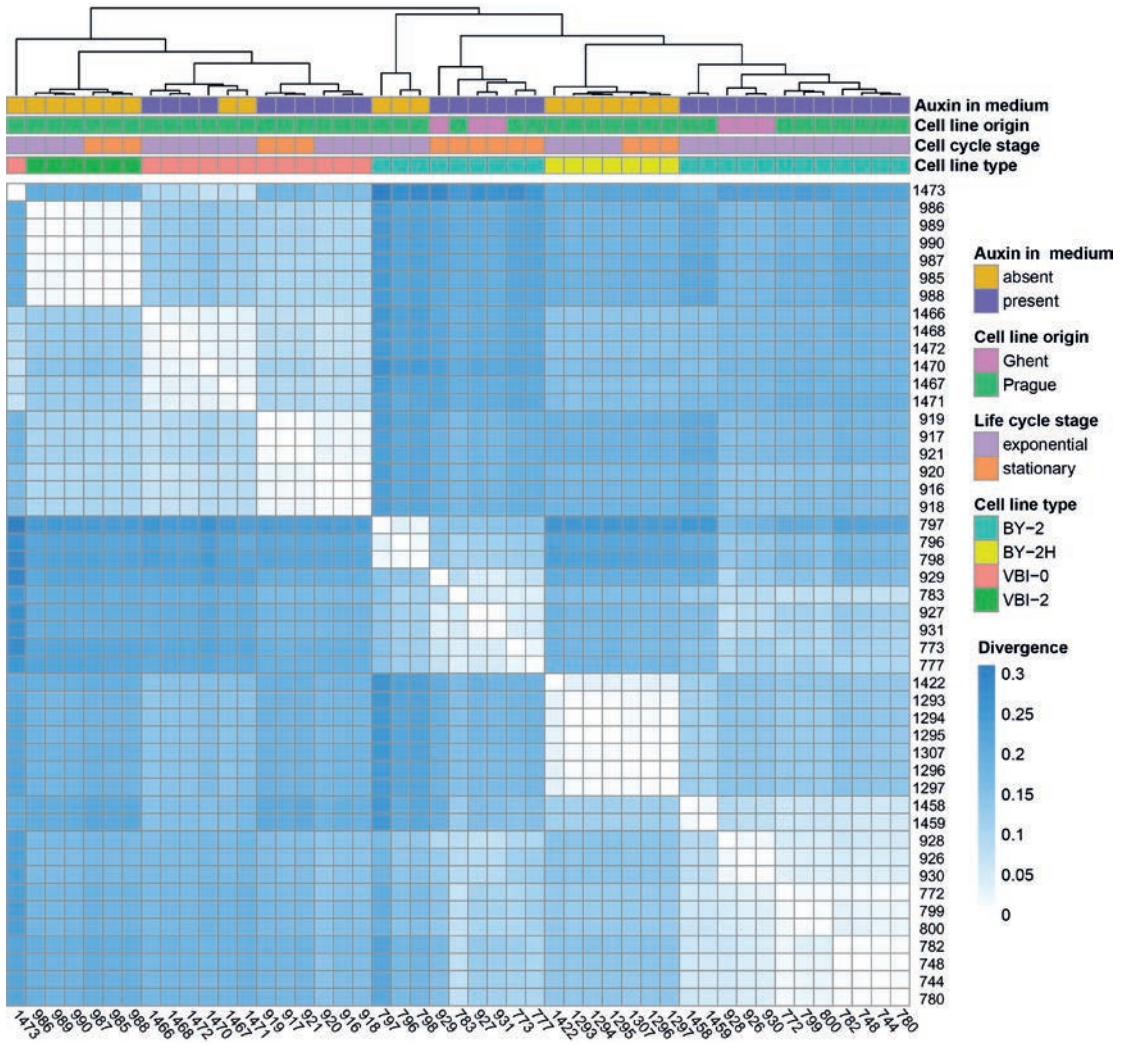


Figure 2. Quality control of RNA-seq data processing represented by the values of Jensen-Shannon divergence coefficients in heatmap representation. RNA-seq was performed for 47 samples collected from four tobacco cell culture types in two different stages of their life cycle and cultured in the presence or absence of exogenous auxin.

Station of Apple Breeding for Disease Resistance

Head of the laboratory:

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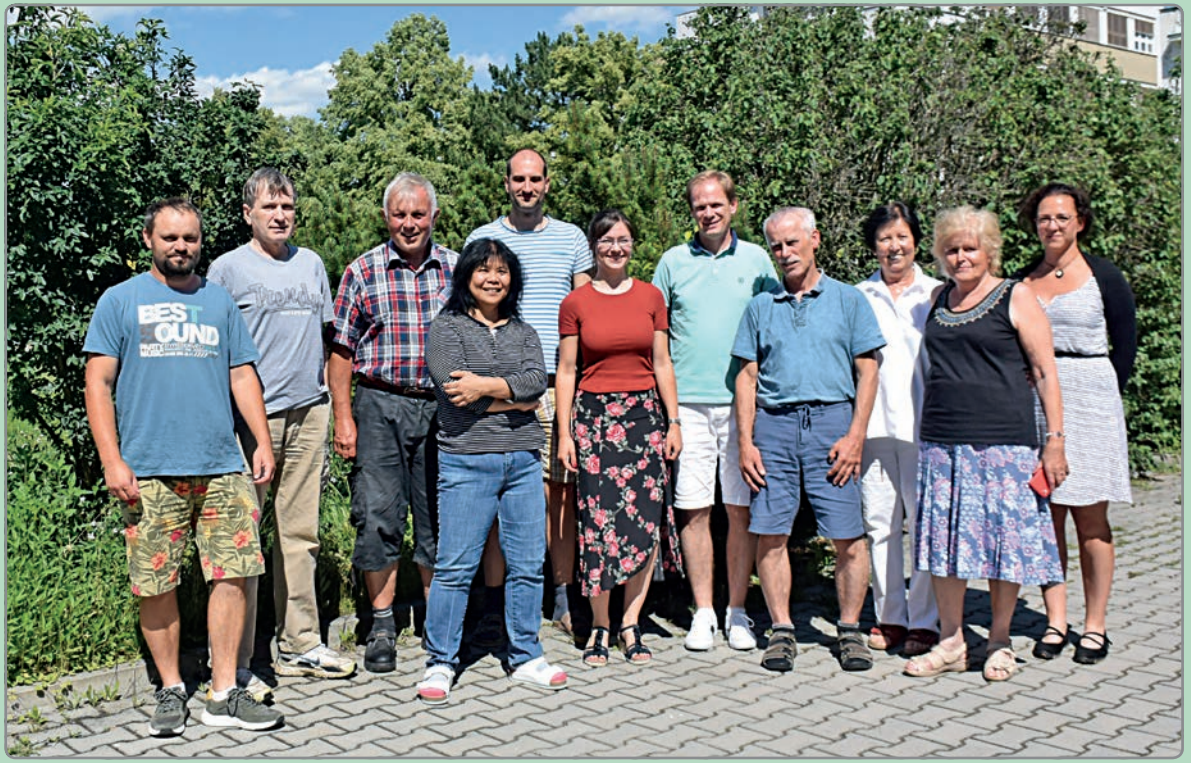
The station continues a long-standing tradition of breeding apple varieties of a high economic quality and with high resistance, especially to scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), and fireblight (*Erwinia amylovora*), the most important apple diseases. Newly developed IEB apple varieties with added value are protected by plant breeding rights or plant patents and find global use in intensive orchards with integrated or organic management, as well as in home gardens all over the world. The cultivation of these new varieties significantly contributes to present environmental and economic demands, consisting of the production of healthy fruit and a decrease in the environmental burden of pesticide application and carbon dioxide production. The high potential for result implementation of applied research into practice is the main domain of the world-renowned IEB apple breeding program, from which about 1.35 million apple trees on average have been sold worldwide annually in recent years.



In the picture (from the left):
Zdeněk Haleš, DiS. / technical assistant, Ing. Miloslav Juříček, Ph.D. / researcher, Ing. Jan Zima / graduated technical assistant, Mgr. Sunee Kertbundit, Ph.D. / former researcher, RNDr. Dimitrij Tyč, Ph.D. / researcher, Mgr. Veronika Janečková / graduated technical assistant, Ing. Radek Černý, Ph.D. / head of the team, Zdeněk Mikula / technical assistant, Květoslava Rabochová / technician, Dagmar Švestková / technician, Ing. Zuzana Krčková, Ph.D. / researcher.

Not pictured:
Ing. Otto Louda / graduated technical assistant, Mgr. Věra Forejtová / graduated technical assistant, Mgr. Jaroslav Kozák / graduated technical assistant.

Breeding for disease resistance

The fungus *Venturia inaequalis* usually harms the host by making grey-black lesions on leaves and scabs on fruits. The tree is thus insufficiently nourished, and the resulting damaged fruits can only be used for processing, not for fresh consumption. The resistance to scab in some apple varieties is most often conditioned by a single gene, *Rvi6*. The first source of scab resistance was found in the crabapple *Malus floribunda* 821. The *Rvi6* gene can be transferred to the progeny simply by

crossing; its presence in offspring can be evidenced by means of molecular markers. By repeated crossing for many generations, we have managed to combine resistance against diseases with growing/bearing characteristics and fruit qualities to fulfil the properties required by growers and consumers.

Monogenic resistance is usually not durable in nature – in some locations it has already been overcome by new races of the fungus. Therefore, the IEB breeding program has been focused for decades on searching for new genetic sources of protection against scab by breeding apple varieties with combined monogenic and polygenic resistance via pyramiding multiple scab resistance genes.

Thus, in our basic research, we are interested in elucidating the elemental mechanisms of the polygenic or *Rvi6* gene-mediated resistance, as well as its breakdown. The completion of the second draft of the genome sequence of *Malus × domestica* 'Golden Delicious' double haploid genome (GDDH), as well as rapid advances in the next generation sequencing (NGS) technology, gave us very promising tools for studying plant pathogen interactions in difficult, non-model species such as the apple tree.

In the past, we developed a method of artificial infection by different conidia isolates, allowing for the monitoring of the host plant response in detail by performing a full quantitative transcriptome sequencing. The RNA-Seq analysis revealed various enzymes and signaling pathways that evidently play important roles in defence responses against scab. Increased expression was found predominantly in two enzyme groups: pathogenesis-related proteins (PR-1, Mal d 1, β-1,3-glucanases, chitinases, thaumatin or thaumatin-like proteins, LRR kinases/lipases, DIR proteins or enhanced disease susceptibility protein, etc.) and reactive oxygen group, incl. antioxidative enzymes

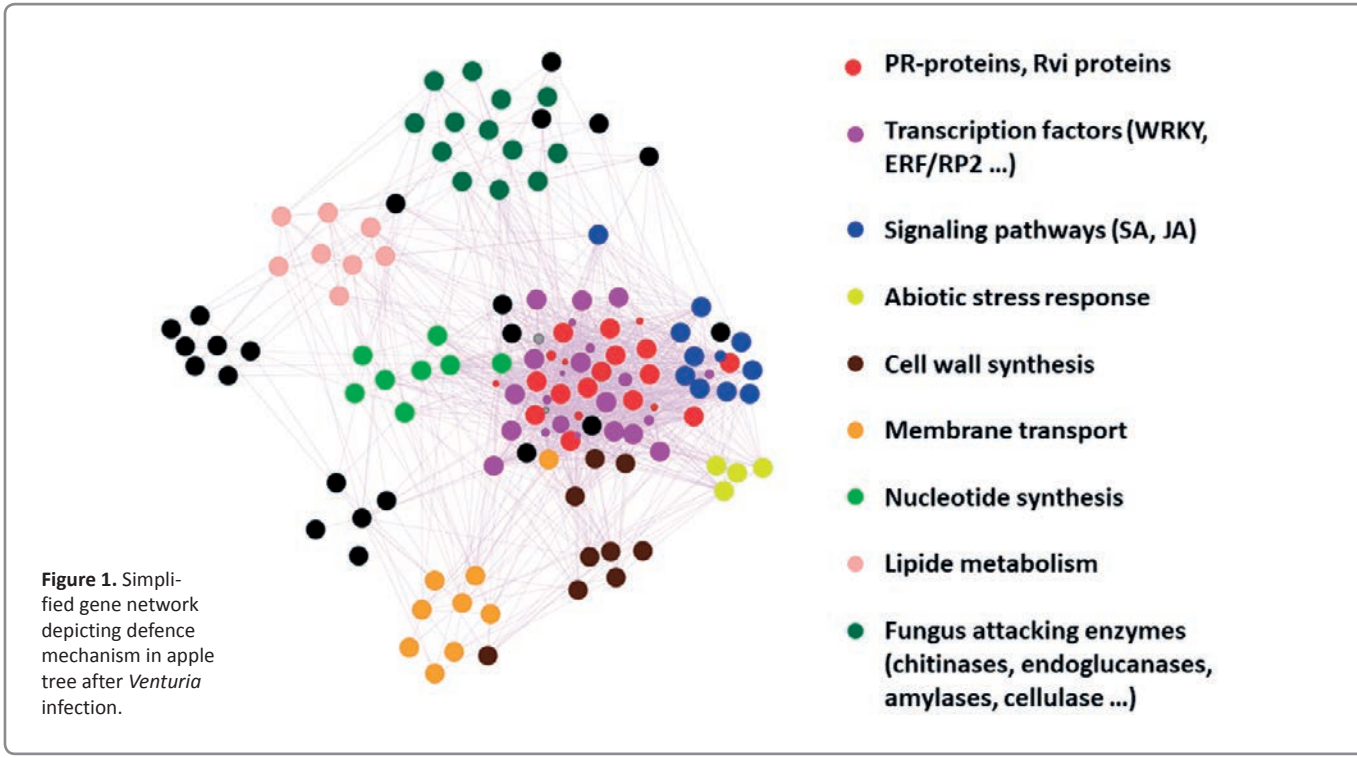


Figure 1. Simplified gene network depicting defence mechanism in apple tree after *Venturia* infection.

(superoxide dismutase, catalases, peroxidases, and glutathione peroxidases). Other enhanced expression was found in the case of metallothionein and genes for lipid transfer. On the contrary, the expression of enzymes promoting infection such as GDSL esterase lipase were greatly reduced.

The key roles in these defence mechanisms have signaling pathways. Gene products involved in the salicylic acid (SA) signaling pathway were strongly enhanced. A similar, but lesser, increase was also observed in jasmonic acid (JA) signaling pathway and mitogen-activated protein kinase signaling pathway, while ethylene signaling pathway was only slightly activated. The activation of these signaling pathways (especially SA) presumably led to the over-expression of the above-mentioned defence-related genes and to the production of antimicrobial compounds, which finally led to resistance against some *Venturia* races (**Fig. 1**).

In our other research work, we used single nucleotide polymorphism (SNP) to identify the markers for polygenic apple scab-resistant genes. Polymorphic SNPs were identified in 'Allegro' crosses bearing polygenic apple scab resistance ('Discovery' origin) and

then converted to CAPS markers for easy detection. Although we were able to detect polygenic resistance in 'Allegro' crosses using one of these markers (**Fig. 2**), the saturation of the population with these SNPs is rather low. Therefore, we also supplemented this method with detection via molecular signature. We employed Weighted Gene Co-expression Network Analysis (WGCNA), together with Gene Set Enrichment Analysis (GSEA) and RNA-Seq Differential Expression Analysis data, to identify crucial genes involved in the apple scab resistance network. With these methods, we are now able to detect and distinguish polygenic and *Rvi6*-based monogenic resistance in apple crosses.

Another important apple disease is powdery mildew (*Podosphaera leucotricha*), which occurs particularly in dry localities. The main symptoms are a whitish coating on the shoots and, indirectly, skin russeting on the fruits. New varieties that are resistant or tolerant to this disease are the result of several years of testing under experimental field conditions.

A further disease, which is even more serious on a worldwide scale, is fireblight, a bacterial disease (caused by *Erwinia amylovora*). It is manifested in the wilting and dying of flowers, the burning of leaves and fruits, and the drying and necrosis of vegetative shoots and branches. The consequences of an attack are very destructive, often ending in the death of the entire tree. Fireblight is quite rare in the Czech Republic; however, it causes serious economic loss in many important growing areas around the world. The new selections chosen at the IEB, or the varieties in an advanced testing stage that show the potential for application in practice, are therefore tested by the method of artificial shoot inoculation with the pathogen in the international research cooperation with Agroscope Wädenswil (Switzerland), the Julius Kühn-Institut (Germany), or by applying a suspension during flowering in cooperation with The Crop Research Institute (Czech Republic).

Intellectual property rights and commercialisation of results

Apart from disease resistance, new varieties must meet stringent requirements in order to be commercially successful. Among these demands are growing characteristics, high and regular productivity, good storability, and fruit quality – such as appearance, flavour, firmness, the crispness and juiciness of the flesh, and even the level of main allergens (Mal d proteins, whose detection was established during the last period). Based on these aspects, the chosen IEB new selections are tested in the Czech Republic, as well as in foreign research centres and by potential business partners such as nurseries, producers, and marketing companies. Commercially promising varieties are legally protected by Community Plant Variety Rights (CPVR) in the EU and by the United States Plant Patent (US PP) in the USA or by other plant breeding rights all over the world. In the period 2021–2023, the new IEB apple varieties 'Magenta', 'Lilac', 'Rubelit', 'Acrobat', and 'UEB 6481' (marketed under the trademark Orange Crisp®) were granted a breeding certificate granting plant variety rights in the Czech Republic, a national plant variety certificate in Switzerland, or CPVR in the EU. All licenced varieties concerned are traded on the market. These varieties are grown predominantly in organic orchards or in integrated production. Their propagation and sales are based on concluded licence agreements. The great ability of the application of new IEB apple

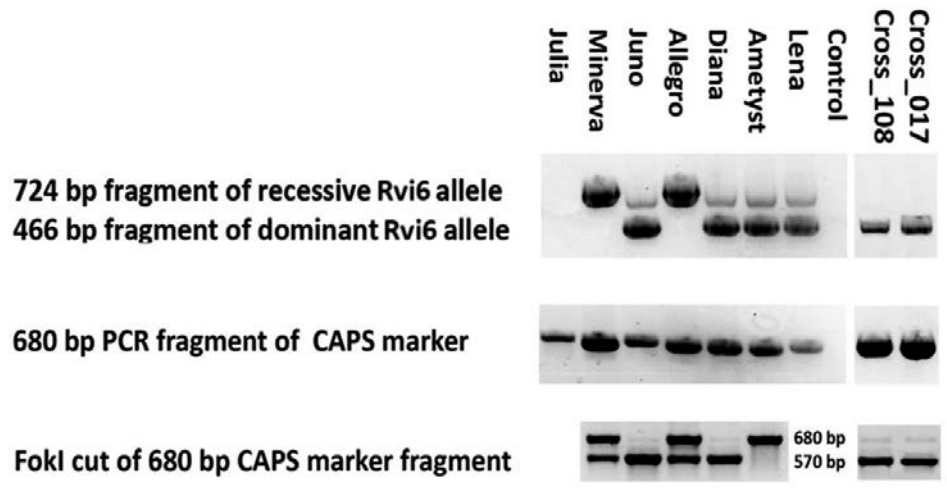


Figure 2. Detection of polygenic resistance against scab using CAPS marker. The 570 bp band appears after cutting the PCR-amplified APS marker with *FokI* only in plants showing polygenic resistance from variety 'Discovery'.

Figure 3. Demonstration of professional integrated orchard of apple variety Opal® in the State of Washington, USA.

varieties in practice is demonstrated by the following in the evaluated period:

- 11 concluded licence agreements (3 Czech and 8 international) and 5 addenda to a licence agreement
- more than 2.6 million trees of IEB apple varieties sold (worldwide) in 2021 and 2022
- almost 25 million CZK of licence income in 2021–2022

One of the most commercially successful varieties of the IEB breeding program is 'Topaz', along with its mutation, 'Red Topaz'. They are the most cultivated scab-resistant apple varieties in the world, and at the same time, the most cultivated varieties grown in organic growing conditions, planted in Europe on an area of approximately 2,000 ha, corresponding in practice to about 6 million planted trees. In the years 2021–2022, over 835,000 'Topaz' and 'Red Topaz' trees were sold under licence all over the world. The varieties are very often used worldwide as a valuable genetic source for further breeding.

Dessert apple varieties

The variety 'UEB 32642', known under the trademark Opal®, which is registered in more than 40 countries and is characterised by a bright yellow skin with crunchy flesh and an aromatic, honey-sweet flavour, is very popular, mainly in the USA. Opal® is suitable mainly for warm, vineyard areas with additional irrigation (**Fig. 3**). Opal® was introduced onto the market according to the worldwide marketing concept managed by the companies Webfruit GmbH, Germany and Varieties International, USA. More than 740,000 trees were sold in 2021–2022. In total, more than 3.4 million Opal® trees have been sold worldwide, which corresponds to plantings in an area of approximately 1,130 ha.

Another IEB apple variety cultivated worldwide is ‘Bonita’. The variety name comes from Portuguese and means “pretty” or “beautiful”, which precisely represents the outstandingly attractive appearance of its bright red fruits. The variety is characterised by high and regular fruit productivity, good tree growth, and fruit quality, including long storability. ‘Bonita’ is protected by plant variety rights and plant patents in the EU, the USA, Switzerland, and South Africa. The variety is commercially applied on the basis of an exclusive licence agreement with Konsortium Südtiroler Baumschuler (KSB), Italy. This agreement allows for the control of the trademark, marketing, growing, propagation, and sales of trees and fruits. In the years 2021 and 2022, more than 320,000 ‘Bonita’ trees were sold in many countries in the EU and in South Africa. Since the variety was introduced onto the market in 2015, more than 1.5 million ‘Bonita’ trees have already been sold. Royalties were contractually agreed to be paid not only from trees sold, but also from fruits sold, i.e. from yields (**Fig. 4**).

Figure 4. Demonstration of professional orchard of apple variety ‘Bonita’ in Vinschgau, Italy at an altitude exceeding 900 m above sea level.

A newcomer to the apple market is the variety UEB 6581, with its first sales in the season 2017/2018. Licence rights for the propagation and sale of trees and trade with fruits were granted to KSB, cooperating with the Italian sales organisation Melinda, which brings together 4,000 growers. During just five seasons, nearly 400,000 trees have been planted, with almost 200,000 of them in 2021–2022. Since 2023, Melinda has been distributing the variety under the trademark Melinda® ‘Dolcevita’. The fruits of ‘UEB 6581’ are characterised by an exceptionally sweet flavour, bringing to mind the tones of tropical fruit (**Fig. 5**).

Columnar and ornamental apple varieties

Apart from dessert apple varieties, our program also includes the breeding of varieties with a compact, columnar growth habit. As a result, a global marketing contract for columnar, scab-resistant varieties was concluded with the American company Varieties International LLC. The released columnar varieties are legally protected in the EU, the USA, and Switzerland. So far, this type of tree is suitable mainly for home gardens as a beautiful solitaire or for the planting of hedges. Thanks to their specific growth habits and low demands for space and pruning, columnar apple varieties may not only contribute to the return of fruit trees in smaller home gardens, but may also show the potential for future cultivation in intensive plantings with the possibility of robotic harvesting. In 2021–2022, nearly 144,000 trees of IEB apple varieties with columnar growth were sold. Considering the fact that they are usually sold in small numbers, we can find IEB colum-

Figure 5. Melinda® 'Dolcevita' apple variety – one of the main sponsors of the cycling race Tour of the Alps 2023.

Figure 6. Demonstration of experimental apple tree plantings equipped with a new support system at the Apple Breeding Station, Střížovice, IEB.

nar apple varieties in tens to hundreds of thousands of home gardens.

In the evaluated period, IEB breeding was also focused on ornamental apple varieties with columnar growth intended for pollination purposes in intensively managed apple orchards. As of yet, there are no such apple varieties intended for pollination on the market. Knowledge of the so-called S-genotype, which is responsible for fertilisation, is key with regard to applying the varieties in practice. In recent years, we have been determining the S-genotype in new selections, and at the same time, we are working on a more processive detection system. During the evaluated period, two new IEB apple varieties, named 'Magenta' and 'Lilac', were introduced onto the EU, US, and Swiss markets. Other new selections with extended value are in trials, particularly in the State of Washington, USA. The determination of the S-genotype in the apple tree, which is also analysed by our research team, is closely related to this issue and is key for optimal fruit set.

Station development

The modernisation of the station in recent years is in line with the trends preceding the effects of climate change (e.g. anti-freeze machines, automatic drip irrigation, increasing the organic content in the soil, etc.) the automation and mechanisation of processes, and a reduction in the application of sprays (mechanical hoeing of the soil, use of bioagens, and stricter monitoring of diseases and pests).

A new, fully regulated greenhouse enables a pre-selection of plants after a previous intentional scab inoculation. Consequently, the team tests only the most robust individuals in the field. Grafting those onto M9 rootstocks allows for the evaluation of the fruit characteristics of new varieties in the fourth year after crossing at the latest, or in the second year after planting. A cold storage, including equipment with ULO (Ultra Low Oxygen) technology, enables the research of optimal storing conditions for new varieties and, at the same time, it preserves fruits at a good quality level for their promotion and presentation in organoleptic evaluations in the late spring. Since 2021, a new support system with intensive tree planting and drip irrigation has been under construction in our experimental orchard (**Fig. 6**). With the support of the CAS, a modern, versatile tractor equipped with a cultivator was purchased in 2022.

Publications: 52, 261–262, 509
Research projects: 10, 108, 114, 117

Additional figures of some apple varieties bred by the station: **1.** Orange Crisp® apple variety. **2.** Ornamental apple variety ´Magenta´. **3.** Promotion of the variety Opal® at Interpoma 2022 international fair, Bolzano, Italy. **4.** Demonstration of professional orchard of apple variety 'Bonita' in Fruit Farm Kindlhof, Merano, Italy. **5.** Market application of Opal® apple fruit in Dubai, UAE. **6.** A truck promoting our variety 'Bonita'. **7.** ´Rubelit´ apple variety. **8.** Example of fruit colour testing of the variety 'Bonita' in South Tyrol, Italy. **9.** Professional orchard of the variety Opal®, The State of Washington, USA. **10.** Promotion of the variety 'Bonita' at Fruchtwelt 2023 international fair, Friedrichshafen, Germany. **11.** A sample of the fruit of the apple variety 'Bonita' intended for the Italian market.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Science Outreach

The institute actively promotes its work, and plant biology in general, among the Czech public of all ages. Public relations and science outreach are coordinated by three professionals:

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

These specialists are assisted by a group of researchers and students who act as presenters at public events and help with event management. Many scientists and other employees also participate in the annual Open Door Days or give public lectures.

A complete list of our activities would be rather long; therefore, we will just review some important examples.

Figure 1. As a part of our day camp activities, children learn the basics of plant *in vitro* cultivation. Here they work in tissue culture hoods with carnivorous plants.

Online communication

The institute's website has a section for journalists and the general public. Here we offer information about new discoveries, research projects, upcoming events, media coverage of our work, etc. We maintain social media accounts on Facebook, Twitter/X and Instagram, which had ca. 6,500, 1,780 and 470 followers, respectively, in December 2023. The Facebook and Instagram accounts are aimed at the Czech general public. The Twitter/X account is also used to communicate with scientists from Czechia and abroad.

Major events

During the Covid-19 pandemic that started in early 2020, public events and group activities were banned or limited for many months. Lockdowns and other restrictions continued into the following year, but in 2022 the situation returned to normal.

We participate in several annual events that promote public interest in science. The largest of these is the Week of the Czech Academy of Sciences (named Science and Technology Week until 2021). During this week, the institute also holds its Open Door Days. School groups, university students, and individuals interested in plant biology visit our laboratories and get involved in hands-on activities. We attract several hundred visitors every year.

The Czech Academy of Sciences (CAS) also organises the Science Fair – a large three-day event at a fairground in Prague. Our programme typically features microscopes, experiments, *in vitro* plants, and workshops with laboratory equipment.

Other annual activities include two events in Prague: VědaFest and Science Festival na Desítce. Our scientists also promote plant biology at Researchers' Night. In 2023, we teamed up with the National Museum of Agriculture for this event. We also participate in the Fascination of Plants Day, held biennially by the European Plant Science Organisation.

Activities in Olomouc

Our Centre of Plant Structural and Functional Genomics is located in the city of Olomouc. Its team is very active in the area of science outreach. The former head of the Centre, Professor Jaroslav Doležel, who holds the country's highest scientific award, the National Government Prize Česká hlava, has also recently received the title *doctor honoris causa* at the Mendel University in Brno and a medal at Lund University in Sweden for his contribution to plant genetics.

Science communication is one of the main objectives of the CAS programme Food for the Future, which is coordinated by Professor Doležel. Most importantly,

Figure 2. During the Science Fair in July 2023, visitors of our booth could learn how to manually pollinate individual Arabidopsis flowers – just like real plant biologists.

the centre opened the Application Laboratory for Agricultural Research, which enables a quick transfer of scientific knowledge to agricultural applications. This laboratory cooperates with breeders, seed producers, farmers, government agencies, and non-profit organisations.

The genomics centre, as well as the Food for the Future programme, team up with Fort Science, an interactive science centre at Palacký University in Olomouc. Our scientists put together a permanent exhibition there that explains genetics, DNA, and related topics.

The scientists also participate in various events, such as science camps for children, Researcher's Night, Week of the Czech Academy of Sciences, etc. In 2021, the team organised a successful online seminar, *Viruses Around Us and Inside Us*, which responded to the current topic of the coronavirus and was viewed by 1,400 people.

Another important event co-organised by the centre in Olomouc is the annual festival The Earth on a Plate. It is aimed at the general public and includes film screenings, practical workshops, lectures, and discussions. We also held a Fair-trade Day that was attended by 150 children and 20 teachers. In August 2023, our genomics centre was visited by teachers who attended science camps for educators organised by the CAS.

The scientists from Olomouc give numerous public lectures and seminars and write articles for the media. Frequent topics are genetic modifications, gene editing, and the potential benefits of these technologies for breeding better crops.

Science for the "youngest researchers"

To give children and teenagers a personal experience with science, we offer many hands-on activities.

Since 2017, we have run a science club for children aged 6-12. Participants learn different methods of growing plants: on solid media *in vitro*, in a hydroponic solution, and in a classical soil substrate. They also try simple experiments and visit our laboratories.

Since 2020, we have organised summer day camps for the children of our employees. In 2023, three camps were run for a total of more than 50 kids. In addition to exciting trips and sports activities, the camp features experiments such as sowing plants on nutrient media in tissue culture hoods, growing plants under different stress conditions, and isolating flower dyes and separating them by paper chromatography.

Families with children can attend our Saturday workshops, held once a month at the institute in Prague. There is a great deal of interest in these events because they always have at least one activity that includes a visit to our laboratories or culture rooms.

Our programme for primary and secondary schools (not only in Prague) is also very popular. We either go to a school or a school visits our institute. The programme usually includes observations of anatomical structures under a binocular microscope, a presentation of basic laboratory instruments, or a simple experiment.

Figure 3. Young participants of science camps regularly visit our laboratories in Olomouc.

Journals

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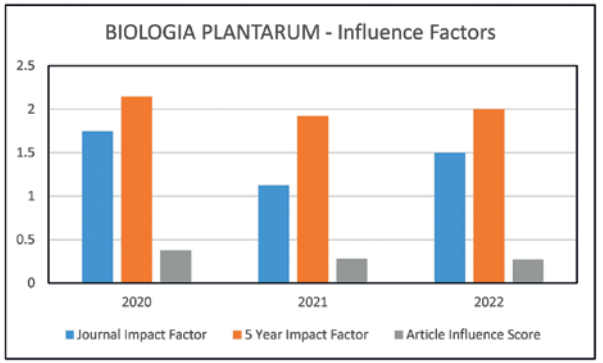
The Institute of Experimental Botany publishes two scientific journals, both with an impact factor. The journals were distributed by Springer Nature until the end of 2018, but since 2019, they have been published electronically in an open-science mode.

Biologia Plantarum is an international journal for experimental botany publishing **original scientific papers** and **brief communications, reviews** on specialised topics, and **hypotheses** in:

- physiology
- biochemistry and biophysics
- biology
- physiological anatomy
- ecophysiology
- genetics
- molecular biology
- biotechnology
- cell biology
- evolution
- abiotic and biotic stress
- plant-insect interactions
- plant-microbe interactions
- phytohormones
- autecology

In 2021, changes in article design and content (e.g. interactive references) were launched.

Biologia Plantarum is included in various scientific databases, e.g.: Cabells Journalytics, CABI, DOAJ, EBSCO, Google Scholar, Scopus, and WOS.

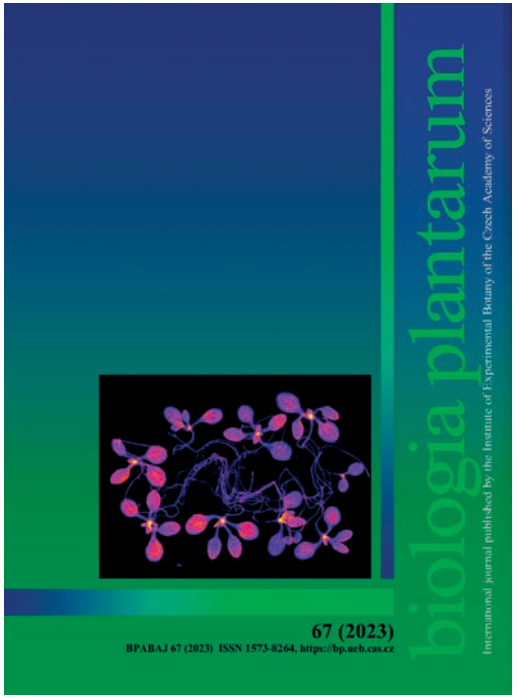


Special issues

- 2023, Special issue on Plant-Microbe Interactions (editors T. Kalachova and M. Janda) focused on molecular mechanisms of plant-microbe interactions, including the functioning of the plant immune system, details of pathogen recognition and biotic stress signaling, the coevolution of plants and pathogens, mechanisms of pathogenesis, the physiology of the infection process and resistance against pathogens
- 2023, Special issue of Czech Society of Experimental Plant Biology (editor M. Janda) intended for members of CSEPB, open to a broad spectrum of plant science topics

Editor-in-Chief

- Dr. rer. nat. Ing. Helena Plchová, Institute of Experimental Botany, Czech Academy of Sciences, Prague (*until May 2023*)
- Prof. RNDr. Viktor Žárský, CSc., Institute of Experimental Botany, Czech Academy of Sciences; Faculty of Science, Charles University, Prague (*since June 2023*)



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5-Year Impact Factor: 2.0

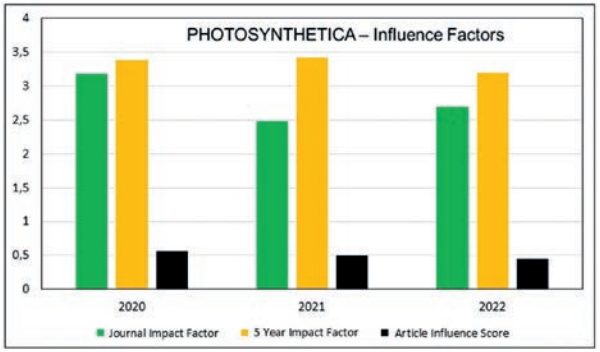
Photosynthetica publishes original scientific papers and brief communications, reviews on specialised topics, announcements, and reports covering a wide range of photosynthesis research and research including photosynthetic parameters of both an experimental and theoretical nature and dealing with physiology, biophysics, biochemistry, and molecular biology on one side and leaf optics, stress physiology, and the ecology of photosynthesis on the other side.

The journal publishes all research as Open Access. The articles are written in English. Four issues per year are produced.

Photosynthetica is directed by an international editorial board composed of Associated Editors.

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

The publisher is the Institute of Experimental Botany, Czech Academy of Sciences, Prague.



In 2019, the new online submission and review system *Actavia* was adopted. In 2021, changes in article design and content (e.g. interactive references) were launched. Starting in 2023, graphical abstracts are optional for our contributors.

Photosynthetica is included in various scientific databases: WOS, Scopus, DOAJ, Google Scholar, EBSCO, Mendeley, and PubMed (pending). Our Facebook and Twitter sites are active.

Special issues

- 2020, Special Issue in honour of Prof. Reto J. Strasser 75 (guest editor Hazem M. Kalaji)
- 2021, Special Issue in honour of Prof. Hartmut Karl Lichtenthaler (guest editor Roland Valcke)
- 2022, Special Issue dedicated to Prof. George C. Papageorgiou (guest editors Ondřej Prášil et al.)
- 2023, Special issue on Recent advances in photomodulation in higher plants, algae, and bryophytes (guest editors Marco Landi et al.)
- 2023, Special Issue in honour of Prof. Győző Garab (guest editors P. H. Lambrev and T. Janda)

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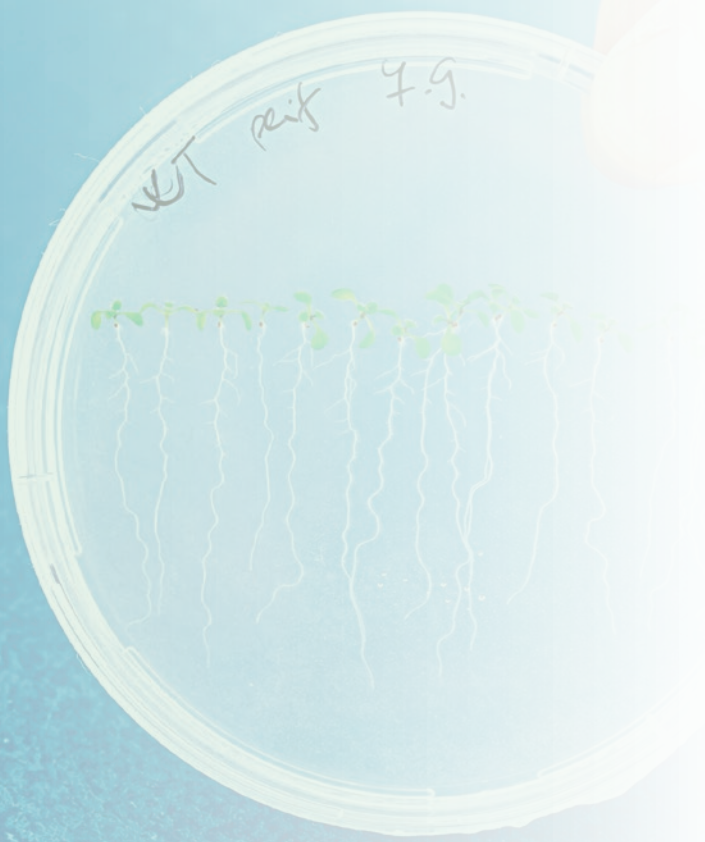
5-Year Impact Factor: 3.2



Research Projects 2021–2023

1. ‘AEGIL101000847, HORIZON 2020: Climate resilient orphan crops for increased diversity in agriculture (CROPDIVA)
2. 101090293, ERA MSCA: Visualization of plant hormone dynamics using bioluminescence during abiotic stress
3. 101094738, HORIZON: Promoting a plant genetic resource community for Europe (PRO-GRACE)
4. 58-8042-7-089-F, Plant tissue analysis for quantification of phytohormones in fruits (tomato)
5. 8J19FR001, ImmuneWall – plant immunity, cell wall and exocyst
6. 8J20FR032, Role of diacylglycerol kinases in the plant unfolded protein response
7. 8J23FR027, Uncovering components of non-host resistance of *Arabidopsis thaliana and Brassica carinata to Leptosphaeria* spp. using a genome-wide association study
8. CZ.02.01.01/00/22_088/0004581, Towards next generation crops (TANGENC)
9. EF16_013/0001775, Modernization and support of research activities of the national infrastructure for biological and medical imaging Czech-BioImaging
10. EF16_019/0000738, Centre for experimental plant biology
11. EF16_019/0000827, Plants as a tool for sustainable global development
12. EF18_046/0016045, Modernization of the national infrastructure for biological and medical imaging Czech-BioImaging
13. F648A74E0957319-C5E09A2--44E98FB0-BC73DE5--2443494EF551D43430BAD8E, Chemical screens of natural products for novel growth regulators – targeting gibberellin biosynthesis and signal transduction
14. GA17-00522S, A new insight into the role of phospholipase in leaf senescence
15. GA17-04607S, Light-cytokinin interactions in contrasting *Arabidopsis thaliana* ecotypes during cold acclimation and their impact on freezing stress responses
16. GA17-05151S, Phospholipid metabolizing enzymes as new components of salicylic acid signalling pathway
17. GA17-06548S, Foreign DNA in barley (*Hordeum spp.*) – are there any genomic enablers of horizontal gene transfer in grasses?
18. GA17-06613S, Phytohormone cross-talk during sub-zero acclimation
19. GA17-10280S, Variability in plant traits as a tool to cope with climate change – from phenotypes to genes and back again
20. GA17-10591S, Definition of physiological, metabolic and adaptation processes in the fern Pteris cretica growing on soils contaminated with arsenic
21. GA17-10907S, Environmental impact of noble metal nanoparticles
22. GA17-14048S, Spatial and temporal characterization of DNA replication in phylogeneticaly related plant species with contrasting genome sizes
23. GA17-23183S, Revealing pollen bZIP transcriptional regulons in *Arabidopsis thaliana*
24. GA17-23203S, mRNA inheritance as a mechanism of parental control over zygotic development
25. GA17-27477S, Multifaceted analysis of diacylglycerol kinase family in plants
26. GA18-02448S, The role of translation initiation factors in transcripts sequestration and activation in the male germline of angiosperm plants
27. GA18-07027S, Involvement of telomerase in the cell interactome

28. GA18-07724S, Circulation of anthelmintics in the environment – does it contribute to drug resistance development in parasitic nematodes?
29. GA18-08452S, Anthelmintics in plants – interactions with polyphenols biosynthesis and antioxidant defence
30. GA18-10349S, Gibberellin biosynthesis and signal transduction – identification of novel targets for plant growth regulation
31. GA18-11688S, Identification and characterization of *T. militinae* gene responsible for wheat APR resistance against powdery mildew
32. GA18-12178S, Unusual light management strategies of Photosystem II in Norway spruce
33. GA18-12197S, Analysis of nuclear organization and dynamics in endosperm tissues of barley
34. GA19-01383S, Modulation of steroid receptors in human cancer cells by brassinosteroids
35. GA19-01639S, Diamonds in the dust. Genetic basis of floral induction in the Chenopodium representatives with the contrasting photoperiodic response
36. GA19-01723S, Revealing the role of the nascent polypeptide associated complex during flower and fruit development of *Arabidopsis thaliana*
37. GA19-02699S, Transcriptome and hormonome of male gametophyte in the evolutionary context
38. GA19-05445S, Study of molecular mechanisms of vernalization in wheat
39. GA19-12262S, Physiological, biochemical, molecular and phylogenic characterization of metabolic pathways and mechanisms of cytokinin down-regulation in plants
40. GA19-13103S, Anatomical and physiological constraints as key factors governing plant vegetative regeneration from roots
41. GA19-13848S, Analyzing repair of toxic DNA-protein crosslinks in Arabidopsis
42. GA19-15609S, Sex-specific proliferation of transposable elements in plants
43. GA19-20303S, Karyotype structure and evolution in the banana family (Musaceae)
44. GA19-21758S, Good-Cop/Bad-Cop: Distinct roles of anionic phospholipids in plant endocytosis
45. GA19-23773S, PIN transporter-mediated auxin sinks in plant development
46. GA20-05095S, Role of the SMC5/6 complex and its interaction partners in DNA damage repair
47. GA20-10019S, Genomic dominance as a force shaping evolution of plant wide hybrids
48. GA20-11642S, Exocyst complex in moss secretory pathway and development
49. GA20-13587S, The evolutionary origin and significance of auxin transport
50. GA20-15621S, Natural agents and their derivatives for the neuroprotective therapy of Parkinson's disease
51. GA20-17984S, Molecular mechanisms of hormone and light signalling in shoots and roots in responses to abiotic stress
52. GA20-21547S, There and back again: the role of phosphatidic acid in the protein transport to and from the nucleus in plant cells
53. GA20-22875S, Organelle-specific gene expression and hormone dynamics during heat stress and high light responses
54. GA20-25308S, Modulation of cyclin-dependent kinases for targeted treatment of tumors with molecularly defined deregulation G1/S phase of cell cycle
55. GA21-02929S, Identification and characterization of imprinted genes during barley seed development
56. GA21-05497S, The role of Lhcb8 protein in the organization and function of the light-harvesting complex of plant photosystem II
57. GA21-07661S, Modulation of plant abiotic stress responses by regulation of cytokinin and purine interconversion enzymes
58. GA21-09254S, Greasing the way: the role of phospholipase A2 and extracellular lipids in pollen germination
59. GA21-15841S, Gene expression in the male germline: regulatory factors and their responsive elements
60. GA21-15856S, Translation regulation in plant dormant reproduction units – pollen and seeds
61. GA21-18794S, Genome-wide mapping of cis-regulatory elements controlling transcription in barley embryo
62. GA22-00204S, Molecular aspects of vernalization in temperate cereals
63. GA22-00301S, Genome dynamics in the context of sex chromosome evolution in the genus *Humulus*
64. GA22-00871S, Role of Condensin II Complex in the DNA Damage Repair of *Arabidopsis thaliana*
65. GA22-02108S, Elimination of the B chromosome in *Sorghum purpureosericeum*
66. GA22-02469S, Ultimate screening of wild barley genomes for panicoid DNA: a further step towards delimiting the foreign-ome in *Hordeum*
67. GA22-03731S, Cytonuclear interactions in plant auto- and allopolyploids
68. GA22-17435S, Mass spectrometry approaches for plant immunity studies
69. GA22-29717S, Unravelling the involvement of PRP8 in mRNA splicing during embryonic development and thermoresponse
70. GA22-35916S, The plasticity of protein-lipid interfaces in plant membranes perceived through evolutionary lenses
71. GA23-04887S, Identification of genes controlling nondisjunction of the maize B chromosome
72. GA23-05389S, Novel CB2 and BChE modulators against Parkinson's disease and related pathologies
73. GA23-05564S, FASS/TON2 functions in moss cell morphogenesis and phylogenetic insights into the TTP complex evolution

74. GA23-06571S, What orang-utans can teach us: plants used for self-medication as a potential source of bioactive substances with amoebicidal and antigiardial effects
75. GA23-07000S, Exploring the regulatory roles of a newly discovered subgroup of bZIP transcription factors in plant development
76. GA23-07363S, Alternative splicing fine-tunes cytokinin perception in planta
77. GA23-07733S, All roads lead to ROS: spatio-temporal regulation of pollen NADPH oxidases
78. GA23-07813S, Revealing the nature of auxin regulation of cell division and expansion
79. GA23-08067S, Modulating ABA signaling pathways by alternative splicing of ABI2
80. GC18-10515J, Mechanisms of parasitic RNA propagation and elimination in male germline studied on economically important viroid species
81. GC18-14450J, MITOCHROM: Three-dimensional organization of nuclear chromatin in plants across the cell cycle
82. GC21-20936J, Development and validation of a suite of imaging reporters for plant hormones based on genetically encoded bioluminescence
83. GF21-08021L, Deep cell biology of plant cell polarity
84. GJ17-21581Y, Auxin homeostasis on subcellular level
85. GJ18-12338Y, B chromosome evolution in the tribe Andropogoneae
86. GJ19-13375Y, The role of actin cytoskeleton in lytic degradation of auxin plasma membrane carriers
87. GM22-35680M, 4D plate – spatiotemporal dynamics of cell plate development
88. LM2018129, National infrastructure for biological and medical imaging Czech-BioImaging
89. LM2023050, National infrastructure for biological and medical imaging
90. LTAIN19030, A study on pollen competition in *Arabidopsis thaliana* hybrids
91. LTAUSA17081, Hormonal mechanisms of plant acclimation to heat and cold stresses
92. LTAUSA18004, Evolution of diploid-polyploid complex of *Chenopodium album* agg. Joint or parallel evolution of North American and Eurasian species?
93. LTAUSA18115, The role of Arabidopsis Lorelei-like GPI anchored proteins (LLGs) in pollen tube reception by the female gametophyte
94. LTC18026, Analysis of 3D organization of nuclear genome in plants with contrasting amount of DNA
95. LTC18034, Characterization of nuclear proteomes in the male germline and their implications under standard and stress conditions
96. LTC18065, Selective COX-1 inhibition as cardioprotective therapeutical target
97. LTC18073, RNA maturation and auxin response – a spot for mRNA methylation
98. LTC20028, Nuclear regulatory landscape of bZIP transcription factors in plant reproductive development (REPROZIP)
99. LTC20050, The application of CRISPR-Cas9 for the creation of multiple mutants in the genes coding for the nascent polypeptide associated complex
100. LTC20066, Genome editing in plants – IEB CAS contribution
101. LTT19007, Collaboration with CIMMYT on the study of diversity and evolution of maize B chromosome
102. LTT19009, Collaboration with the International Institute of Tropical Agriculture on comparative genetic and epigenetic analysis of plantains (AAB bananas)
103. LUAUS23236, Early auxin response – the role of m6A methylation in mRNA?
104. QK1710302, Improvement of common wheat tolerance to drought, frost, *Phytophthora infestans* and *Fusarium* head blight using genomics and proteomics approaches
105. QK1710397, Compatibility characterization of the relationship between originators of phoma blackening of the stem and varieties of winter oilseed rape as a basis for increasing the profitability of that crop in the Czech Republic
106. QK1910290, Development and application of molecular genetic methods for the rationalization of sweet cherry breeding practices (*Prunus avium L.*)
107. QK21010207, Diversification and strengthening the competitiveness of aquaculture by promoting aquaponics as an innovative technology for agricultural food production
108. QK21010390, Modern breeding using molecular-genetic methods to make selection and practical application of new apple tree varieties with high resistance to significant apple diseases faster and more effective
109. QK22010268, Development of systems for fruit crops genotyping and their implementation to the practice
110. QK22010293, Genomic and proteomic characteristics of wheat resistance to selected abiotic and biotic stresses
111. QK22020062, Identification of surviving individuals of forest tree species in calamity areas, their rescue and research of their resistance
112. SS06020173, Methods reducing the risks of circulation of veterinary drugs in the environment
113. TH80020004, Semitransparent PV coatings for greenhouse application
114. TJ04000490, New apple varieties suitable not only for organic production
115. TM04000026, Effective diagnostic methods for tobamoviruses and tospoviruses, improving disease surveillance and tomato resistance to these pathogens
116. TN01000062, Biotechnological centre for plant genotyping
117. TP01010037, Support for the process of commercializing the results of research and development at the Institute of Experimental Botany AS CR v.v.i. from 2020
118. UNDP-IRH-00048, Introduction of transcriptomic analysis through the Challenge Fund: advancing biological insights on plant resistance to candidatus *Phytoplasma solani*

Publications 2021–2023

Authors in **bold** are from the Institute of Experimental Botany, Czech Academy of Sciences. Corresponding authors are marked with an asterisk.

2021

1. Ackerman-Lavert M, Fridman Y, Matosevich R, Khandal H, Friedlander-Shani L, Vragovic K, Ben El R, Horev G, **Tarkowská D**, Efroni I, Savaldi-Goldstein S* (2021) Auxin requirements for a meristematic state in roots depend on a dual brassinosteroid function. CURRENT BIOLOGY 31: 4462-4472.
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6. Ameztoy K, Sánchez-López AM, Muñoz FJ, Bahaji A, Almagro G, Baroja-Fernández E, Gámez-Arcas S, De Diego N, **Doležal K**, **Novák O**, **Pěnčík A**, Alpizar A, Rodriguez-Concepción M, Pozueta-Romero J* (2021) Proteostatic regulation of MEP and shikimate pathways by redox-activated photosynthesis signaling in plants exposed to small fungal volatiles. FRONTIERS IN PLANT SCIENCE 12: 637976.
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9. Bai YC, Fernández-Calvo P, Ritter A, Huang AC, Morales-Herrera S, Bicalho KU, **Karady M**, Pauwels L, Buyst D, Njo M, Ljung K, Martins JC, Vanneste S, Beeckman T, Osbourn A, Goossens A*, Pollier J (2021) Modulation of *Arabidopsis* root growth by specialized triterpenes. NEW PHYTOLOGIST 230: 228-243.
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12. **Bednářová M**, **Karafiátová M**, **Hřibová E**, **Bartoš J*** (2021) B chromosomes in genus *Sorghum* (Poaceae). PLANTS 10: 505.
13. Bekturova A, Oshanova D, Tiwari P, Nurbekova Z, Kurmanbayeva A, Soltabayeva A, Yarmolinsky D, Srivastava S, **Turečková V**, **Strnad M**, Sagi M* (2021) Adenosine 5’phosphosulfate reductase and sulfite oxidase regulate sulfite-induced water loss in *Arabidopsis*. JOURNAL OF EXPERIMENTAL BOTANY 72: 6447-6466.
14. Belser C, Baurens FC, Noel B, Martin G, Cruaud C, Istace B, Yahiaoui N, Labadie K, **Hřibová E**, **Doležel J**, Lemainque A, Wincker P, D’Hont A, Aury JM* (2021) Telomere-to-telomere gapless chromosomes of banana using nanopore sequencing. COMMUNICATIONS BIOLOGY 4: 1047.
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17. **Blavet N**, Yang H, Su HD, **Solanský P**, Douglas RN, **Karafiátová M**, **Šimková L**, Zhang J, Liu YL, Hou J, Shi XW, Chen C, El-Walid M, McCaw ME, Albert PS, Gao Z, Zhao CZ, Ben-Zvi G, Glick L, Kol G, Shi JH, **Vrána J**, **Šimková H**, Lamb JC, Newton K, Dawe RK, **Doležel J**, Ji TM, Baruch K, Cheng JL, Han FP*, Birchler JA*, **Bartoš J*** (2021) Sequence of the supernumerary B chromosome of maize provides insight into its drive mechanism and evolution. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 118: e2104254118.
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20. Camalle MD, Sikron N, Zurgil U, Khadka J, Pivonia S, **Pěnčík A**, **Novák O**, Fait A, Tel-Zur N* (2021) Does scion-rootstock compatibility modulate photoassimilate and hormone trafficking through the graft junction in melon-pumpkin graft combinations? PLANT SCIENCE 306: 110852.
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26. **Diachkov MV**, **Ferrer K**, **Oklešťková J**, **Rárová L**, **Bazgier V**, **Kvasnica M*** (2021) Synthesis and biological activity of brassinosteroid analogues with a nitrogen-containing side chain. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 22: 155.

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28. **Doležel J***, Lucretti S, **Molnár I**, **Cápal P**, Giorgi D (2021) Chromosome analysis and sorting. CYTOMETRY PART A 99: 328-342.

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Patents 2021–2023

2021

1. **Havlíček L,** Šturc A, **Řezníčková E, Jorda R, Kryštof V, Strnad M.** 5-Alkythio-7[(4-arylbenzyl) amino]-1(2)H-pyrazolo[4,3-D]pyrimidines treatment of lymphoma.
   Issue date: 06.10.2021. **Patent No. EP3749670B1.**
2. **Plíhalová L, Zatloukal M, Doležal K, Strnad M, Walla J, Voller J.** Mesylátová sůl para-topolinu, přípravky ji obsahující, a její použití. [Para-topolin mesylate salt, preparations containing it, and its use.]
   Issue date: 10.06.2021. **Patent No. CZ308865.**
3. **Havlíček L,** Šturc A, **Řezníčková E, Jorda R, Kryštof V, Strnad M.** 5-Alkythio-7[(4-arylbenzyl) amino]-1(2)H-pyrazolo[4,3-D]pyrimidines treatment of lymphoma.
   Issue date: 30.11.2021. **Patent No. US11,186,583 B2.**

2022

4. Cankař P, Tomanová M, Jedinák L, **Kryštof V, Jorda R, Řezníčková E, Strnad M.** Substituované purinové sloučeniny jako inhibitory proteinkináz, jejich použití jako léčiva a farmaceutické přípravky obsahující tyto deriváty. [Substituted purine compounds as protein kinase inhibitors, their use as medicaments and pharmaceutical preparations containing these derivatives.]
   Issue date: 17.08.2022. **Patent No. CZ 309356.**

Apple Varieties 2021–2023

2021

1. **Černý R**, **Zima J**, **Tupý J**, **Louda O**. Variety of *Malus domestica Borkh.*, **RUBELIT**.
   Issue date: 30.06.2021. Identification No. CH 21.2929.
   A new and distinct late dessert apple variety which is characterized by medium to strong vigor, medium to large, red colored fruits with globose shape and over color with strongly defined stripes, very good eating qualities, long storability, scab resistance based on Vf gene, resistance to fire blight and tolerance to powdery mildew. The variety is without special agrotechnical requirements, especially suitable for organic cultivation as well as integrated production.
2. **Černý R**, **Zima J**, **Tupý J**, **Louda O**. Variety of *Malus domestica* Borkh., **MAGENTA**.
   Issue date: 30.06.2021. Identification No. CH 21.2930.
   A new and distinct ornamental apple variety with narrow columnar growth, attractive purple-red color of flowers, greyish-purple, later green leaves and on average 25 mm, broadly globose, purple-red fruits. These ornamental fruits remain on the tree over the winter and are valuable food for birds. The variety is, apart from ornamental purposes, suitable for pollination of apple tree plantings with early to medium time of flowering.
3. **Černý R**, **Zima J**, **Tupý J**, **Louda O**. Variety of *Malus domestica* Borkh., **LILAC**.
   Issue date: 30.06.2021. Identification No. CH 21.2931.
   A new and distinct ornamental apple variety with columnar growth, characterized by pink to light purple color of flowers and greyish-purple, later green leaves. Ornamental fruits with mean size of 33 mm are flat globose, slightly ribbed, with red over color on yellow background. Fruits of this variety remain on the tree over the winter as food for birds. Apart from ornamental purposes, this variety is suitable for pollination of apple tree plantings with early time of flowering.
4. **Černý R**, **Zima J**. Variety of *Malus domestica* Borkh., **ACROBAT**.
   Issue date: 20.09.2021. Identification No. EU 59370.
   A new and distinct columnar apple variety with autumn term of harvest maturity and resistance to scab and powdery mildew. It is characterized by orange-red fruits of a very good balanced acid/sugar flavor. Fruits are suitable for direct consumption or for medium long storage. The variety is particularly suitable for home gardens as a solitaire or for narrow hedges. It does not have any special growing requirements.

2022

5. **Černý R**, **Zima J**, **Tyč D**. Variety of *Malus domestica* Borkh., **UEB 6481**.
   Issue date: 15.11.2022. Identification No. UKZUZ 220242/2022.
   A new and distinct late dessert apple variety is characterized by medium vigor and orange-red, medium-sized globose fruits with firm, very juicy flesh, excellent taste and long storability. The variety is thanks to its robustness against scab, mildew and fire blight particularly suitable for organic cultivation as well as integrated production.

2023

6. **Černý R, Zima J, Tyč D.** Variety of Malus domestica Borkh., **UEB 6481**.
   Issue date: 6.2.2023. Identification No. EU 63132.
   A new and distinct late dessert apple variety is characterized by medium vigor and orange-red, medium-sized globose fruits with firm, very juicy flesh, excellent taste and long storability. The variety is thanks to its robustness against scab, mildew and fire blight particularly suitable for organic cultivation as well as integrated production.

Scientific Report
2021–2023

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