Description of the main research directions investigated by the institute

To highlight the main research directions of IEB during the evaluated period 2015-2019, I will first present a snapshot summary of key data describing the institute overall activities during the last five years. In total, scientists of the institute published 778 articles in journals with impact factors. This corresponds to almost 38 % increased activities compared to the previous evaluation periods (2010 - 2014: 565 articles, 2005 -2009: 377 articles). As such, for the available space, the description of the main directions of the institute will be limited to only the most important and highest quality matters, while briefly highlighting many other results that are undeniably also noteworthy and made significant contribution to the institute outstanding activities.

Majority of our articles were published in collaboration with our international colleagues (almost 69 % of articles). Corresponding author affiliated with IEB were present in 275 articles (35.3 %, a slightly increase compared to the previous evaluation). During the evaluation period, we have published our results in the most prestigious journals, including *Nature* (1), *Nature Biotechnology* (1), *Nature Communications* (4), *Nature Genetics* (1), *Nature Plants* (6), *Nature Protocols* (1), *Annual Rev. Plant Biol.* (1), *Crit. Rev Plant Sci.* (1), *Trends in Cell Biol.* (1), *Trends in Plant Sci* (1), *Plant Physiology* (31), *New Phytologist* (13), *Molecular Plant* (6), *Current Biol*. (2), *Developmental Cell* (1), *PNAS* (9), *Plant Cell* (8), *Genome Biol*. (4), *Microbiome* (1), *J. Am. Chem Soc.* (1), and many others.

Among the institutes of the Czech Academy of Sciences, the IEB represents the absolute top in the number of filed patents and Plant Variety Rights. In the evaluated period 2015 - 2019, IEB obtained 24 patents (13 x Czech Republic, 4 x European Union, 2 x USA, 2 x Canada, 2 x South Africa, and 1 x South Korea). Some of these patents have already been licensed, and other licensing agreements are currently being negotiated. The IEB Station of Apple Breeding alone obtained a total of 49 Plant Variety Rights certificates for varieties bred at the IEB spanning four continents (16 x Czech Republic, 14 x European Union, 9 x USA, 6 x Switzerland, and 1x South Africa, Ukraine, Armenia and Morocco). The vast majority of these Plant Variety Rights have reached commercial license agreements, which generate a significant financial return for the IEB.

Due to the limited scope of the part, the achieved results cannot be described in more detail, so the following part is instead summarises a general institute research directions. More detailed information can be obtained in Section *3-4 Report on the Research Activity* of individual teams. It must be noted that the research directions of individual teams (and thus of the whole institute) is strongly dependent on the financial resources provided. With few exceptions, the obtained funds are short-term, typically for three years. Thus, medium or even long-term planning of research directions is thus only theoretical.

Main research directions

IEB research can be broadly divided into five research directions. These include research on structural and functional genomics, phytohormones, pollen biology, and polar growth, and research into the interactions of plants with the external environment. These directions only partially overlap between the research teams. Some research directions have a long tradition in IEB while others have been established relatively recently mostly in collaboration with international colleagues and are associated with individual team leaders. The main research directions are completed by applied

science. This is represented by the activity of the Station of Apple Breeding for Disease Resistance.

Research direction concerning structural and functional genomics is represented mainly by the work of two teams, the larger team at the Centre of Plant Structural and Functional Genomics and the smaller team of Plant Reproduction Laboratory. Part of the Laboratory of Pollen Biology also contributed to this IEB direction.

The research of the *Centre of Plant Structural and Functional Genomics* centered on the nuclear genome organization, its evolution, and function. While this fundamental research unravels the molecular mechanisms of inheritance and the way plant phenotype is determined, this work also delivers knowledge and concepts to support crop breeding. Most of the work concentrated on agronomically important species and the emphasis was on polyploids and, in particular, those which originated by interspecific hybridization. A majority of the research involved cereals from the family *Triticeae*, forage and amenity grasses, as well as bananas.

The most prestigious research project was the **sequencing of the huge genome and complex genome of hexaploid bread wheat**. IEB was one of the key partners of the global effort managed by the International Wheat Genome Sequencing Consortium (IWGSC 2018). Our main tasks were the coordination of sequencing wheat chromosome 4A (Balcárková et al. 2017), and sequencing the short arms of chromosome 3D (3DS) (Cviková et al. 2015, Holušová et al. 2017) and chromosome 7D (7DS) (Tulpová et al. 2019a). We developed a protocol for the construction of optical maps from particular chromosome arms (Staňková et al. 2016). We participated in two other prestigious international projects that produced **reference genomes of barley** (Mascher et al. 2017) **and pea** (Kreplak et al. 2019). The newly generated genome sequences of barley and wheat and chromosome-based experimental approaches were utilised to **identify and clone agronomically important genes** (Ivaničová et al. 2016, 2017, Shorinola et al. 2016, 2017, Janáková et al. 2019, Tulpová et al. 2019b). We collaborated with several foreign research groups on cloning important genes acting in phytopathological resistance in wheat and barley, which included stem rust resistance gene Sr50 (Mago et al. 2015), leaf rust resistance gene Lr22a (Thind et al. 2017) and the powdery mildew resistance gene Pm21 (Xing et al. 2018).

Focused primarily on *Festulolium* (*Festuca* x *Lolium*) **grass hybrids of agricultural interest**, we made significant advances in understating the genome organisation and stability in plant interspecific hybrids (Kopecký et al. 2017, 2019, Perničková et al. 2019ab).

Significant advances were made in the **analysis of the three-dimensional organisation of plant genomes** (Němečková et al. 2019, Koláčková et al., 2019), enormous progress was also achieved during the analysis of **the mechanisms of genome stability maintenance** in the model plant species *Arabidopsis thaliana* (Díaz et al. 2019).

In collaboration with the Bioversity International (France), we characterised the **genetic diversity of** all accessions stored in the International **Musa** Germplasm Transit Centre (Belgium) (Christelová et al. 2017), characterised genotypes of wild relatives of cultivated banana (Čížková et al. 2015; Němečková et al. 2018) and new *Musa* accessions collected during exploratory missions organised by the Bioversity International (Sardos et al. 2018). Moreover, in collaboration with the International Institute of Tropical Agriculture (Nigeria), we cooperated on breeding materials genotyping and participated in association gene mapping projects (Nyine et al. 2018).

These studies were complemented by the analysis of evolutionary chromosome rearrangements in *Musa* using the method for chromosome-specific oligo painting FISH (Šimoníková et al. 2019).

The *Laboratory of Plant Reproduction* is interested in two aspects of functional genetics, the **genetic background of flowering in the species of *Chenopodium*** (Štorchová et al. 2015; Drabešová et al. 2016; Mandák et al. 2018; Štorchová 2019) **and mitochondrial genomes and transcriptomes of *Silene vulgaris* in the context of cytoplasmic male sterility** (CMS) (Stone et al. 2017; Štorchová et al. 2018). Both topics have been very attractive and important lately. *Chenopodium quinoa* has attracted attention as a promising crop in semi-desert regions without sufficient water resources. The knowledge on the floral induction in close relatives of *C. quinoa* helps to understand this essential process in the crop. The genus *Silene* is known for the largest mitochondrial genomes in the plant kingdom and for the extreme rate of mitochondrial genomic rearrangement, which makes the investigation of the *S. vulgaris* organelles highly topical.

Our experience gained in the transcriptomic studies of *S. vulgaris* we applied to the analysis of the mitochondrial transcriptome of *Silene noctiflora*, the species with an extremely large mitogenome (Wu et al. 2015). Much less is known about plastid transcriptomes. We used the same RNAseq data, which were analysed for mitochondrial transcriptomes, in the construction of plastid transcriptomes in female and hermaphrodite individuals of *S. vulgaris*. We found no significant differences between the two genders (Krüger et al. 2019).

On similar grounds, the Group of DNA Repair, part of *Laboratory of Pollen Biology* is for a long time interested in DNA stability research. We analysed single and double-strand break (SSB, DSB) repair using a "Comet assay", a microscopic-based electrophoretic analysis of single cell genomic DNA damage. The use of this innovative technique opened up wide possibilities of collaboration, which has produced a fruitful number of excellent works published in renowned high impacted journals. (e.g., Goffova et al. 2019).

Plant hormone research direction has a long tradition at IEB. The consequence of this tradition is that two large laboratories, the *Laboratory of Growth Regulators (LGR)* and the *Laboratory of Hormonal Regulation (LHR)*, investigate preferentially the spatio-temporal dynamics of phytohormones in several model species. However, other teams (e.g. *Laboratory of Biologically Active Substances (LBAS)* and *Laboratory of Plant Biotechnologies (LPB)*) also contribute to phytohormone research. IEB has thus become an important player in the field of hormone research, which is reflected, for example, by the fact that it has been regularly organising Auxin and Cytokinin international conferences in Plant Development for almost half a century. Over time, the need for accurate quantification of phytohormone levels has emerged in both main laboratories, and both have reached world standards in the field. The LGR bioanalytical subgroup is a recognised world leader in the field. Both laboratories developed unique methods, in which a large number of hormones in small plant samples could be measured (Dobrev et al. 2017, Novák et al. 2017, Šimura et al. 2018, Nahar et al. 2019). Detection of specific hormones is also possible at the level of organ or even individual cells, as pioneered by tissue- and cell-specific developed approaches (Antoniadi et al. 2015, Novák et al. 2017, Pařízková et al. 2017). Currently, the most suitable and most used analytical technology for phytohormone analysis is based on liquid chromatography-tandem mass spectrometry ((U)HPLC-MS/MS). This methodology was gradually developed for virtually all plant hormones (cytokinins,

auxins, strigolactones, JAs, ABAs, gibberellins, brassinosteroids) and some other related substances (tryptophan-related neuroactive substances, ecdysteroids, phenolics, isoflavonoids, tocopherols, ingenol, phytocannabinoids, karrikins and other phenylpropanoids). Excellently mastered bioanalytical methods naturally led to many very productive collaborations, from which world-class publications arose (their list and a more detailed description can be found in the chapters dedicated to individual teams).

In addition to advanced analytical methods, it is mainly the research of auxin and cytokinins, and to a lesser extent other phytohormones, on the level of mechanisms of regulation for plant development and responses to abiotic and biotic changes.

Research on auxin has produced significant outputs, especially in the field of **regulation of auxin transport and homeostasis, the evolution of auxin transport mechanisms, and cellular biology of auxin transporters**.

We revealed a **new way of regulation of the amount of auxin carriers on cell membranes** based on auxin-driven gene expression and cooperation between auxin influx and efflux transporters (Müller et al. 2019). We characterised t**he role of the nitrate transceptor NRT1.1** by testing the auxin transport of mutated versions carrying point mutations in the phosphorylation site at amino acid residue T101 (Bouguyon et al. 2015). Auxin transport assays in tobacco cell lines were used to present the first 3-dimensional atomic resolution **atlas of the substrate requirements of AUX1 transporter** (Hoyerová et al. 2018). We significantly contributed to the identification of **a novel crosstalk between auxin transport and brassinosteroids**, based on their inhibitory effect on the endocytosis of the auxin efflux carrier PIN2 (Retzer et al. 2019), and to the characterisation of the noncanonical member of the PIN family of auxin transporters, PIN6 (Simon et al. 2016). We provided the first experimental evidence on **the evolutionary origins of PIN-mediated carrier-driven auxin efflux** (Skokan et al. 2019). In the field of cell biology of auxin transporters, we have uncovered their differential dynamics within the plasma membrane, with **the pioneering usage of raster image correlation spectroscopy (RICS) in plants** (Laňková et al. 2016). We identified a novel action of ARF-GEF GNOM-LIKE protein1a, NtGNL1a from tobacco, which was shown to be involved in the endocytosis of PIN proteins (Jelínková et al. 2015). We identified **the ROTUNDA3 protein as a regulator of the protein phosphatase 2A-driven PIN recycling** and revealed its importance in auxin transport during plant developmental (Karampelias et al. 2016). We discovered that the **DIOXYGENASE AtDAO1** enzyme represents the major pathway for auxin oxidation in *Arabidopsis* (Porco et al. 2016). The unexpected capacity of local **auxin metabolism** to modulate homeostasis and spatial distribution of free auxin was shown in specialised organs such as hypocotyls in response to shade and high temperature (Zheng et al. 2016). We described that maternal auxin supply contributes to early embryo patterning in *Arabidopsis* (Roberts et al. 2018) and selective auxin agonists induce specific AUX/IAA protein degradation to modulate plant development (Vain et al. 2019). Finally, our new high-resolution confocal microscopy platform for in vivo observations of *Arabidopsis* seedling roots in natural gravitational vectors allowed to provide *in vivo* evidence for **the presence of unique organisation of PIN1 auxin efflux carrier in dividing provasculature cells** (Marhavá et al. 2019).

Research on cytokinins has been focused on their **biosynthesis and metabolism in a range of developmental and environmental contexts**. We also addressed the evolution of their biosynthesis, including relation to other phytohormones.

We identified **new components of the hormonal crosstalk network** as potential candidates explaining the regulation of bud outgrowth by sucrose (Barbier et al. 2015) and the REPRESSOR OF CYTOKININ DEFICIENCY 1 (ROCK1) as an Er-localised transporter (Nieman et al. 2015). We also showed that CKX1 is a type II single-pass membrane protein that localises predominantly to the endoplasmic reticulum (ER) in *Arabidopsis* (Nieman et al. 2018) and discovered that responses to systemic nitrogen signalling in *Arabidopsis* roots involve trans-zeatin in shoots (Poitout et al. 2018). We continued in our effort with the characterisation of so far overlooked cytokinin forms, **cis-zeatins**. We have shown that cis-zeatin-type cytokinins occur in cyanobacteria and algae (Žižková et al. 2017) as well as in mosses (Záveská Drábková et al. 2015). The involvement of *cis*-zeatins in the modulation of plant defence responses against pathogen infections (Trdá et al. 2017), during somatic embryogenesis in conifers (Vondráková et al. 2018) and during phosphate starvation in *Arabidopsis* roots (Přerostová et al. 2018). We have shown **cytokinin *N*-glucosides** to be subject to metabolic conversions that differ between *N*-glucosides of N^6-(Δ^2-isopentenyl)adenine and *trans*-zeatin in *Arabidopsis* (Hošek et al. 2019). We have significantly contributed to the understanding of **the molecular mechanisms engaged in hormonal control of plant development and plant responses to salinity**. Using cultured and wild relative tomato species (*Solanum lycopersicum, Solanum chilense*), we have demonstrated the involvement of phytohormones in these processes and specified the roles of *SlIPT3* and *SlIPT4* genes coding for CK biosynthesis (Žižková et al. 2015). Levels of cytokinins have been shown to be upregulated during autotetraploidization of energy willow *Salix viminalis* (Dudits et al. 2016).

Besides auxin and cytokinin studies, we are globally renowned for the contribution to the expansion of a number of cytokinins**,** especially the aromatic cytokinins **topolins** and their **olomoucine**-derived derivatives. Recently, we discovered **several new natural phytohormones** (Arnold et al. 2016, Floková et al. 2016, Wasternack and Hause 2016) and we have continued to develop increasingly effective **cyclin-dependent-kinase inhibitors**, leading to the discovery of several other potent compounds with various structural motifs (Jorda et al. 2015; Vymětalová and Kryštof, 2015; Baltus et al. 2016; Ajani et al. 2018). Cytokinins also have multiple activities in animals. Kinetin and trans-zeatin can reduce the levels of several ageing markers in human fibroblasts. Kinetin can also protect mice against oxidative and glyoxidative stress and prolong fruit fly lifespan. Additionally, several cytokinins are currently used in cosmetics (Hönig et al. 2018; Kadlecová et al. 2019; Voller et al. 2019). Discovery of cytokinin activities in animals supported the interest of LGR in medicinal chemistry. This direction developed enormously within the last five years and nowadays includes the development of effective cyclin-dependent-kinase (CDK) inhibitors, a study of triterpene-based compounds for anticancer activity, adaptogenic effects and supramolecular characteristics and searching for new anti-inflammatory and anticancer substances. Moreover, anti-proliferative, anticancer, anti-angiogenic, anti-viral and anti-bacterial properties of brassinosteroids and ecdysteroids and their derivatives were tested (for details see the profile of LGR).

Additionally, strigolactone mimics based on triazolide scaffold were prepared using a straightforward three-step reaction procedure, which notably simplifies their synthesis when compared to natural strigolactones. (Dvořáková et al. 2017, 2019). Similarly, a simple two-step synthesis of a new type of strigolactone mimics based on resorcinyl scaffold was published (Dvořáková et al. 2018).

So far, the most detailed **analysis of endogenous phytohormones over the course of somatic embryogenesis** (SE) in Norway spruce (Vondráková et al. 2018)

was performed. Here we have presented for the first time in conifer SE both the evidence for the involvement of the non-indole auxin phenylacetic acid, *cis*-zeatin and dihydrozeatin-type cytokinins, or patterns of jasmonates and salicylic acid. However, phytohormones remain the central regulators of SE, other substances (e.g. **polyamines**) are also active in the process. We described the effect of elevated putrescine levels on somatic embryo development (Vondráková et al. 2015).

Excellent research on phytohormones is made possible by the availability of radiolabelled phytohormones (and other related compounds) whose synthesis is performed in the *Isotope Laboratory.*

Molecular basis of pollen biology and polar tip growth are research directions of two teams, *Laboratory of Pollen Biology* and *Laboratory of Cell Biology*. Both build on the long tradition of pollen research at IEB, which was founded by Dr. Tupý in the late fifties of the last century.

Laboratory of Pollen Biology is focused mainly on several aspects of pollen development and pollen communication with female tissues. We applied the Agilent 44K tobacco gene chip to conduct **the first comprehensive developmental transcriptomic analysis of the tobacco male gametophyte** representing the first plant species shedding bicellular pollen (Bokvaj et al. 2015). In the follow-up projects, they focused on the **identification of pollen-expressed transcription factors** involved in the regulation of male gametophyte development. Phenotype screen was enabled by the optimisation of our previously published protocol for large-scale separation of developing spores (Dupľáková et al. 2016). We further functionally analysed the **regulatory network of bZIP transcription factors** (Gibalová et al. 2017). In our analysis of fully sequenced plant proteins, we identified **new evolutionary lineages of callose synthase subfamilies** and established a basis for understanding their functional evolution in terrestrial plants (Záveská Drábková and Honys 2017). Since 2015, we have applied microarray and LC-MS/MS to **analyse the transcriptome and proteome covering three cytoplasmic sub-fractions containing mRNAs** at different translational status. This comprehensive analysis led to the isolation of genes whose transcripts enter the large ribonucleoprotein particles storage compartment during pollen maturation associated with "ready-to-translate" complete translation initiation machinery and later shift to actively translating polysomes upon pollen hydration and activation of pollen tube growth (Hafidh et al. 2018). We showed **the first phosphoproteomics data on activated pollen**, where the position of the respective phosphorylation sites was demonstrated (Fíla et al. 2016). Pollen tube secretomics has uncovered **novel pistil-dependent pollen tube-secreted proteins critical for establishing male-female signalling interaction** for successful sperm cells delivery and fertilisation and as means to overcome interspecific pre-zygotic barriers (Hafidh et al. 2016).

The *Laboratory of Cell Biology* significantly expanded **understanding of the exocyst role in polar expansion** of several plant cell types. We also linked the specific exocyst localisation with a **distinct distribution of anionic membrane lipids** in the plasma membrane. In collaboration with the laboratory of Prof. Shaul Yalovsky (Tel-Aviv University), we performed a **detailed characterisation of the molecular function of SEC3 exocyst subunit** during pollen tube tip growth (Bloch et al. 2016). Along the same lines, we performed a comparative **functional studies of multiple exocyst subunit EXO70** in Arabidopsis and tobacco pollen (Sekereš et al. 2017, Synek et al. 2017). We have participated in the **pioneering research describing the phospholipid-based identity of plant membranes** (Platre et al. 2018) and described

the **link between phospholipid localisation and targeting of exocyst subunits** (Pleskot et al. 2015, Kubátová et al. 2019). We have described the **role of specific exocyst subunit EXO70H4** in the maturation of *Arabidopsis* trichomes, where it regulates the deposition of distinct cell wall components (Kulich et al. 2015, 2018). Since the exocyst subunit EXO70 evolved from three conserved clades with assumed distinct roles, we also studied the function of the moss P**hyscomitrella patens exocyst subunit PpEXO70.3d** from the yet poorly characterised EXO70.3 clade (Rawat et al. 2017). Another role for the exocyst was found in tracheary elements, where we showed that microtubule-dependent targeting of the exocyst complex is necessary for xylem development in *Arabidopsis* (Vukašinović et al. 2017). We also described a novel and unexpected **role of exocyst complex in the development and organisation of the root-shoot junction** in *Arabidopsis* (Janková-Drdová et al. 2019) and participated in a multi-lab study that led to the **discovery of exocyst-targeting inhibitor blocking exocytosis** (Zhang et al. 2016).

We continued in the **characterisation of the formin family** of cytoskeleton-membrane organisers. **FH1**, the major member of the formin family, was found to regulate the morphogenesis of pavement cells and we described its dynamic localisation pattern during cell ontogeny (Rosero et al. 2016, Oulehlová et al. 2019). Our research also led to the **development of new methodical approaches** for the quantification of cytoskeletal dynamics in plants (Cvrčková and Oulehlová 2017) and the **role of reactive oxygen species in polar growth of pollen tubes** (Jiménez-Quesada et al. 2019).

The research direction dealing with the interactions between plants and the external environment is the most multi-team at IEB. Some teams deal predominantly with this topic, e.g. Laboratory of Signal Transduction, Laboratory of Pathological Plant Physiology or Laboratory of Plant Biotechnologies, while others touch on this topic in terms of their primary research direction (e.g. the role of hormones in stress responses). Very often, the outputs mentioned below are the results of inter-team collaboration at IEB. The sharing of this topic is also due to its scientific appeal and social significance.

The approach to this research direction at IEB is divided based on the type of stress under studied or according to the underlying molecular mechanisms in response to the specified stress.

Phytohormones, as already mentioned, are widely studied at IEB and are represented in responses to virtually all types of biotic and abiotic stress response. Phospholipids as a part of the phospholipid signalling system that is studied by two IEB teams, are also widely involved in the mechanisms involved in responses to abiotic and biotic stresses. Exocyst, cytoskeleton and flotillins are also studied at the level of response mechanisms.

As for specified stress factors, a wide range of abiotic stresses are represented. The role of phytohormones has been described in responses to heat stress (Dobrá et al., 2015; Skalák et al., 2016) drought stress (Přerostová et al., 2017; Přerostová et al., 2018, Gruszka et al. 2016, Korovetska et al. 2016, Pavlovič et al. 2018b), salt stress (Pavlovič et al. 2018a, 2019; Formentin et al. 2018; Keshishian et al. 2018) and phosphate deficiency (Přerostová et al. 2018). Another abiotic stress studied was UV-B irradiation. Detailed anatomic and microscopic changes and polyamine involvement in stress responses of somatic embryos were described (Cvikrová et al. 2016); Eliášová et al. 2017; Eliášová et al. 2018). Involvement of non-specific phospholipase C1 (NPC1) in heat stress response was shown as well. The knockout T-DNA insertion

line NPC1 (*npc1*) exhibited significant decreases in survival rate, and chlorophyll content after heat stress (HS) compared to wild-type. Conversely, plants overexpressing NPC1 (NPC1-OE) were more resistant to HS compared to WT (Krčková et al., 2015).
Toxicity mechanism of nanomaterials to plants was revealed as well. We tried to uncover **the mechanism responsible for the toxicity of ZnO** (Landa et al. 2015) and **CuO nanoparticles** (NPs) (Landa et al. 2017) for plants, including the effect of ZnO nanoparticles (NPs) on phytohormone pools (Vaňková et al. 2017). Furthermore, we demonstrated that isotopically-labelled nanoparticles (NPs) in combination with single particle ICP-MS provide a useful tool for the study of NP uptake by plants (Nath et al. 2018). We studied the **mechanism of metal uptake by plants**, which may be utilised in practical applications, such as phytoremediation or biofortification (Soudek et al. 2016). We also investigated the transcriptomic response of tobacco plants to the presence of thorium (Mazari et al. 2017, Soudek et al. 2019). Impact of aluminium (Al) on the expression, activity, and function of the non-specific phospholipase C4 (NPC4) suggests a role of NPC4 in both early and long-term responses to Al stress (Pejchar et al., 2015a; Pejchar et al. 2015b).

Plant metabolism of widely used drugs such as ibuprofen, fenbendazole or **praziquantel** was studied in detail. It was shown using LC/MS HRAM that the **contaminant ibuprofen** was metabolised *in vitro* by *A. thaliana* cell suspension into more than 300 metabolites, including oxidation products, conjugates with amino acids and saccharides, were detected (Maršík et al. 2017a). **Concentration of main non-steroidal antiflogistics in the various water streams** was mapped in Elbe river basin during the vegetation period using LC/MS and GCxGC/MS. Concentrations of the majority of the drugs were higher in small local streams than rivers and ibuprofen was found as the most abundant drug (Maršík et al. 2017b). **Anthelmintic drug, fenbendazole** (FBZ) was taken up into plants and metabolised in 12 different metabolites, which influenced both gene expression and protein abundance. FBZ thus represents a risk for ecosystems (Syslová et al. 2019). **Antiparasitic drug praziquantel** was studied in suspension and intact plants of *Phragmites australis*. The results showed the ability of plants to uptake and metabolise the drug (Maršík et al. 2017c).

Concerning biotic stress, several outputs addressed the role of phytohormones in the reaction plants to biotic stress. Firstly, the role of phytohormone-based defence responses was elucidated at the onset of cyst nematode below ground pathogen *Heterodera schachtii* in *Arabidopsis* (Kammerhofer et al. 2015). Secondly, we have contributed to the elucidation of phytohormone responses that accompany the efficient defence of *Brassica napus* plants against *Plasmodiophora brassicae* infection (Přerostová et al. 2018). Manipulation of plant immune system by a pathogen and related signalling pathways were also studied in a **pathosystem *Brassica napus* – *Leptosphaeria maculans***. We published findings on the function of the effector AvrLm4-7 (Nováková et al. 2016a). *L. maculans* produces a wide spectrum of phytohormones. Induced changes in cytokinin profile in *L. maculans* colonised tissues, as well as biosynthetic pathways of cytokinins in this fungus were described (Trdá et al. 2017). Besides signalling molecules, protein secretome of this pathogen was also investigated (Nováková et al. 2016b).

The role of phospholipid in plant defence was investigated. We demonstrated a crosstalk between salicylic acid (SA) and phospholipid signalling (Janda et al., 2015a) as well as interrelation among actin cytoskeleton, phospholipid signalling and plant immunity during pathogen infection (Leontovyčová et al., 2019;

Kalachova et al., 2019). Moreover, our results suggest that Arabidopsis NPC2 function play no role in response to *Pseudomonas syringae* attack (Krčková et al. 2018).

Induced plant resistance against pathogens is additional topic related to plant defence and signalling. We investigated both elicitors originating from microorganisms (Nováková et al. 2016a) and other natural sources (Jindřichová et al. 2018). Among them, saponins as plant-originating compounds were studied in more detail (Trdá et al. 2019).

We also investigated a crosstalk between abiotic-biotic stress response. We propose that short exposure to high temperature is a crucial abiotic stress factor that suppresses PAMP-triggered immunity, which subsequently leads to the higher susceptibility of plants to pathogens (Janda et al. 2019).

We also studied **the role of plant flotillins in stress responses** (Daněk et al. 2016). We screened knockout flotillin mutants for phenotype alteration in response to heat/biotic stress (Kroumanová et al. 2019), identified FLOT2-interacting partners in *Arabidopsis* plasma membrane (Junková et al. 2018), and showed that cell wall contributes to the stability of plasma membrane (Daněk et al. 2020).

The Station of Apple Breeding for Disease Resistance (SABDR) is engaged in both applied research and an oriented one. The station has focused its efforts on the breeding of apple trees with complex resistance to the most significant diseases; scab caused by *Venturia inaequalis*, powdery mildew caused by *Podosphaera leucotricha* and quarantine bacterial disease fireblight caused by *Erwinia amylovora.* Complex resistance must resonate with good economic characteristics, especially optimal vigorous growth, high and regular productivity, attractive fruit appearance, good taste, and long storability. Certificate granting Plant Variety Rights (PVR) is usually acquired for commercially promising apple varieties. SABDR sells licenses for the cultivation of bred varieties to Czech and foreign partners. For the evaluated period 2015-2019, 24 licensing agreements were concluded, 49 PVRs were obtained, and 5.9 million trees of our varieties were planted worldwide.

For many years, SABDR study the mechanism of action of resistance genes and other genes during scab disease on the molecular level. The recent implementation of a new NGS technology called genome-by-sequencing (GBS) allows efficient search for molecular markers to isolate polygenic resistance to scab.

For more details, see parts *3-3 Research for practice* and *3-4 Report on the Research Activity of TEAM 9*.

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Research activity and characterisation of the main scientific results

The largest and most prestigious research project of the Team was the sequencing of the huge and complex genome of hexaploid bread wheat. The Team was one of the key partners of the global effort managed by the International Wheat Genome Sequencing Consortium (IWGSC 2018) and the main tasks of the Team were the coordination of the sequencing wheat chromosome 4A, and sequencing the short arms of chromosome 3D (3DS) and chromosome 7D (7DS). They contributed to sequencing of chromosome 4A by developing its radiation hybrid map (Balcárková et al. 2017). To obtain a sequence of the 3DS arm, they developed a sequencing-based strategy to anchor physical maps (Cviková et al. 2015) and used it to construct a physical map of 3DS (Holušová et al. 2017). Subsequently, they clone-by-clone sequenced this chromosome arm (IWGSC 2018). To obtain the sequence of chromosome arm 7DS they developed its physical map and then clone-by-clone sequenced it (Tulpová et al. 2019a). Another important contribution to the IWGSC project was the development of a protocol for construction of optical maps from particular chromosome arms (Staňková et al. 2016), whose availability supported the development and validation of the reference genome sequence of bread wheat. The optical maps were also used to position and analyze ribosomal DNA loci in bread wheat and barley genomes (Kapustová et al. 2019).

The Team participated in three other prestigious international projects that produced reference genomes of barley (Mascher et al. 2017), rye (Rabanus-Wallace et al. 2020, in press) and pea (Kreplak et al. 2019). Apart from the previous production of chromosome survey sequences of barley and rye, the Team produced optical genome maps as the contribution to all three projects. In the case of pea, they also flow-sorted chromosomes whose DNA was sequenced and used to validate the genome assembly. Moreover, sequencing chromosomes flow-sorted from wild relatives of pea allowed the identification of evolutionary chromosome translocations (Kreplak et al. 2019).

The Team utilized the newly generated genome sequences of barley and wheat and chromosome-based experimental approaches to identify and clone agronomically important genes. Among the main targets were genes controlling flowering time in wheat, and the Team identified a new *Vrn-A1f-like* allele (Ivaničová et al. 2016) and described the influence of *Ppd-B1* gene copies on wheat heading date (Ivaničová et al. 2017). They assessed the role of epigenetic modifications on the vernalization phenomenon and characterized the Polycomb repressive complexes 1 and 2 in bread wheat (Strejčková et al. 2020). Another important target was the pre-harvest sprouting resistance gene *Phs-A1* (Shorinola et al. 2016, 2017). The Team also made a significant advance in cloning a gene underlying resistance to Russian wheat aphid in wheat. Combining a chromosome-based approach with marker development (Staňková et al. 2015), optical mapping and long-read sequencing of BAC clones, they identified *EPOXIDE HYDROLASE 2* as the candidate for the *Dn2401* resistance gene (Tulpová et al. 2019b). They also identified a candidate gene for powdery mildew resistance *QPm.tut-4A* in wheat (Janáková et al. 2019).

The Team collaborated with several foreign research groups on cloning important genes in wheat and barley, which included stem rust resistance gene *Sr50* (Mago et al. 2015), leaf rust resistance gene *Lr22a* (Thind et al. 2017) and powdery mildew

resistance gene *Pm21* (Xing et al. 2018). To simplify gene cloning in barley and wheat the Team jointly with the group of B. Wulff (JIC, Norwich, UK) developed a new method, which is based on flow sorting and sequencing of mutant chromosomes to identify induced mutations by comparison to parental chromosomes (Sánchez-Martín et al., 2016). The Team used the method called MutChromSeq in collaboration with other groups to clone and characterize important genes such as barley genes *Eceriferum-q* (Sánchez-Martín et al. 2016) and *Rph1* (Dracatos et al. 2019), and wheat genes *Pm2* (Sánchez-Martín et al. 2016) and a semi-dwarfism gene (Ford et al. 2018).

The Team made significant advances in understating the genome organization and stability in plant interspecific hybrids. They focused primarily on Festulolium (*Festuca* x *Lolium*) grass hybrids of agricultural interest, which display homoeologous chromosome pairing and recombination. This work revealed a dominance of the ryegrass (*Lolium*) genome over that of fescue (*Festuca*) in successive generations, which results in the replacement of *Festuca* chromosomes by those of *Lolium*. In introgression cultivars this leads to elimination of *Festuca* chromatin within 3 - 4 generations (Kopecký et al. 2019). On the contrary, amphiploid cultivars reach a stable genome constitution in F5 - F6 generations (Kopecký et al. 2017). Similarly, the analysis of gene expression revealed the dominance of the *Lolium* genome when more genes exhibit the *Lolium*-expression level dominance (Glombik et al., in preparation). The Team also studied genome composition in Triticale (wheat-rye hybrids) with strict homologous (diploid-like) meiotic pairing. It was found that the lower transmission of rye chromosomes is a consequence of reduced meiotic chromosome pairing, which in turn is due to improper positioning of rye chromosomes in interphase nuclei at the onset of meiosis, and presumably also in somatic tissues (Perničková et al. 2019a, b).

Important advances were made in the analysis of the three-dimensional organization of plant genomes. Thus, the organization of interphase chromosomes and replication dynamics was characterized in closely related *Poaceae* species (Němečková et al. 2019, 2020). The analysis of the replication dynamics at chromosome level in two phylogenetically related species differing in genome size (*Hordeum vulgare* and *Brachypodium dystachion*) using the RepliSeq analysis revealed conserved dynamics of DNA replication and a similar replication timing order for telomeres and centromeres, as well as for euchromatin and heterochromatin regions. Moreover, stable chromosome positioning was observed while transitioning through different stages of interphase (Koláčková et al. 2019). In order to improve spatial resolution of the analyses, the Team developed protocols for super-resolution STED microscopy that will be used to analyze chromatin organization in 3D nuclei and mitotic metaphase chromosomes. To study 3D structure of chromatin in interphase nuclei and of metaphase chromosomes the Team adopted chromatin-conformation-capture-based technique Hi-C.

Important results were also achieved during the analysis of the mechanisms of genome stability maintenance in model plant species *Arabidopsis thaliana*. The Team has successfully established a forward-directed genetic screen for the identification of the DNA-protein crosslink (DPC) repair factors. The screen yielded several novel candidates (unpublished) and also pointed to an important role of the enigmatic SMC5/6 complex in DPC repair. Therefore, the Team functionally characterized the kleisin subunits of the SMC5/6 complex (Díaz et al. 2019). In addition, they opened a new research line focusing on functional genomics of barley with the primary focus on epigenetic and chromatin-related processes during seed development. To this end,

endoreduplication dynamics in developing barley grain was analyzed (Nowicka et. al. 2020), with a special attention to the interphase chromosome organization in seed tissues and exploration of seed transcriptome by RNA-sequencing.

In collaboration with the Bioversity International (Montpellier, France) the Team characterized genetic diversity of all accessions stored in the International Musa Germplasm Transit Centre (Leuven, Belgium), which is the world's largest collection of banana germplasm (Christelová et al. 2017). The Team has also characterized genotypes of wild relatives of cultivated banana (Čížková et al. 2015; Němečková et al. 2018) and new *Musa* accessions collected during exploratory missions organized by the Bioversity International (Sardos et al. 2018). Moreover, in collaboration with research stations of the International Institute of Tropical Agriculture in Uganda and Tanzania, they genotyped breeding materials and participated on association gene mapping projects (Nyine et al. 2018). These studies were complemented by the analysis of evolutionary chromosome rearrangements in *Musa* using the method for chromosome-specific oligo painting FISH, which the Team has developed for *Musa* (Šimoníková et al. 2019).

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Research activity and characterisation of the main scientific results

Overall research summary

In the years 2015-2019 we made significant progress in all main research topics studied in the lab, resulting in a total 35 impacted articles, a 20% increase to the last evaluation period and the same trend can be seen from citation data. We maintained the quality of scientific outputs that was set in the previous period (median IF of all LCB papers 5.36 vs 5.79) and we improved substantially the quality of the "in-house" publications, where first and/or corresponding authors are from LCB (median IF 5.95 vs 4.86, see Fig. 1). We significantly expanded our understanding of the exocyst role in polar expansion of several plant cell types and we linked the specific exocyst localization with distinct distribution of anionic membrane lipids in plasma membrane. In collaboration with the laboratory of Prof. Shaul Yalovsky from the Tel-Aviv University we performed detailed characterization of molecular function of SEC3 exocyst subunit during pollen tube tip growth (Bloch, Pleskot, Pejchar et al., 2016). Along the same lines, we performed comparative functional study of multiplied exocyst subunit EXO70 in Arabidopsis and tobacco pollen (Synek et al., 2017; Sekereš et al., 2017). We have participated in the pioneering study describing the phospholipid-based identity of plant membranes (Platre et al., 2018) and described the link between phospholipid localization and targeting of exocyst subunits (Pleskot et al., 2015; Kubátová et al., 2019). We have described the role of specific exocyst subunit EXO70H4 in the maturation of Arabidopsis trichomes, where it regulates the deposition of distinct cell wall components (Kulich et al. 2015; 2018). Since the exocyst subunit EXO70 evolved from three conserved clades with assumed distinct roles, we also studied the function of moss *Physcomitrella patens* exocyst subunit PpEXO70.3d from the yet poorly characterized EXO70.3 clade (Rawat et al. 2017). Another role for exocyst was found in tracheary elements, where we showed that microtubule-dependent targeting of the exocyst complex is necessary for xylem development in Arabidopsis (Vukašinović et al., 2017). We also described a novel and unexpected role of exocyst complex in the development and organization of the root-shoot junction in Arabidopsis (Janková-Drdová et al., 2019) and participated in a multi-lab study that led to the discovery of exocyst-targeting inhibitor blocking exocytosis (Zhang et al., 2016).

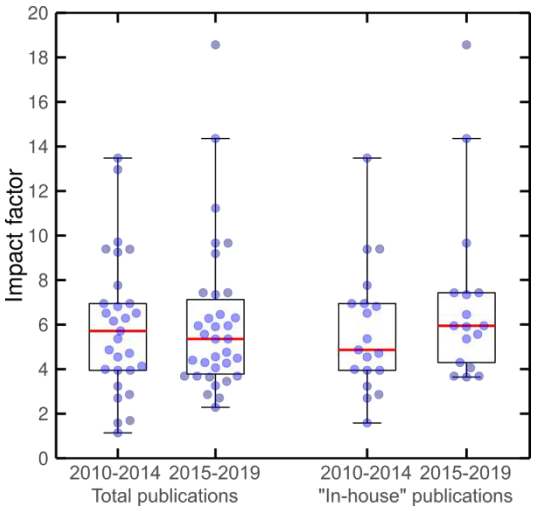

Fig. 1. Overview of LCB publication activity in current and last evaluation period.

We continued in the characterization of the formin family of cytoskeleton-membrane organizers. FH1, the major member of the formin family, was found to regulate the morphogenesis of pavement cells and we described its dynamic localization pattern during cell ontogeny (Rosero et al., 2016; Oulehlová et al., 2019). Our research led also to development of new methodical approaches for quantification of cytoskeletal dynamic in plants (Cvrčková and Oulehlová, 2017). Tangentially to the exocyst-phospholipids-cytoskeleton framework, we continued revealing the relationship between cell polarity, membrane traffic and plant-pathogen interactions (Pečenková et al., 2017a; 2017b) and the role of reactive oxygen species in polar growth of pollen tubes (Jiménez-Quesada et al., 2019).

During the evaluation period we were also active in summarizing and discussing our research in a broader context and we wrote six invited review or opinion articles published in impacted journals. Here we highlight the comprehensive review on membrane-lipid interactions and domains in plants (Sekereš et al. 2015), and two reports summarizing our work on exocyst, autophagy and plant defense - a hypothesis paper proposing the involvement of autophagy in R proteins regulation (Pečenková et al. 2016) and a review article describing the role of exocyst in autophagy and unconventional secretion in plants (Pečenková et al. 2018).

Research highlights

The role of exocyst complex in Arabidopsis and tobacco pollen tube germination and growth

We revealed that pollen-expressed SEC3a exocyst subunit is an essential gene in plants, i.e. loss of its function results in zero male function/transmission (Bloch, Pleskot, Pejchar et al., 2016). We found that the localization of SEC3a to the pollen tube tip is very dynamic and predicts the future direction of pollen tube exocytosis and growth. Using combination of biochemical and computational approaches, we characterized the lipid-binding properties of N-terminal PH-like domain in SEC3a and proved that the interaction of PH domain with phosphoinositides is required for its proper localization. However, the loss of membrane binding ability of SEC3a PH domain is not affecting the function of the whole exocyst complex suggesting that SEC3-phospholipid interaction plays only auxiliary role in the targeting of the whole complex. This study was a joint effort whit the Yalovsky group in Tel Aviv University and we also collaborated with J Heyrovsky Institute of Physical Chemistry, CAS, who helped with the molecular dynamics studies.

In parallel we studied the functional diversity of the EXO70 exocyst subunit, which was proposed to target the whole exocyst complex to the membrane and which is represented by many isoforms in most plant genomes. This diversity could be partly responsible for the establishment and maintenance of membrane domains with different composition. To address this hypothesis, we performed large-scale expression, localization, and functional analysis of tobacco (Nicotiana tabacum) and Arabidopsis EXO70 isoforms in pollen (Sekereš et al., 2017, Synek et al., 2017). In tobacco, various isoforms localized to different regions of the pollen tube plasma membrane, apical vesicle-rich inverted cone region, nucleus, and cytoplasm. The overexpression of major pollen-expressed EXO70 isoforms resulted in growth arrest and characteristic phenotypic deviations of tip swelling and apical invaginations. NtEXO70A1a and NtEXO70B1 occupied two distinct and mutually exclusive plasma membrane domains. Both isoforms partly colocalized with the exocyst subunit NtSEC3a at the plasma membrane, possibly forming different exocyst complex subpopulations. NtEXO70A1a localized to the small area previously characterized as the site of exocytosis in the tobacco pollen tube, while NtEXO70B1 surprisingly colocalized with the zone of clathrin-mediated endocytosis (Sekereš et al., 2017). In contrast, members of the EXO70C class, which are specifically expressed in tip-growing cells, exhibited exocytosis-related functional effects in pollen tubes despite the absence of apparent plasma membrane localization. This was further corroborated by genetic study in Arabidopsis, where loss-of-function EXO70C2 allele resulted in a significant male-specific transmission defect due to aberrant pollen tube growth. Mutant Arabidopsis pollen tubes grown *in vitro* exhibited an enhanced growth rate and a decreased thickness of the tip cell wall, causing tip bursts. However, *exo70C2* mutant pollen tubes could frequently recover and restart their speedy elongation, resulting in a repetitive stop-and-go growth dynamics (Synek et al., 2017). Taken together, our data support the existence of multiple membrane-trafficking domains regulated by different EXO70-containing exocyst complexes within a single cell and suggest that members of EXO70C subfamily are negative exocyst regulators of tip growth in pollen tubes.

Specific Arabidopsis EXO70 paralogs are required for regulated deposition of secondary cell wall in different cell types

Arabidopsis leaf trichomes are unicellular outgrowths with very specific polarized shape and usually three sharp branches. Their shape is easily and visibly distorted by numerous mutations, which, together with their large size and good accessibility, led to their popularity as a model for studies of plant cell morphogenesis and for the regulated deposition of secondary cell wall. In collaboration with the partner Laboratory of Cell Morphogenesis (Department of Experimental Plant Biology, Faculty of Science, Charles University), we have identified exocyst subunit EXO70H4 as the highly upregulated gene in mature trichome. We revealed that EXO70H4 is required for the general deposition of secondary cell wall and for the development of novel, callose-rich cell wall structure - Ortmannian ring (termed after our

pHD student who observed it first), which is formed between the apical and the basal parts of mature trichome. We also found that EXO70H4-dependent secondary cell wall deposition is involved in the plant response to UV radiation and herbivore attack (Kulich et al., 2015). In a follow-up study we demonstrated that the specific function of EXO70H4 is to facilitate the local secretion of callose synthase PMR4 and revealed that callose is indispensable for silica deposition in Arabidopsis trichomes. We also found that the expression of EXO70H4 can be strongly activated outside trichomes upon pathogen attack (Kulich et al., 2018).

The xylem vessel system is essential for water and nutrient transport of vascular plants and their mechanical stability. Tracheary elements (TEs), mature xylem vessels, display structured cell wall morphology with diversity of secondary thickening patterns. We have shown that exocyst is important for the proper deposition of secondary cell wall during TE development and demonstrated that EXO70A1 is involved in this process (Vukašinović et al., 2017). Importantly, the recruitment of exocyst to the secretion sites in developing xylem vessels is microtubule-dependent, in contrast to microtubule-independent exocyst dynamics in epidermal cells, showing that, the role of the cytoskeleton in the targeting and spatio-temporal dynamics of the exocyst complex can be strikingly different in different cell types. Our data also pinpointed secondary cellulose synthase as the probable cargo for exocyst in TE development.

Distinct anionic phospholipids define the identity of plasma membrane domains and control the localization of EXO70 exocyst subunits

A conserved feature of endomembrane organelles is their distinct phospholipid composition, which was proposed to specify membrane identity and function. We participated in the large study, which revealed that in plant cells, each endomembrane organelle has a distinct electrostatic signature created by a combination of various anionic phospholipids (our contribution was the generation of genetically-encoded sensor for phosphatidylserine and the detailed microscopic analysis of its dynamics in tip-growing cells). We proposed that this "electrostatic code" represents a fundamental patterning principle of the endomembrane system and acts as a key determinant of protein subcellular targeting (Platre et al., 2018). This hypothesis was independently corroborated by our initial in silico and in planta studies exploring the exocyst function at the membrane-cytoplasm interface, with the special focus on the interaction of EXO70 subunits with the specific components of lipid bilayer. Molecular dynamics (MD) simulations have been shown to play an invaluable role in the molecular-level description of dynamic interaction of membrane proteins with phospholipids, which could be hardly obtained by experimental approaches. In Pleskot et al. (2015), we utilized coarse-grained MD simulations to reveal details of the specfic interactions of yeast EXO70 with PIP_2. Surprisingly we observed that the interaction of EXO70 with the membrane causes clustering of PIP_2 in the adjacent leaflet. For this work we benefited again from fruitful collaborated with Lukasz Cwiklik (J Heyrovsky Institute of Physical Chemistry, CAS) and Pavel Jungwirth (Institute of Organic Chemistry and Biochemistry, CAS), who helped with the molecular dynamics studies and analyses (first author Roman Pleskot was a joint postdoc between our lab and the team of Pavel Jungwirth).

To test whether distinct EXO70 isoforms have specific interactions with plasma membrane- defining phospholipids, we used Arabidopsis trichomes, where two markedly different domains—the basal domain, with a thin cell wall, and the apical domain, with an extremely thick cell wall, are formed. We showed that the mature trichome contains, along with two cell wall domains, two distinct plasma membrane domains that differ in their phospholipid composition and also in their ability to recruit different EXO70 proteins. While the apical domain, which is phoshatidylserine- and phosphatidic acid- rich, recruits EXO70H4, the basal domain recruits PIP2-rich EXO70A1, which corresponds to the biochemically determined lipid-binding capacities of these two paralogs (Kubátová et al., 2019).

Identification and initial characterization of small-compound exocytosis inhibitor

We were the integral part of a large international multidisciplinary team (lead by Natasha Raikhel - University of California, Riverside) that identified the small molecule Endosidin2,

which binds to the EXO70 exocyst subunit in different organisms and cell types, ultimately resulting in inhibition of exocytosis and endosomal recycling in both plant and human cells and enhancement of plant vacuolar trafficking (Zhang et al., 2016). Our contribution was detailed microscopic study of the localization and dynamics of EXO70A1 exocyst subunit in Arabidopsis roots after Endosidin2 treatment.

Unexpected role of exocyst complex in the developmental plasticity of Arabidopsis root-shoot transition zone

The collet, root–hypocotyl junction, is an important transition zone in angiosperms and its correct organization is crucial for plant fitness. We uncovered a new role of the exocyst complex in the collet formation and hypocotyl ontogeny reprogramming (Janková-Drdová et al., 2019). We found that exocyst mutants can form ectopic collet hair-like structures that are located above the normal collet region. This defect, which, to our knowledge, has never been observed in any Arabidopsis WT or mutants, is accompanied by changes in auxin response, impaired PIN3 localization and ectopic starch accumulation in the affected region. These observations suggest an important role of the exocyst in environmentally regulated hypocotyl developmental plasticity related to polar auxin transport and signaling.

First experimental study of exocyst outside angiosperm plants

Our previous phylogenetic analyses led to the discovery of major multiplication of land plant EXO70 subunits during evolution and to their classification into three distinct, ancient subfamilies—EXO70.1, EXO70.2, and EXO70.3. In this study we performed the experimental study of the exocyst complex in the moss *Physcomitrella patens*, focusing on one of the 13 paralogs of the EXO70 subunit, PpEXO70.3d, that belongs to experimentally poorly characterized EXO70.3 clade. We showed that the knock-out mutant of PpEXO70.3d displays pleiotropic developmental defects and that this exocyst subunit is necessary for female gametogenesis and therefore for completion of the moss life cycle. Despite the presence of multiple EXO70 genes, PpEXO70.3d is thus crucial for Physcomitrella patens development and morphogenesis (Rawat et al., 2017). This study represented a significant milestone for the team as it was the first experimental study of exocyst subunit in bryophytes and it paved the way to multiple efforts we are currently undertaking in the area of evolutionary-developmental biology of secretory pathway.

Functional and microscopic analysis of FH1, major member of formin family of cytoskeleton regulator

Formins are an evolutionarily ancient family of proteins that can nucleate, bundle and cap actin filaments, but also bind microtubules, resulting in an ability to modulate actin and microtubule organization and dynamics, well documented also in plants. Angiosperms have two formin clades with multiple paralogs; typical plant Class I formins are integral membrane proteins that can anchor cytoskeletal structures to membranes. In close collaboration with the partner Laboratory of Cell Morphogenesis (Department of Experimental Plant Biology, Faculty of Science, Charles University), we analyzed the function of Arabidopsis FH1 gene, which codes for the most abundantly expressed Class I formin in Arabidopsis and found that loss-of-function mutant shows minor but significant phenotypic changes, including cotyledon epinasty, as well as changes in pavement cell shape and in actin and microtubule dynamics (Rosero et al., 2016). In a follow-up study, we used complementation of *fh1* mutant phenotype to prove functionality of GFP-tagged FH1 construct expressed from the FH1 promoter to achieve close to native protein levels, and characterized localization of this construct in detail, revealing a surprisingly complex pattern of formin relocation between a variety of membrane compartments and domains. FH1-GFP also colocalizes with plasmodesmata, suggesting that formins might have a shared (ancestral or convergent) role at cell–cell junctions.

We also developed a new freeware-based, operational system-independent semi-manual technique for analyzing cytoskeleton dynamics, which we termed QuACK (Quantitative Analysis of Cytoskeletal Kymograms), and validated it on data from Arabidopsis

thaliana *fh1* formin mutants. Besides confirming the published mutant phenotype, QuACK was used to characterize surprising differential effects of various fluorescent protein tags fused to the Lifeact actin probe on actin dynamics in A. thaliana cotyledon epidermis. In particular, Lifeact-YFP slowed down actin dynamics compared to Lifeact-GFP at marker expression levels causing no macroscopically noticeable phenotypic alterations (Cvrčková and Oulehlová., 2017).

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Research activity and characterisation of the main scientific results

The research running in the LGR and IL during the period 2015-2019 in the frame of the Research Programme and grant projects can be divided into two main parts reflecting the structure and subject. The original results obtained during the last five years document the high scientific quality of the teams both in the national as well as in the international context (see also List of publications and patents). The research is oriented to biomolecules, their structure and analysis, activity, molecular and cellular mechanism of action as well as applications in different fields. The compatibility of the approaches of participating laboratories including the cooperation of members of the team has been successfully proven by their previous experience, patents and publications. In the following text, a more detailed description of results achieved is divided into two main research areas and these are sub-divided into smaller thematic parts for better orientation. **The main contribution and output of the LGR are of course professional publications in high quality, internationally recognized journals.** These enhance the prestige of both the LGR and the parent organizations, i.e. IEB and UP. The LGR publishes its results in good scientific journals (see below), and contributes to the popularization of its results and science in general. The number of publications of the research team is continuously increasing as is the quality (see Fig. 2). The dynamics of the publishing activity is due to the increasing productivity and number of team members. Publication activity was also evaluated using the RIV database, where it showed similar tendencies. As the research team works on science and medicine-related issues, they incorporated more internationally recognized databases such as WOS. Due to the high professional quality of the team, we have taken the liberty of using the WOS database to assess our scientific standard, including impact factor. We especially appreciate the **constant growth of citations** to our articles – an encouraging indication is the linear growth of publications against the almost exponential growth of citations.

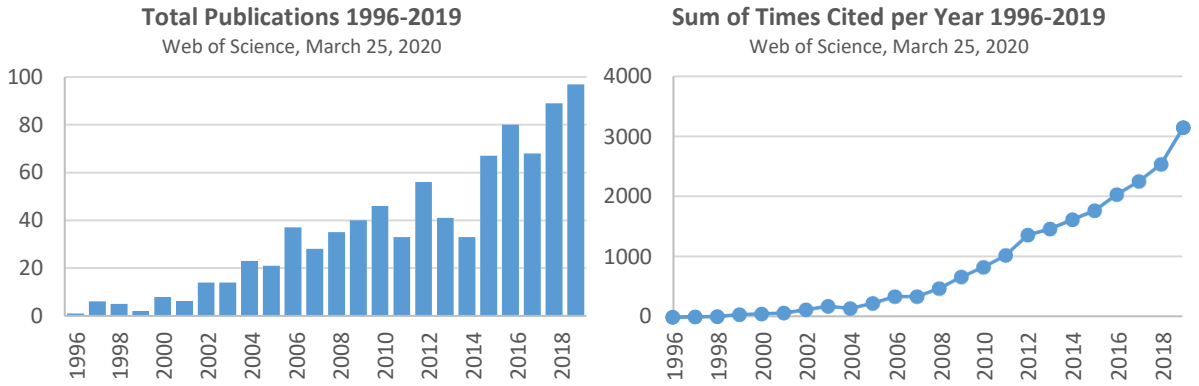

Fig. 1. Publication and citation activity of LGR according to ISI Web of Knowledge.

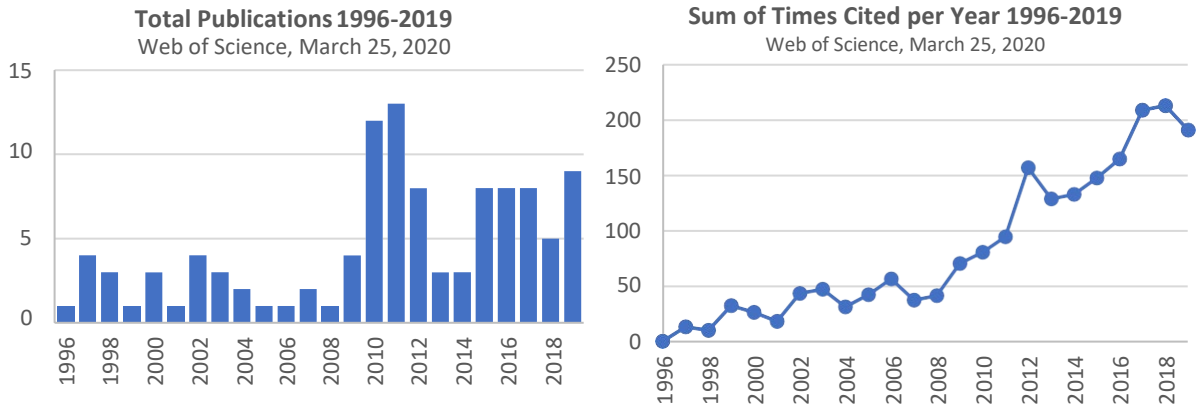

Fig. 2. Publication and citation activity of the Isotope Laboratory according to the ISI Web of Knowledge.

I. NEW PHYTOHORMONES, BIOSTIMULANTS AND BIOMOLECULES

New phytohormones, their derivatives and probes, biostimulants, and labelled derivatives for basic research, biotechnological and agricultural applications

Our laboratories have long-standing experience in organic synthesis, labelling of phytohormones and production of heavy and radioactively labelled phytohormones and probes [Amoo *et al.* 2015; Aremu *et al.* 2015a; Kumar *et al.* 2015; Bruňáková et al 2015; Petřík *et al.* 2016; Mik *et al.* 2017; Zhabinskii *et al.* 2017; Bon *et al.* 2018; Buček *et al.* 2018; Konrádová *et al.* 2018; Kubiasová *et al.* 2018; Medvedev *et al.* 2018; Milosavljevic *et al.* 2018; Bieleszová *et al.* 2019; Hanuš *et al.* 2019; Zielke *et al.* 2019]. There are also several reviews on this topic from our laboratory [Plíhalová *et al.* 2016; Tarkowská, Strnad 2016; Wasternack, Strnad 2016; Wasternack 2016; Zwanenburg *et al.* 2016a,b; Pospíšil *et al.* 2017; Skalický *et al.* 2018; Tarkowská, Strnad 2018; Wasternack, Feussner 2018; Wasternack, Strnad 2018, 2019; Podlešáková *et al.* 2019; Tarkowská *et al.* 2019]. We also studied their biological activities *in vitro* and in vivo [Lacuesta *et al.* 2018, Nisler *et al.* 2018; Ugena *et al.* 2018].

We also discovered several new natural phytohormones. For example, the recently identified OPDA-Ile [Floková *et al.* 2016]. suggests that OPDA specific responses might be mediated upon formation of OPDA-Ile. Thus, we tested OPDA-Ile-induced gene expression in wild type and JA-deficient [Arnold *et al.* 2016; Wasternack, Hause 2016]. A novel enzymatic activity dependent on NADP+ converting *trans*-zeatin to 6-(3-methylpyrrol-1-yl)purine (MPP) was detected. MPP shows weak anticytokinin properties and inhibition of CK dehydrogenases due to its ability to bind to an active site in the opposite orientation to substrates [Hluska *et al.* 2016]. Furthermore, we developed and examined the effect of *meta*-topolin and several novel aromatic cytokinin (CK) derivatives on *in vitro* adventitious shoot production, rooting and photosynthetic pigment content of different regenerated plants *in vitro*. Its carry-over effect on *ex vitro* growth, photosynthetic performance and the antioxidant enzyme system of different plants was also investigated. The treatments with some of the aromatic CK (ARCK) gave the highest number of adventitious shoots when compared to thidiazuron (TDZ) application and the control. In many cases, we also evaluated the distribution pattern of cytokinins (CKs), other phytohormones and phenolic compounds in different organs of *in vitro* propagated plants to better understand their physiology which can provide an essential basis for coherently achieving a conservation/ micropropagation driven strategy for valuable plant species [De Diego *et al.* 2015; Pěnčík *et al.* 2015; Plačková *et al.* 2015; Aremu *et al.* 2016c, 2017; Vylíčilová *et al.* 2016; Madzikane-Mlungwana *et al.* 2017; Kumari *et al.* 2018a; Matsuura *et al.* 2018; Hudzieczek *et al.* 2019; Murvanidze *et al.* 2019; Smýkalová *et al.* 2019;]. We also investigated the effect of many different biostimulants of (un)defined chemical characteristics (eckol, phloroglucinol, phenolic compounds, algae leachate, seaweed extracts, silicon, Kelpak®, smoke-derived karrikinolide, volatiles, allelochemicals, silver nanoparticles, …) on the growth,

phytochemical and phytohormone content of many different plant species [Aremu *et al.* 2015b,c; 2016a,b; 2019; Rengasamy *et al.* 2015; Van Bockhaven *et al.* 2015; Sánchez-López *et al.* 2016a,b; Steenackers *et al.* 2016; Vinković *et al.* 2017; Dube *et al.* 2018; Moncaleán *et al.* 2018; Moyo *et al.* 2018b; Pecková *et al.* 2018; Fajinmi *et al.* 2019; Gupta *et al.* 2019; Kulkarni *et al.* 2019; Masondo *et al.* 2019; Stirk *et al.* 2019;]. Finally, we provided an overview and the results of the outcomes from pot and field experiments using exogenous treatments with growth regulators, summarized the modes of application and pointed to the affected traits in various field crops, vegetables, cotton and fruit trees [Koprna *et al.* 2016].

New phytohormone molecular targets and compounds modulating phytohormone perception, biosynthesis and degradation

Cytokinins, a class of phytohormones, are adenine derivatives common to many different organisms. In plants, these play a crucial role as regulators of plant development and the reaction to abiotic and biotic stress. Key enzymes in cytokinin synthesis and degradation in today' s land plants are the **isopentyl transferases (IPT)** and the **cytokinin dehydrogenases (CKX)**, respectively. For example, we showed that the CK profile of ergot-infected rye plants is altered, although no pronounced changes occur in the expression of the host plant's CK biosynthesis genes. We also reported on the first fungal *de novo* CK biosynthesis genes, proved their functions and constituted a biosynthetic pathway - the ergot fungus *Claviceps purpurea* produces substantial quantities of CKs in culture and, like plants, expresses enzymes containing the isopentenyl transferase and lonely guy domains necessary for de novo isopentenyladenine production [Hinsch *et al.* 2015]. The identification and functional characterization of sensor histidine kinases, homologous to Arabidopsis CK receptors AHK2 and AHK3 in winter oilseed rape were also presented. Five CHASE-containing His kinases were identified in *Brassica napus* var. Tapidor (BnCHK1–BnCHK5) by heterologous hybridization of its genomic library with gene-specific probes from Arabidopsis [Kuderová *et al.* 2015]. We also showed that auxin activates root formation, whereas cytokinins mediate early loss of the root identity, primordia disorganisation and initiation of shoot development. We proposed an important role for endogenous cytokinin biosynthesis and AHK4-mediated cytokinin signalling in the control of de novo-induced organ identity [Pernisová *et al.* 2018]. We identified the REPRESSOR OF CYTOKININ DEFICIENCY 1 (ROCK1) as an ER-localized transporter of UDP-GlcNAc and UDP-GalNAc in plants. We demonstrated that ROCK1-mediated transport plays a role in the ER-associated protein quality control and loss of ROCK1 enhances cytokinin responses by suppressing the activity of cytokinin-degrading CKX proteins [Niemann *et al.* 2015]. Using a forward genetic approach, we isolated constitutively active gain-of-function variants of the *AHK2* and *AHK3* genes, named *repressor of cytokinin deficiency2* (*rock2*) and *rock3*, respectively. It is hypothesized that the structural changes caused by these mutations in the sensory and adjacent transmembrane domains emulate the structural changes caused by cytokinin binding, resulting in domain motion propagating the signal across the membrane [Bartrina *et al.* 2017]. We also showed that the *Lotus japonicus* CKX3 gene is induced by the Nod factor during the early phase of nodule initiation [Reid *et al.* 2016]. To identify the source, timing, and effect of this accumulation, we followed transcript levels of the cytokinin biosynthetic pathway genes in a sliding developmental zone of *L. japonicus* roots. *LjIpt2* and *LjLog4* were identified as the major contributors to the first cytokinin burst [Reid *et al.* 2017]. Transgenic barley overexpressing a CKX gene also showed a greater tolerance to drought stress [Pospíšilová *et al.* 2016]. The full maize CKX family's subcellular localization was identified. ZmCKX1 was the only isoform to control extracellular cytokinin homeostasis while isoform ZmCKX9 is associated with the endoplasmic reticulum. The majority of ZmCKX isoforms are targeted to vacuoles [Zalabák *et al.* 2016]. Furthermore, utilizing the overproduction of single-chain Fv antibodies selected for their ability to bind trans-zeatin riboside and targeted to the endoplasmic reticulum, we post-synthetically modulated cytokinin ribosides, the proposed transport forms of cytokinins [Gelová *et al.* 2018]. In this study, we showed that CKX1 is a type II single-pass membrane protein that localizes predominantly to the endoplasmic reticulum (ER) in Arabidopsis. This indicates that this CKX isoform is a bona fide ER protein directly controlling the cytokinin, which triggers the signalling from the ER. By

using various approaches, we demonstrated that CKX1 forms homodimers and homooligomers *in vivo* [Niemann *et al.* 2018]. Here, we showed that class I *KNOX* gene activity is necessary and sufficient for axis extension from an intercalary region of determinate moss shoots. Class I *KNOX* activity can promote cytokinin biosynthesis by the *PpIPT3* gene. *PpIPT3* promotes axis extension, and *PpIPT3* and exogenously applied cytokinin can partially compensate for loss of class I *KNOX* function. By outgroup comparison, the results suggest that a pre-existing *KNOX*-cytokinin regulatory module was recruited into vascular plant shoot meristems during evolution to promote indeterminacy, thereby enabling the radiation of vascular plant shoot forms [Coudert *et al.* 2019]. Our recent findings demonstrate the usefulness of ectopic *CKX* gene expression for achieving root enhancement in oilseed rape and this underpins the functional relevance of a larger root system. Furthermore, the lack of major developmental consequences on shoot growth in cytokinin-deficient oilseed rape indicates species-specific differences of *CKX* gene and/or cytokinin action. [Nehnevajova *et al.* 2019]. Here we showed that cytokinin signalling functions as a lateral root specific anti-gravitropic component, promoting the radial distribution of the root system. We performed a genome-wide association study and reveal that signal peptide processing of CKX2 affects its enzymatic activity and, thereby, determines the degradation of cytokinins in natural *A. thaliana* accessions [Waidmann *et al.* 2019]. Novel thidiazuron (TDZ)-derived inhibitors of CKX have been developed. Two new TDZ derivatives are very potent inhibitors of CKX and are promising candidates for *in vivo* studies and agricultural applications [Nisler *et al.* 2016]. Here, we described synthetic auxins, RubNeddins (RNs), which act as selective auxin agonists. The RN with the greatest potential for dissecting auxin perception was RN4, which we used to reveal a role for the chromatin remodelling ATPase BRAHMA in apical hook development [Vain *et al.* 2019].

II. BIOANALYTIC CHEMISTRY OF PHYTOHORMONES AND ITS APPLICATION

In order to understand better the network regulation of hormone action, there is need of measuring multiple hormone concentrations simultaneously, i.e. characterize the 'hormone-metabolome'. Mostplant hormones are found in plant tissues at extremely low concentrations (in general pmol/g of fresh weight) which makes qualitative and quantitative analysis difficult and therefore very sensitive analytical tools are required. Moreover, with regard to the complexity of the matrix, the need of thorough isolation and high enrichment of these substances as the analytes, is essential prior to the detection by standard analytical techniques. Immunoaffinity chromatography was developed for fast and selective phytohormone purification [Oklešťková *et al.* 2017]. We have developed a simple microscale magnetic immunoaffinity-based method for selective one-step isolation of CKs from very small amounts of plant tissue [Plačková *et al.* 2017] as well as the micro-SPE method [Vlčková *et al.* 2017]. Last but not least, the throughput is also an important factor for fully realizing the technology of 'hormonomics', in which the hormone content of a large number of small amounts of plant samples has to be measured. The analysis of plant hormones is challenging as these compounds are present in trace amounts and because many other substances in plant extracts interfere with the analysis, such as pigments, lipids, phenolics and proteins. Currently, the most suitable and most used analytical technology for phytohormone analysis is based on liquid chromatography-tandem mass spectrometry ((U)HPLC-MS/MS) as evidenced in our reviews [Novák *et al.* 2016; Nahar *et al.* 2019]. Since 2008, this methodology was gradually developed in LGR for plant hormone analyses (cytokinins, auxins, tryptophan-related neuroactive substances, JAs, ABAs, gibberellins, brassinosteroids, ecdysteroids, phenolics, isoflavonoids, tocopherols, ingenol, phytocannabinoids, karrikins and other phenylpropanoids) [Kamlar *et al.* 2015; Eyer *et al.* 2016; Hényková *et al.* 2016; Kučera *et al.* 2016; Pilařová *et al.* 2016; Tarkowská *et al.* 2016; Vlčková *et al.* 2016; Guo *et al.* 2017; Roche *et al.* 2017; Béreš *et al.* 2018; de la Fuente *et al.* 2018; Pěnčík *et al.* 2018; Pilařová *et al.* 2018; Béréš *et al.* 2019; Hrdlička *et al.* 2019;]. Tissue- and cell-specific approaches in plant hormone analysis have also been recently introduced [Antoniadi *et al.* 2015, Novák *et al.* 2017; Pařízková *et al.* 2017;]. Here also, electrochemistry combined with mass spectrometry represents an emerging

analytical technique used to study the oxidation pathway of various drugs and *in vivo* occurring compounds. An on-line HPLC/EC/HR ESI-MS method had been developed to investigate the oxidation of selected cytokinin compounds. This setup allowed rapid identification and general structure elucidation of the obtained products [Karády *et al.* 2015]. Another aim was to acquire an understanding of the dynamics of pericarp growth and development and, of the molecular factors involved. Since the tissue organization of the pericarp is heterogeneous and varies between different regions, magnetic resonance imaging (MRI) was introduced to analyse this organ during development in order to identify cell regions which are responsible for critical growth periods and thus for defining grain shape and size. These studies were combined with the analysis of differential gene expression and the measurement of the distribution of auxin and GAs along the developing pericarp [Pielot *et al.* 2015]. Furthermore, the method of protoplast and vacuole isolation combined with precise cytokinin analysis and recovery assay of a vacuolar marker protein were used to quantify the contents of individual cytokinin forms in the leaf extracellular space, cell interior and vacuole. The data obtained for wild type plants and in each case a specific mutant line, allow comparison of the effect of genetic manipulation on the hormone distribution and homeostatic balance of cytokinins in the modified plants [Jiskrová *et al.* 2016]. The aim of this study was to synthesize the deuterated derivatives of 7α-hydroxy-dehydroepiandrosterone (DHEA) and 7-oxo-DHEA and test them in liquid chromatography–tandem mass spectrometry (LC-MS/MS) in order to enhance the performance characteristics of this method [Kolatorova-Sosvorova *et al.* 2016]. We also developed a method for the simultaneous targeted profiling of 101 phytohormone-related analytes from minute amounts of fresh plant material (less than 20 mg). Rapid and nonselective extraction, fast one-step sample purification, and extremely sensitive UHPLC-MS/MS enable concurrent quantification of the main phytohormone classes: cytokinins, auxins, brassinosteroids, gibberellins, jasmonates, salicylates, and abscisates [Šimura *et al.* 2018].
The application of new technologies led to several good papers and also important discoveries [Humplík *et al.* 2015a,b; Janezko *et al.* 2015; Martín-Rodríguez *et al.* 2015; Novák *et al.* 2015; Sebastian *et al.* 2015; Stolárik *et al.* 2015; Leljak-Levanić *et al.* 2016; Steiner *et al.* 2016; Šimura *et al.* 2016; Edlund *et al.* 2017; Karuppanapandiana *et al.* 2017; Krausko *et al.* 2017; Steenackers *et al.* 2017; Ševčíková *et al.* 2017; Žižková*et al.* 2017; Wei *et al.* 2017; Bahaji *et al.* 2018; Cucinotta *et al.* 2018; Edwards *et al.* 2018; Ferrero *et al.* 2018; Florencio-Ortiz *et al.* 2018; Gigli-Bisceglia *et al.* 2018; Hoyerová *et al.* 2018; Janečková *et al.* 2018; Ma *et al.* 2018; Minina *et al.* 2018; Orman-Ligeza *et al.* 2018; Plackett *et al.* 2018; Poitout *et al.* 2018; Prát *et al.* 2018; Přerostová *et al.* 2018; Racca *et al.* 2018; Stirk *et al.* 2018; Bahaji *et al.* 2019; Široká *et al.* 2018; Godzien *et al.* 2019; Hradský *et al.* 2019; Müller *et al.* 2019; Sadura *et al.* 2019; Schubert *et al.* 2019; Skalák *et al.* 2019; Starý *et al.* 2019; Tarkowská *et al.* 2019; Wang *et al.* 2019;]. For example: to monitor sugar availability to buds, we cultivated bud-bearing stem segments *in vitro* and supplied them with different sugar conditions, including different sucrose concentrations and non-metabolizable sucrose analogues. To assess the sequence of events, we monitored the temporal patterns of bud outgrowth and hormonal state. We used several techniques to characterize hormonal state, including the determination of hormone levels and gene expression, and imaging of reporter genes. We demonstrated that sucrose availability modulates the entrance of buds into sustained growth, and that this effect is conserved across species. This impact is preceded by an early modification of hormonal homeostasis. We also identified some components of the hormonal network as potential candidates explaining the regulation of bud outgrowth by sucrose [Barbier *et al.* 2015]. We further showed that phosphoglucose isomerase is an important determinant of photosynthesis, energy status, growth and starch accumulation in mesophyll cells, likely as a consequence of its involvement in the production of oxidative pentose phosphate pathway/glycolysis intermediates necessary for the synthesis of plastidic MEP-pathway derived hormones such as CKs [Bahaji *et al.* 2015]. We also showed that in gametophytic shoots of *Physcomitrella*, lateral branches arise by re-specification of epidermal cells into branch initials. A simple model co-ordinating the activity of leafy shoot tips can account for branching patterns, and three known and ancient hormonal regulators of sporophytic branching interact to generate the branching pattern- auxin, cytokinin

and strigolactone. The mode of auxin transport required in branch patterning was discovered to be a key divergence point from known sporophytic pathways [Coudert *et al.* 2015]. We also investigated the function of the low-duplicated CYP715 cytochrome P450 gene family that appeared early in seed plants and evolved under strong negative selection. Comparative expression analysis revealed the downregulated expression of genes involved in pollen development, cell wall biogenesis, hormone homeostasis, and floral sesquiterpene biosynthesis, especially TPS21 and several key genes regulating floral development such as MYB21, MYB24, and MYC2. Flower hormone profiling, in addition, indicated a modification of gibberellin homeostasis and a marked disturbance of the turnover of jasmonic acid derivatives [Liu *et al.* 2015]. To elucidate the link between proteasome function, NO resistance, and pathogenesis of *Mycobacterium tuberculosis,* we screened for suppressors of NO hypersensitivity in a mycobacterial proteasome ATPase mutant and identified mutations in Rv1205. We determined that Rv1205 encodes a pupylated proteasome substrate. Rv1205 is a homolog of the plant enzyme LONELY GUY, which catalyzes the production of hormones called cytokinins. Remarkably, we report that an obligate human pathogen secretes several cytokinins. Finally, we determined that the Rv1205-dependent accumulation of cytokinin breakdown products is likely responsible for the sensitization of *Mycobacterium tuberculosis* proteasome-associated mutants to NO [Samanovic *et al.* 2015, 2018]. PIN-FORMED (PIN) proteins actively transport the plant hormone auxin, whosedirectionality, referred to as polarity, steers developmental processes throughout the plant's lifecycle. We identified the ROTUNDA3 protein as a regulator of the protein phosphatase 2A-driven PIN recycling and revealed its importance in auxin transport-related plant developmental programs [Karampelias *et al.* 2016]. In this study, we generated functional, fluorescent protein-tagged PIN6 proteins and performed comprehensive analysis of their subcellular localization and also performed a detailed functional characterization of PIN6 and its developmental roles [Simon *et al.* 2016]. The recent isolation of mutants of the model grass species *Brachypodium distachyon* with dramatically enhanced root cell elongation due to increased cellular auxin levels has allowed us to address question of the long-standing Acid Growth Theory. We found that the primary transcriptomic effect associated with elevated steady state auxin concentration in elongating root cells is upregulation of cell wall remodelling factors, notably expanses, while plant hormone signalling pathways maintain remarkable homeostasis [Pacheco-Villalobos *et al.* 2016]. We also reported that the *DIOXYGENASE FOR AUXIN OXIDATION 1* (*AtDAO1*) enzyme represents the major pathway for auxin oxidation in *Arabidopsis*. Disrupting *AtDAO1* function elevates levels of auxin conjugates between ~50- and 280-fold [Porco *et al.* 2016]. Here, we showed that loss of function of VAS2 (IAA-amido synthetase Gretchen Hagen 3(GH3).17) leads to increase in free IAA at the expense of IAA-Glu (IAA-glutamate) in the hypocotyl epidermis. This active IAA elicits shade- and high temperature-induced hypocotyl elongation largely independently of 3-IPA-mediated IAA biosynthesis in cotyledons. It revealed the unexpected capacity of local auxin metabolism to modulate the homeostasis and spatial distribution of free auxin in specialized organs such as hypocotyls in response to shade and high temperature [Zheng *et al.* 2016]. Our work also revealed how the PLETHORA (PLT) transcription factor gradients can regulate cell state by region-specific induction of cell proliferation genes and repression of differentiation [Santuari *et al.* 2016]. Here, we showed that modulating the expression of the maize *PLASTOCHRON1* (*ZmPLA1*) gene, encoding a cytochrome P450 (CYP78A1), results in increased organ growth, seedling vigour, stover biomass and seed yield. Transcriptome studies, hormone measurements and the expression of the auxin responsive DR5rev:mRFPer marker suggest that PLA1 may function through an increase in auxin [Sun *et al.* 2017]. AtNHX5 and AtNHX6 are endosomal Na^+,K^+/H^+ antiporters that are critical for growth and development in *Arabidopsis.* We found that *nhx5 nhx6* exhibited growth variations of auxin-related defects because it was affected in auxin homeostasis. Genetic analysis showed that AtNHX5 and AtNHX6 were required for the function of the endoplasmic reticulum (ER)-localized auxin transporter PIN5 [Fan *et al.* 2018]. Here we investigated the role of PIN phosphorylation during gravitropic response. We discovered that loss- and gain-of-function mutants in PINOID and related kinases but not in D6PK kinase or mutations mimicking the constitutive

dephosphorylated or phosphorylated status of two clusters of predicted phosphorylation sites partially disrupted PIN3 phosphorylation and caused defects in gravitropic bending in roots and hypocotyls [Grones *et al.* 2018]. Here we investigated the source of auxin for early embryogenesis and provided evidence that the mother plant coordinates seed development by supplying auxin to the early embryo from the integuments of the ovule. We showed that the auxin response increases in ovules after fertilization, due to upregulated auxin biosynthesis in the integuments, and this maternally produced auxin is required for proper embryo development [Robert *et al.* 2018]. We showed that AA rescues root gravitropic growth in the anthranilic acid (AA)-deficient mutant at concentrations that do not rescue IAA levels. Overproduction of AA affects root gravitropism without affecting IAA levels. Treatments with, or deficiency in, AA result in defects in PIN polarity and gravistimulus-induced PIN re-localisation in root cells [Doyle *et al.* 2019]. Here, using loss-of-function mutants we showed that three Aux/IAA genes, *IAA6, IAA9*, and *IAA17*, act additively in the control of adventitious root (AR) initiation. These three IAA proteins interact with ARF6 and/or ARF8 and likely repress their activity in AR development. We showed that *TIR1* and *AFB2* are positive regulators of AR formation and TIR1 plays a dual role in the control of jasmonic acid (JA) biosynthesis and conjugation, as several JA biosynthesis genes are up-regulated in the *tir1-1* mutant [Lakehal *et al.* 2019 a,b]. Using the *Arabidopsis* model, we showed that the chromatin-modifying enzyme HISTONE DEACETYLASE 9 (HDA9) is essential for promoting an open plant architecture that allows for efficient mitigation of the impact of warm temperature. HDA9 does not affect hypocotyl elongation in response to different light conditions, setting it apart from the shade-avoidance response that phenotypically resembles acclimation to warmth. We demonstrated that HDA9 is required for transcriptional activation of YUCCA8 [van der Woude *et al.* 2019]. Here, we analysed the auxin distribution and expression of PIN auxin efflux carriers from early phases of germination, and showed that bending of the root in response to gravity is the crucial initial cue that governs the hypocotyl bending required for apical hook formation. Importantly, polar auxin transport machinery is established gradually after germination starts as a result of tight root-hypocotyl interaction and the proper balance between abscisic acid and gibberellins [Zhu *et al.* 2019].

III. MEDICINAL CHEMISTRY

Chemical modulators of kinases

During the last five years, we have continued development of increasingly effective cyclin-dependent-kinase (CDK) inhibitors, leading to the discovery of several other potent compounds with various structural motifs [Jorda *et al.* 2015; Vymětalová and Kryštof, 2015; Baltus *et al.* 2016; Ajani *et al.* 2018;]. The potential of CDK inhibitors in different therapeutic areas was also reviewed several times [Malínková *et al.* 2015; Jorda *et al.* 2018b; Kubczak *et al.* 2019;]. New generations were prepared following well-established methods, including our previously described syntheses of purines, pyrazollo[4,3-d]pyrimidines, 8-azapurines, fluoroaryl benzimidazoles and arylazopyrazoles (for patents see [Branná *et al.* 2015; Havlíček *et al.* 2015; Lamie *et al.* 2015; Vanda *et al.* 2015; Wróbel *et al.* 2015; Xavier *et al.* 2015; Bhambra *et al.* 2016; Jedinák *et al.* 2016; Tenora *et al.* 2016; Vymětalová *et al.* 2016; Hylsová *et al.* 2017;] and www.espacenet.com). Selected examples are enclosed: We described new 4-arylazo-3,5-diamino-1H-pyrazole derivatives developed from CAN508, one of the first inhibitors to show preference for transcriptional regulator cyclin-dependent kinase 9 [Jorda *et al.* 2015]. We also described the leishmanicidal activities of a library of 2,6,9-trisubstituted purines that were screened for interaction with Cdc2-related protein kinase 3 (CRK3) and subsequently for activity against parasitic *Leishmania* species [Řezníčková *et al.* 2015a]. We also prepared and studied a series of 3,5,7-trisubstituted pyrazolo[4,3-d]pyrimidines, a new CDK inhibitor scaffold, to assess their CDK2 inhibitory and antiproliferative activities. A new compound, which preferentially inhibits CDK2, CDK5 and aurora A was also discovered [Řezníčková *et al.* 2015b]. In this study, the effect of a novel exogenous cyclin-dependent kinase inhibitor, BP-14, was investigated in three human anaplastic thyroid carcinoma (ATC) cell lines. ATC is an extremely aggressive human malignancy characterized by a marked

degree of invasiveness, absence of features of thyroid differentiation and resistance to current medical treatment. Our data indicated that BP-14 is a potential new compound effective against ATC. Combined treatment with BP-14 and the mTOR inhibitor Everolimus, had strong synergistic anticancer effects, suggesting that the combined used of CDK and mTOR inhibitors may be a useful strategy for ATC treatment [Allegri *et al.* 2016]. We have developed a new series of inhibitors that show a preference for inhibiting CDK5. Inhibition of CDK5 has been shown to have anti-angiogenic effects *in vitro* and *in vivo*. The new series showed a preference for *in vitro* and *in vivo* cytotoxicity in a murine model of hepatocellular carcinoma [Zhang *et al.* 2016]. We reported an alternative synthetic approach for selected 2,6,9-trisubstituted purine CDK inhibitor conjugates with folic acid as a drug-delivery system targeting folate receptors [Krajčovičová *et al.* 2016]. We described a collection of novel 2,6,9-trisubstituted purine derivatives with nanomolar inhibitory activities against PDGFRα, a receptor tyrosine kinase often found constitutively activated in various tumours. The compounds demonstrated strong and selective cytotoxicity in the human eosinophilic leukemia cell line EOL-1 [Malínková *et al.* 2017]. A series of pyrrolo[1,2-b]pyrazoles was synthesized and their ALK5 inhibition activity by kinase profiling was discovered [Řezníčková *et al.* 2017]. We also reported on the synthesis, activity testing, docking, and quantum mechanical scoring of novel imidazo[1,2-c]pyrimidin-5(6H)-one scaffold for cyclin-dependent kinase 2 (CDK2) inhibition [Ajani *et al.* 2018]. A novel series of 4,6-disubstituted pyrazolo[3,4-d]pyrimidines have been designed and synthesized by molecular hybridization. All the synthesized compounds were evaluated for *in vitro* CDK2/cyclin E and Abl kinase inhibitory activity as well as anti-proliferative activity [Cherukupalli *et al.* 2018]. FLT3 tyrosine kinase is a potential drug target in acute myeloid leukemia (AML) in patients with FLT3-ITD mutations. Recently, we presented novel 2,6,9-trisubstituted purine derivatives with potent FLT3 inhibitory activity [Gucký *et al.* 2018]. We also investigated the selectivity of commercially available CDK inhibitors and identified compounds that will be suitable for further studies to identify the CDKs responsible for S81-AR phosphorylation [Jorda *et al.* 2018a]. 3,5,7-substituted pyrazolo[4,3-d]pyrimidine inhibitors of CDK and their evaluation in lymphoma models was recently reported [Jorda *et al.* 2019a]. The activity of 2,6,9-trisubstituted purines as potent PDGFRα kinase inhibitors with anti leukaemic activity was also described [Řezníčková *et al.* 2019].

Natural phytochemicals as potential drug candidates

1. Triterpenoid Compounds with Anticancer Activity - Triterpenoids are natural compounds with a variety of biological activities and are usually produced in plants as secondary metabolites. In recent decades, scientists have focused on the properties of triterpenoids and have discovered a number of activities, including anti-tumor, anti-viral, anti-microbial, anti-inflammatory and others [Kvasnica *et al.* 2015; Urban *et al.* 20105;]. For example, concise synthesis of 28a-homolupane triterpenes and the corresponding saponins containing d-mannose, d-idose, d-arabinose, and l-rhamnose moieties was elaborated. Several triterpenes and the corresponding saponins exhibited an interesting cytotoxic activity profile against human cancer cell lines [Sidoryk *et al.* 2015]. A series of lupane-type saponins bearing the OSW-1 disaccharide unit as well as its regio- and stereoisomers was prepared and used for structure–activity relationships (SAR) study [Kuczynska *et al.* 2016]. Several other homolupane and homobetulin derivatives have been synthesised and their boiological activites examined [Sidoryk *et al.* 2016a,b,c]. New cytotoxic triterpenoid esters were prepared from 5 triterpenic acids. Some of the compounds had high cytotoxicity on at least 3 of 4 tested cancer cell lines and induced apoptosis via caspase-3 activation [Eignerova *et al.* 2017]. A series of picolyl amides of betulinic acid was prepared and subjected to cytotoxicity screening tests. Structure-activity relationship studies, resulted in finding differences in biological activity [Bildziukevich *et al.* 2018]. An enhancing effect of cystamine in its amides with betulinic acid as antimicrobial and antitumor agent *in vitro* was also reported [Bildziukevich *et al.* 2019]. We reported a concise synthesis of solasodine analogues containing the seven-membered F ring from diosgenin and their biological activity [Kiełczewska *et al.* 2019]. Model synthesis of oleandrigenin from androstenolone was developed. The Na^+/K^+-ATP-ase inhibitory and cytotoxic activities *in vitro* of new compounds were compared with that of oleandrin [Michalak

et al. 2019]. 2-Deoxyglycoside conjugates of lupane triterpenoids with high cytotoxic activity were also developed [Perlíková *et al.* 2019].

2. Triterpene-Based Compounds with Supramolecular and Pharmacological Characteristics – A number of triterpene-based compounds display supramolecular characteristics resulting in the formation of self-assembly systems and even supramolecular gels. From this point of view, hydrogels are of top pharmacological importance. Supramolecular characteristics can be studied by several different methods (UV-VIS spectrometry, DOSY-NMR spectroscopy, (cryogenic) transmission electron microscopy etc.). These physico-chemical and imaging methods enable investigation of the supramolecular structures that are formed. We have found and proven that these systems are dynamic where supramolecular systems change from one to another over time intervals from the beginning of experiments, which are usually several day-lasting processes. Irregularities observed with several of these compounds in cytotoxicity sceening tests resulted in a new finding that dynamic supramolecular systems formed during cytotoxicity screening tests in cell lines may influence their pharmacological activity [Bildziukevich *et al.* 2020; Özdemir *et al.*, 2020a].

3. Triterpene- and Steroid-Based Adaptogens – We have focused our attention on adaptogenic compounds as well. A number of plant products, including several natural plant triterpenes, and natural plant steroids display adaptogenic effects. Generally, these plant products affect the central nervous system (CNS). They may thus be of benefit in Alzheimer and Parkinson-type diseases, and they have other overall health improving qualities as well [Özdemir *et al.* 2018, Bildziukevich *et al.* 2019]. As a result of this general investigation, we have developed conjugates of diosgenin, a CNS active aglycone of a plant drug, with a triterpenoid acid. The synthesis resulted in developing new cytotoxic compounds. The described structural modification appears to be another important finding showing us how to modify the type of a pharmacological activity and/or revealing a way of making the effect of modified compounds more controlled. If a potentially cytotoxic drug of this type is metabolized in the human body, a CNS active compound appears as a metabolite in the human body, capable of augmenting health [Özdemir *et al.* 2020b].

4. Natural cytokinins and their derivatives – Cytokinins are phytohormones that are involved in many processes in plants, including growth, differentiation and leaf senescence. However, they also have various activities in animals. Kinetin and trans-zeatin can reduce levels of several aging markers in human fibroblasts. Kinetin can also protect mice against oxidative and glyoxidative stress, and prolong fruit fly lifespan. Additionally, several cytokinins are currently used in cosmetics [Hönig *et al.* 2018a; Kadlecová *et al.* 2019; Voller *et al.* 2019]. Here, we reported the synthesis and *in vitro* biological testing of thirty-one cytokinin derivatives substituted at the C8 position of the adenine skeleton and twenty-seven compounds which served as their N9-tetrahydropyranyl protected precursors [Zahajská *et al.* 2017]. To extend the breadth of knowledge of cytokinin activities, we examined the effects of natural cytokinin bases on the model nematode *Caenorhabditis elegans*. We found that kinetin, para-topolin and meta-topolin prolonged the lifespan of *C. elegans* [Kadlecová *et al.* 2018]. New cytokinin derivatives possessing UVA and UVB photoprotective effect on human skin cells and that prevent oxidative stress were also described [Hönig *et al.* 2018b]. We developed new 6-substituted purines as ROCK inhibitors with anti-metastatic activity [Voller *et al.* 2019]. Novel dodecyl-containing azido and glucuronamide-based nucleosides exhibiting anti-cancer potential are also reported [Xavier *et al.* 2019].

5. New anti-inflammatory and anti-cancer substances - We have developed a number of new anti-inflammatory compounds over the last years which have been tested in different bioassays [Mistry *et al.* 2015; Mojzych *et al.* 2015; Mrozek-Wilczkiewicz *et al.* 2015; Solichová *et al.* 2015; Páchniková *et al.* 2016; Patel *et al.* 2016; Wróbel *et al.* 2016; Cavallaro *et al.* 2017; Jorda *et al.* 2017b; Mistry *et al.* 2017; Morrogh-Bernard *et al.* 2017; Moyo *et al.* 2017; Xavier *et al.* 2017a,b; Lamie *et al.* 2018; Lasák *et al.* 2018; Liu *et al.* 2018; Milišiūnaitė *et al.* 2018; Abo-Ashour *et al.* 2019; Krajčovičová *et al.* 2019; Kumar *et al.* 2019; Milišiūnaitė *et al.* 2019; Pokorná *et al.* 2019; Rárová *et al.* 2019; Shulha *et al.* 2019;]. For example, sixteen new phthalimide derivatives were synthesized and evaluated for their *in vitro* anti-microbial, anti-

oxidant and anti-inflammatory activities [Lamie *et al.* 2015]. Novel N-substituted indole Schiff bases as dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase enzymes were synthesized and their anti-inflammatory activity was evaluated [Lamie *et al.* 2016]. New retinoids and curcuminoids with a broad spectrum of anti.oxidant, anti-inflammatory and anti-tumor activity were prepared and tested [Morzycki *et al.* 2016]. We also designed and synthesized new salicylamide derivatives with dipeptide moieties, which were tested for their anti-proliferative effect against three leukaemia cell lines *in vitro* and they displayed GI50 values in the mid-micromolar range [Dušek *et al.* 2017; Jorda *et al.* 2017a]. We further reported that the natural cytokinin 2OH3MeOBAR induces cell death by a mechanism that is different from that of the "classical" cytokinin ribosides [Voller *et al.* 2017].

6. Steroid growth regulators (steroids brassinosteroids, and ecdysteroids) - Brassinosteroids (BRs) are a relatively recently discovered group of phytohormones that are essential for normal plant growth and development. They participate in the regulation of numerous vital physiological processes in plants, such as elongation, germination, photomorphogenesis, immunity and reproductive organ development and stress responses [Filek *et al.* 2018, 2019; Janeczko *et al.* 2018, 2019; Oliwa *et al.* 2019] and also in ethylene production [Jiroutová *et al.* 2016, 2019]. Recent studies have indicated that BRs have anti-proliferative, anti-cancer, anti-angiogenic, anti-viral and anti-bacterial properties in animal cell systems, and thus have potential medical applications [see our reviews Oklešťková *et al.* 2015, 2017; Jiroutová *et al.* 2018;]. We have prepared and studied a series of new brassinosteroid (BR) derivatives with a p-substituted phenyl group in the side chain. Several very potent compounds were prepared [Kvasnica *et al.* 2016]. Structure-activity relationship analysis and profiling of a library of AB-functionalized cholestane derivatives closely related to BRs were performed to examine their anti-proliferative activities and activities on steroid hormone receptors [Rárová *et al.* 2016]. A series of phenyl and seco analogues of brassinosteroids was prepared and tested using the pea inhibition biotest, Arabidopsis root sensitivity and cytotoxicity assay [Kořínková *et al.* 2017; Kvasnica *et al.* 2019]. Synthesis and *in vitro* anti-cancer activity of 23(23′)E-benzylidenespirostanols derived from steroid sapogenins is also described [Ramos-Enríquez *et al.* 2017, 2018]. It was also reported that the novel brassinosteroid analogue BR4848 inhibits angiogenesis in human endothelial cells and induces apoptosis in human cancer cells *in vitro* [Rárová *et al.* 2018]. Synthesis of novel galeterone derivatives and tetrahydropyrazolo[1,5-a]pyridine-fused steroids and evaluation of their *in vitro* activity against prostate cancer cell lines was also reported [Jorda *et al.* 2019b,c].

7. Chemopreventive phytochemicals, redox-reactive antioxidants, phenolics – We have published a number of papers on this topic related to chemopreventive phytochemicals and phenolics [Ndhlala *et al.* 2015; Šamec *et al.* 2015a,b; 2016; Colak *et al.* 2016a,b, 2017, 2019; Kumari *et al.* 2018b; Moyo *et al.* 2018a; Bhattacharyya *et al.* 2019; Linič *et al.* 2019; Milanović *et al.* 2019a,b;]. In one publication, there is a structure-based overview of lignans and neolignans as well as a bioactivity-based overview of compounds [Zálešák *et al.* 2019]. Hydroxycinnamates are common phenolic compounds of plants and plant foods, often found in substantial quantities. Due to their high *in vitro* antioxidant activity they can easily be oxidized under oxidative conditions. For example, we found that *in vitro* oxidation of coumaric, ferulic and sinapic acids resulted mainly in dimeric compounds. By applying the sensitive UHPLC–MS/MS method, we were able to identify and quantify four free hydroxycinnamic acid dimers for the first time, namely 8-8′-disinapic, 8-5′-diferulic, 8-O-4′-diferulic and 8-3′-dicoumaric acids, in wheat sprouts, Chinese cabbage, millet sprouts, light beer and parsley [Grúz *et al.* 2015]. We developed a one-pot microwave-assisted method for the synthesis of cinnamic acid and coumarin derivatives. This approach provides high product yields and selectivities without the need of a phenol protecting group [Konrádová *et al.* 2017]. Furthermore, a novel approach to the neolignan-core skeletons of boehmenan natural products and to dehydrodiconiferyl alcohol glucosides based on the Fe(III)-promoted oxidative coupling has been achieved [Barbuščáková *et al.* 2018]. We also identified and characterized potential bioactive compounds from the leaves of *Leucosidea sericea* [Pendota *et al.* 2018].

8. Natural and synthetic alkaloids - With over 500 individual compounds, the Amaryllidaceae alkaloids represent a large and structurally diverse group of phytochemicals. Coupled to this structural diversity is a significant array of biological properties manifested by many of its members, whose relevance in motor neuron disease and cancer chemotherapy has attracted considerable attention. Given this background, we have been attempting to uncover the various mechanisms which have been invoked to corroborate their different biological effects [Nair *et al.* 2015; Makong *et al.* 2019]. We observed that there were significant variations in alkaloid levels for different months of the year in the genus *Cyrtanthus* which were also affected by the environment that has a significant role on the quality of plant extracts [Ncube *et al.* 2015]. Work on *S. puniceus* led to the isolation of haemanthamine (1), haemanthidine (2), and a rare chlorinated amide, metolachlor (3), the natural occurrence of which is described for the first time [Naidoo *et al.* 2018].

Molecular docking and quantum modelling - Molecular docking is a powerful tool for theoretical prediction of the preferred conformation and orientation of small molecules within protein active sites. The obtained poses can be used for estimation of binding energies, which indicate the inhibition effect of designed inhibitors, and therefore might be used for in silico drug design. For example, we presented implementation and testing of a scoring function based on more physically justified exponential repulsion instead of the standard Lennard–Jones potential. We found that this scoring function significantly improved prediction of the native binding modes in proteins bearing narrow active sites such as serine proteases and kinases [Bazgier *et al.* 2016]. By using an extensive computational chemistry approach, the affinities of the inhibitors to CDK2 are determined as calculated binding scores of complexes of the inhibitors with the protein. The interactions of the inhibitors with CDK2 are computationally described using a hybrid quantum mechanics/semi-empirical quantum mechanics method (QM/SQM), which combines the DFT-D method for the QM part and the PM6-D3H4X method for the SQM part. The solvent effect is described by the COSMO implicit solvation model at the SQM level for the whole system [Nekardová *et al.* 2017].

IV. PLANT MOLECULAR PHYSIOLOGY

This session has been developing smoothly over the last five years. It originates from the transfer of some groups to LGR from other university departments and their subsequent unification (prof. Fellner, Dr. Ohnoutková a Dr. Plíhal) but also due to the establishment of brand-new research groups (prof. P. Hedden). The research program focuses on understanding cross-talk between light and hormonal signalling pathways, molecular mode of action oh phytohormones and their signalling as well as signalling involved in the growth and development of plants and in plant responses to abiotic stress. They also study phytohormone molecular mode of action using transcriptomic a and next-generation sequencing approaches. The genetic approach involves mutant collections including Arabidopsis, tomato and maize. In their work, they use a number of physiological, genetic and molecular methods. There are also several reviews related to the topics mentioned above [Wasternack, Song 2017].

Hormones and light

The group for example, showed that ABA promotes DNA endoreduplication by enhancing the expression of the genes encoding inhibitors of cyclin-dependent kinases *SlKRP1* and *SlKRP3* and by reducing cytokinin levels. The data were supported by expression analysis of the genes which encode enzymes involved in ABA and CK metabolism. It seems that ABA is essential for the process of hypocotyl elongation and that appropriate control of the endogenous level of ABA is required in order to drive the growth of etiolated seedlings [Humplik *et al.* 2015a; 2017]. Furthermore, in dark-grown seedlings, the ABA accumulation was maximal in the cotyledons and elongation zone of hypocotyl, whereas under blue-light, the ABA content was distinctly reduced. Thus, ABA promotes the growth of etiolated seedlings and the results suggest that ABA plays an inhibitory role in de-etiolation and photomorphogenesis in tomato [Humplik *et al.* 2015b]. We also reported that the 7B-1 mutant in tomato (*Solanum lycopersicum* L., cv. Rutgers), an ABA overproducer, is defective in blue light (B) signalling leading to B-specific resistance to abiotic and biotic stresses. Using a methylation-sensitive

amplified polymorphism (MSAP) assay, a number of genes were identified, which were differentially methylated in the 7B-1 mutant and its wild type (WT) seedlings. The data showed that DNA methylation remodelling is an active epigenetic response to different lights and stresses in 7B-1 and WT, and it highlighted the differences in epigenetic and transcriptional regulation of light and stress responses in 7B-1 and WT. Furthermore, it shed light on the crosstalk between DNA hypomethylation and miRNA regulation of ARFs expression [Omidvar and Fellner, 2015]. Using sRNA sequencing, we further identified miRNAs that are potentially involved in another development and regulation of male-sterility in the 7B-1 mutant [Omidvar a*et al.* 2015a]. Subsequent data also suggests that miR159, miR166, miR472, miR482, miR#A, and miR#D probably facilitate the blue-light-specific enhanced tolerance of 7B-1 to abiotic stress [Omidvar *et al.* 2015b]. The study provides insight into the transcriptome of the *7B-1* mutant. We identified several genes with altered expression level in *7B-1* (including *beta-1,3 glucanase, GA2oxs, cystatin, cysteine protease, pectinesterase, TA29*, and *actin*) that could potentially regulate anther developmental processes, such as meiosis, tapetum development, and cell-wall formation/degradation [Omidvar *et al.* 2017]. Our data also indicated the existence of a link between osmotic stress and BL signalling and the involvement of the 7B-1 mutation in this crosstalk [Balarynová *et al.* 2018, Balarynová, Fellner 2019]. We further demonstrated that an age-regulated microRNA, miR156, regulates shoot regenerative capacity. As a plant ages, the gradual increase in miR156-targeted SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors leads to the progressive decline in shoot regenerative capacity [Zhang *et al.* 2015]. Our results also indicated that some acyltransferases associated with cutin formation are involved in CK responses and skotomorphogenesis in Arabidopsis [Wu *et al.* 2015a]. Through the use of the photosynthesis inhibitor, norflurazon and of masking experiments, evidence is given here that light acts mainly as a morphogenic signal in the triggering of bud outgrowth and that initial steps in the light signalling pathway involve cytokinins [Roman *et al.* 2016]. We also used two species of the genus *Geranium* to study the involvement of auxin, brassinosteroids (BRs), and gibberellins (GAs) in supplemental far-red-induced elongation growth [Gommers *et al.* 2018]. In order to study whether blue light (BL) suppression accelerated senescence rate of wheat leaves, involves the alteration of different cytokinin (CK) metabolites, CK-degradation, as well as the expression CK-perception, -inactivation, -reactivation and/or -turnover genes, leaf segments of 30 day-old plants were placed in boxes containing bi-distilled water and covered with blue (B) or green (G) light filters, which supplied a similar irradiance but differed in the percentage of BL transmitted (G << B) [Marchetti *et al.* 2018]. We identified leaves as a site for perception of seasonal shifts and revealed that components of floral transition such as FLOWERING LOCUS T (FT) and plant hormone GA have been recruited to function as long-range signals to communicate seasonal changes perceived in leaves to the shoot apical meristem to control its activity to synchronize bud set with the change of seasons in perennials [Miskolczi *et al.* 2019].

Phytohormones and stress responses

The aim of this study was to characterize phytohormonal homeostasis in barley (*Hordeum vulgare*) in reaction to drought and validate the role of brassinosteroids in the regulation of this process [Gruszka *et al.* 2016b]. The aim of our work was also to explore the dynamics of changes in ABA metabolites as well as other stress-induced phytohormones such as JA, IAA, and their respective metabolites in hop [*Humulus lupulus* (L.)] plants during drying and to identify among them potential signals involved in drought signalling [Korovetska *et al.* 2016]. We provided genetic and molecular evidence that a component of the decapping machinery, the LSM1-7 complex, plays a critical role in plant tolerance to abiotic stresses. Depending on the stress, the complex from *Arabidopsis thaliana* interacts with different selected stress-inducible transcripts, targeting them for decapping and subsequent degradation [Perea-Resa *et al.* 2016]. GAs and BRs are important phytohormones that control plant development and responses to environmental cues by involving DELLA proteins and BRASSINAZOLE-RESISTANT1 (BZR1) respectively, as key transcription factors. Here, we revealed a new role for JUNGBRUNNEN1 (JUB1) as a transcriptional regulator of GA/BR signalling in *A. thaliana*

[Shahnejat-Bushehri *et al.* 2016]. We investigated strigolactones (SL). This can be transported acropetally as a systemic indication of stress by analysing molecularly and physiologically wild-type (WT) tomato (*Solanum lycopersicum*) scions grafted onto SL-depleted rootstocks, compared with self-grafted WT and SL-depleted genotypes, during a drought time-course [Visentin *et al.* 2016]. We monitored movement, electrical signalling, jasmonate accumulation and digestive enzyme secretion in local and distal (systemic) traps in response to prey capture, mechanical stimulation of trigger hairs and wounding [Pavlovič *et al.* 2017]. We also found that short-term salt stress in *Brassica rapa* seedlings causes alterations in auxin metabolism [Pavlovič *et al.* 2018a, 2019], while drought stress causes other responses on physiological, biochemical and hormonal levels [Pavlovič *et al.* 2018b]. A genome-wide transcriptional analysis was also performed on leaves and roots of three-day salt treated and untreated plants of two rice varieties, which differ in salt sensitivity. Genes correlated with hormonal pathways were identified and analysed [Formentin *et al.* 2018]. This study examined how two different stresses, salt, and oxidative stress, affect changes in both cytokinin levels and whole plant transcriptome response in *Solanum lycopersicum* seedlings [Keshishian *et al.* 2018]. The content of endogenous brassinosteroids together with various aspects of plant morphology, water management, photosynthesis and protection against cell damage were also assessed in two maize genotypes that differed in drought sensitivity [Tůmová *et al.* 2018]. Singlet oxygen produced from triplet excited chlorophylls in photosynthesis is a signal molecule that can induce programmed cell death (PCD) through the action of the OXIDATIVE STRESS INDUCIBLE 1 (OXI1) kinase. Here, we identified two negative regulators of light-induced PCD that modulate OXI1 expression: DAD1 and DAD2, homologs of the human antiapoptotic protein DEFENDER AGAINST CELL DEATH [Beaugelin *et al.* 2019].

New phytohormone mutants

We hypothesized that a potentially suitable phenotypic marker is root curling induced by CK, as observed in the auxin biosynthesis mutant *CK-induced root curling 1/tryptophan aminotransferase* of Arabidopsis 1 (*ckrc1/taa1*). Phenotypic observations, genetic analyses and biochemical complementation tests of Arabidopsis seedlings displaying the trait in large-scale genetic screening showed that it can facilitate isolation of mutants with perturbations in auxin biosynthesis, transport and signalling [Wu *et al.* 2015b]. The identification of the *HvDWARF* genomic sequence, its mutational and functional analysis and characterization of new brassinosteroid biosynthetic mutants are reported [Gruszka *et al.* 2015a].

Phytohormones in plant-pathogen/insect interaction

Phytohormone levels and the expression of genes encoding key enzymes participating in hormone biosynthetic pathways involved in plant-pathogen/insect interactions were investigated in many different plant species [Dziurka *et al.* 2016]. Furthermore, we provided genetic evidence that a nematode-derived cytokinin is involved in activating the host cell cycle during infection. Our findings confirmed the ability of an animal to synthesize and secrete a functional plant hormone to establish long-term parasitism [Siddique *et al.* 2015]. Phytohormone production by microorganisms is not yet considered as a biocontrol mechanism. We identified the ability of *Pseudomonas fluorescens* G20-18 to efficiently control *P. syringae* infection in *Arabidopsis*, allowing maintenance of tissue integrity and ultimately biomass yield [Großkinsky *et al.* 2016]. In this study we used transcriptomics and metabolomics to investigate changes in cytokinin metabolism during gall formation of clubroot-infected *A. thaliana* [Malinowski *et al.* 2016]. We also examined the role played by ABA/GA interactions regulating the formation of arbuscular mycorrhizal in tomato. We reported differences in JA, ABA and GA metabolism and signalling between control and mycorrhizal roots [Martín-Rodríguez *et al.* 2016; Adolfsson *et al.* 2017]. Here, we provided draft genome sequences of three more members of the *Fusarium fujikuroi* species complex. The comparisons to publicly available genome sequences revealed species-specific and isolate-specific differences in the composition and expression of genes involved in secondary metabolite production including those for phytohormone biosynthesis [Niehaus *et al.* 2016]. We also reported that the rhizobacterium *B. amyloliquefaciens* subsp. plantarum UCMB5113 stimulated the growth of *A. thaliana* by increasing lateral root outgrowth and elongation and

root-hair formation, although primary root elongation was inhibited. Specific hormone reporter gene lines were tested which suggested a role for at least auxin and cytokinin signalling during rhizobacterial modulation [Asari *et al.* 2017]. We also discovered that infection by *Rhodococcus fascians* maintains cotyledons as a sink tissue for the pathogen [Dhandapani *et al.* 2017, Jameson *et al.* 2019]. In addition, while both the virulent strain and the epiphytic strain impacted the expression of transporter genes in the shoots and roots, only the virulent strain affected morphology. The inhibited root growth, the greening of the roots, and the expression of the pea response regulators in the infected roots were indicative of a response to cytokinin, but a role for the 'classical' cytokinins as virulence determinants was not established [Dhandapani *et al.* 2018]. In this study, we showed that strigolactone(SLs)-deficient tomato plants (*S. lycopersicum*; SlCCD8 RNAi lines), infected with pre-germinated *Phelipanche ramosa* seeds, display an increased infection level and faster development of the parasite, which suggests a positive role for SLs in the host defence against parasitic plant invasion [Cheng *et al.* 2017]. We also reported that two mutations in the truncated *Rep* gene RBR domain delayed the *Wheat dwarf virus* infection in transgenic barley plants [Cejnar *et al.* 2018]. This study examined the temporal changes in the leaf content of defence-involved phytohormones in pepper plants responding to the green peach aphid (*Myzus persicae* Sulzer) infestation, at both local and systemic level [Florencio-Ortiz *et al.* 2018]. Recombinant expression of limen, a small cysteine-rich peptide isolated from lima beans with activity against Micromycetes in both a prokaryotic and plant system was performed to demonstrate the wide range of its activities with potential for further use [Řehořová *et al.* 2018]. In this work, we evaluated and reconsidered IAA metabolism in *Bradyrhizobium japonicum* E109, one of the most widely used strains for soybean inoculation around the world [Torres *et al.* 2018]. We also showed that Mal de Río Cuarto virus infection causes hormone imbalance and sugar accumulation in wheat leaves [de Harro *et al.* 2019]. This study describes how one pectin-modifying enzyme, *PECTIN ACETYLESTERASE 9 (PAE9),* affects the Arabidopsis transcriptome, secondary metabolome, and aphid performance [Kloth *et al.* 2019]. The effect of plant heat-shock pre-treatment on *Pseudoidium neolycopersici* development in the susceptible and moderately resistant *Solanum* spp. genotypes was studied together with biochemical responses (endogenous concentrations of salicylic, jasmonic, abscisic acid, and peroxidase activity) [Nožková *et al.* 2019]. In this work, we explored changes in the levels of phytohormones in maize and mango plant tissues infected with *Fusarium* [Vrabka *et al.* 2019].

Seed dormancy and germination

In seeds, the transition from dormancy to germination is regulated by abscisic acid (ABA) and gibberellins (GAs), and involves chromatin remodelling [Urbanová, Leubner-Metzger 2016]. We showed that DOF AFFECTING GERMINATION1 (DAG1) expression is controlled at the epigenetic level through the H3K27me3 mark during the seed-to-seedling transition, and that DAG1 also directly represses the ABA catabolic gene CYP707A2; consistently, the ABA level is lower while the GA level is higher in dag1 mutant seeds. Furthermore, both DAG1 expression and protein stability are controlled by GAs [Boccaccini *et al.* 2016]. We used RT-qPCR to determine the expression of multiple cytokinin gene family members, and LC-MS/MS to ascertain endogenous cytokinin levels in germinating Pisum sativum L. We showed that genes that are actively expressed when the seed is a strong sink during its development, are also expressed when the seed is in the reverse role of being an active source during germination and early seedling growth [Jameson *et al.* 2016]. We also used phylogenetic and comparative analyses of fruit and seed anatomy, biomechanics, physiology, and environmental responses to study fruit and seed heteromorphism, a typical morphological basis of the bet-hedging strategy of plants, in the annual Brassicaceae species *Aethionema arabicum* growth [Lenser *et al.* 2016; Lenser *et al.* 2018; Arshad *et al.* 2019; Mérai *et al.* 2019; Wilhelmsson *et al.* 2019]. We also found that polishing and washing of the sugar beet fruits had both a positive effect on germination performance and seedling phenotype, and when combined, this positive effect was stronger. Polishing as well as washing removed germination inhibitors from the pericarp, specifically, ABA, ABA metabolites, and ions [Ignatz *et al.* 2019]. This study provides a comparative analysis of the dormancy and germination mechanisms of the indehiscent fruits

of hoary cress (*Lepidium draba* L.) and hairy whitetop (*L. appelianum* Al-Shehbaz), two invasive weeds of the Brassicaceae [Mohammed *et al.* 2019].

Research activity and characterization of the main scientific results

With the financial support of numerous grant projects, research activities of the laboratory in the period 2015-2019 included studies of the biological role of auxin and cytokinin and the interaction of phytohormonal pathways in response to the environment. In total, between 2015 and 2019, members of the laboratory co-authored 91 original contributions and four reviews in impacted journals, six contributions in books and non-impacted journals and three editorial materials in impacted scientific journals. Here, a brief summary of the major scientific achievements of laboratory members is described. In these contributions, members of the laboratory were first, joint first and/or corresponding authors and they represented a significant part of the author's collective. Besides these major achievements, researchers in the laboratory were involved in many other works and joint outputs with other laboratories within the institute and from abroad.

Research on auxin produced significant outputs mainly in the field of regulation of auxin transport and homeostasis, the evolution of auxin transport mechanisms and cellular biology of auxin transporters.

Utilizing primarily the model of tobacco cell lines, well established in the laboratory, we revealed a new way of regulation of the number of auxin carriers on cell membranes based on auxin-driven gene expression and co-operation between auxin influx and efflux transporters (Müller et al., Plant Journal 100, 627-640, 2019). Auxin transport characteristics predicted by the mathematical model for the most potent auxin efflux carrier NtPIN11 were confirmed by qRT-PCR and cultivation in auxin-free conditions, justifying the modelling approach developed in our laboratory. In the frame of this work, we have also successfully initiated extensive RNAseq profiling of tobacco cell lines BY-2 and VBI-0 and made them accessible for the community in Genevestigator.

Model of tobacco cell lines was very significant in our characterization of the nitrate transceptor (transporter&receptor/sensor) NRT1.1. In the continuation of our previous collaborative work with CNRS/INRA/SupAgro-M/UM, Montpellier, France showing the auxin-transporting activity of NRT1.1, we have characterized the role of NRT1.1 by testing the auxin transport of mutated versions carrying point mutations in the phosphorylation site at amino acid residue T101 (Bouguyon et al., Nature Plants 1, 15015, 2015). We have shown by ^{3}H-IAA uptake assays that it is the phosphorylated form of NRT1.1 (T101D) that dominates in its auxin-uptake activity and that this function is needed for the repression of lateral root emergence under low NO_3.

Auxin transport assays in tobacco cell lines were used to present the first 3-dimensional, atomic resolution atlas of the substrate requirements of AUX1 transporter, explaining why many commercially-successful auxin herbicides are not good substrates for this transporter (Hoyerová, Hošek, Quareshy et al., New Phytologist 217, 1625-1639, 2018). Commercial auxin use is rising, as is the probability of field resistance. In this collaborative work with the University of Warwick, UK, we discuss the role of uptake carriers in auxin herbicide action and relate this to likely mechanisms of resistance arising in the fields. Auxin-like herbicidal effects were also s hown for a quinoline carboxamide compound ACCERBATIN (AEX), which was selected in chemical screen based on its exacerbation of the triple response in the collaborating laboratory at University Ghent, Belgium (Hu et al., Journal of Experimental Botany 68, 4185-4203, 2017). Results from our team allowed to proposed that AEX interferes with auxin transport either as a direct consequence of poor basipetal transport from the shoot meristematic region, or indirectly, through excessive IAA oxidation and ROS accumulation. We also provided novel evidence on the mechanism of action of silver ions in plants cells (Klíma, Laňková et al., Plant Cell Reports 37: 809-818, 2018). Using tobacco cells, we show that silver ions, previously known to specifically target ethylene signalling, primarily act through the modification of the activity of calcium channels with downstream effects on the permeability of the plasma membrane for small compounds, including auxins and cytokinins. Finally, characterization of modulation of auxin transport and auxin metabolic profiles in relation to the mode of action of selected intermediates of phenylpropanoid metabolic pathway, namely

derivatives of cinnamic acid, was performed in detail in our co-operation with PSB VIB Belgium. We have shown that cis-cinnamic acid (Steenackers et al., Plant Physiology, 173: 552-565, 2017) inhibits auxin efflux from cells, representing a novel compound among native, endogenous auxin transport inhibitors and shedding light on allelochemical properties of such substances.

In collaboration with laboratory from BOKU, Vienna, Austria, we significantly contributed to the identification of novel cross-talk between auxin transport and brassinosteroids, based on their inhibitory effect on the endocytosis of auxin efflux carrier PIN2 (Retzer et al., Nature Communications 10, 5516, 2019). Using roots of *Arabidopsis thaliana*, the brassinosteroid-induced stabilization of PIN2 at the plasma membrane was shown to be instructive for the tuning of differential cell elongation during a bending response of roots to gravity.

With colleagues from IST Austria, we have contributed fundamentally to the characterization of the non-canonical member of the PIN family of auxin transporters, PIN6 (Simon et al., New Phytologist 211, 65-74, 2016). The evidence from our experiments indicates that PIN6 has dual localization at the plasma membrane and endoplasmic reticulum and mediates auxin transport across the PM and intracellular auxin homeostasis, including the regulation of free auxin and auxin conjugates levels. Our results point to the role of PIN6 during lateral and adventitious root organogenesis.

We brought the first experimental evidence on the evolutionary origins of PIN-mediated carrier-driven auxin efflux (Skokan, Medvecká et al., Nature Plants 5, 1114-1119, 2019). By testing auxin transport activities in several heterologous models, including tobacco cells, we show that PIN homolog from *Klebsormidium flaccidum* is capable of auxin transport at the plasma membrane, which is typical for PIN proteins of land plants. Unlike these canonical PINs, *Kf*PIN is not localized in polar domains, suggesting its evolutionary basal role in the non- directional auxin export. This work has been coordinated by our laboratory in collaboration with laboratory from IST, Austria.

In the field of cell biology of auxin transporters, we have uncovered their differential dynamics within the plasma membrane (Laňková et al., Microscopy and Microanalysis 22: 290-299, 2016). In this collaborative work with J. Heyrovský Institute of Physical Chemistry CAS, we have pioneered the usage of raster image correlation spectroscopy (RICS) in plants. We showed that the faster mobility of PIN1 auxin efflux carrier depends on the cytoskeleton, while significantly lower mobility auxin influx carrier AUX1 is determined by sterol composition of the membrane. Introducing method of SRRF-based super-resolution microscopy into *in vivo* analysis of root-specific PIN2 auxin efflux carrier allowed us to unravel the importance of cysteine residues for their PM localization (Retzer, Lacek et al., IJMS 18, 2274, 2017). This work has been published in collaboration with laboratory from BOKU, Vienna, Austria and the University of Warwick, UK. In combination with site-directed mutagenesis and pioneering molecular modelling approach, we suggested that cysteine residues are essential for regulatory redox-based post-translational modifications of PIN2. Based on the systematic screening of the effects of a set of intracellular trafficking inhibitors in tobacco cells, we identified a novel action of ARF-GEF GNOM-LIKE protein1a NtGNL1a from tobacco in the endocytosis (Jelínková et al., BMC Plant Biology 15, 272, 2015). Using confocal microscopy in lines carrying engineered versions of the protein, NtGNL1a was shown to be involved in the endocytosis of PIN proteins. Our data demonstrate the potential of tobacco BY-2 cells for selective mapping of ARF-GEF-regulated endomembrane trafficking pathways. By the combination of confocal microscopy and auxin transport assays in *Arabidopsis thaliana* inflorescence stems we contributed to the evidence on the role of actin-nucleating complex ARP2/3 in the control of cell wall synthesis and auxin-controlled morphogenesis in plants. In collaboration with Faculty of Science, Charles University, we discovered that stem vascular tissues in plants lacking a functional ARP2/3 complex have decreased basipetal auxin transport and decreased presence of AUX1 auxin influx carriers (Pratap Sahi et al., Annals of Botany 122: 777-789, 2018). Finally, our new high-resolution confocal microscopy platform for *in vivo* observations of Arabidopsis seedling root in natural gravitational vector allowed to provide *in vivo* evidence for the presence of unique organization of PIN1 auxin efflux carrier in dividing provasculature cells (Marhavá et al., Developmental Cell 52, 223-235, 2019/20). We have complemented the work colleagues from the University of Lausanne, who provided

evidence from fixed preparations.

Research on cytokinins has been focused on their biosynthesis and metabolism in a range of developmental and environmental contexts. We also addressed the evolution of their biosynthesis, including the relation to other phytohormones.

Integrating phylogenetic, physiological, biochemical and molecular approaches, we continued in our effort to the characterization of so far somewhat overlooked cytokinin forms, *cis*-zeatins. We have shown that *cis*-zeatin-type cytokinins occur in cyanobacteria and algae (Žižková et al., Annals of Botany 119, 151-166, 2017) as well as in mosses (Záveská Drábková et al., PLOS One 12, e0187331, 2015). We have also demonstrated the involvement of *cis*- zeatins in the modulation of plant defence responses against pathogen infections (Trdá et al., Frontiers in Microbiology 8, 1374, 2017), during somatic embryogenesis in conifers (Vondráková et al., Frontiers in Plant Science 9, 1283, 2018) and during phosphate starvation in Arabidopsis roots (Přerostová, Kramná et al., Environmental and Experimental Botany 153, 198-208, 2018). These all outputs implied that the *cis*-zeatins have a much higher impact for cytokinin biology being more relevant and prevalent in plants than previously supposed. The papers of Žižková et al. (Annals of Botany 119, 151-166, 2017) and Záveská Drábková et al. (PLOS One 12, e0187331, 2015) represent one of the most comprehensive surveys of cytokinin and auxin hormonome so far. Using our analytical approach, we have suggested here for the first time evolutionary early-diverging conjugation mechanisms for cytokinin and auxin homeostasis as well as differences in metabolic strategies of these two phytohormones between liverworts and mosses. The collaborative effort with the Laboratory of Pathological Plant Physiology of IEB CAS (Trdá et al., Frontiers in Microbiology 8, 1374, 2017) provided original results on cytokinin metabolism in oilseed rape and its reaction to the infection with a filamentous fungus, *Leptosphearia maculans*. Here for the first time in the fungal kingdom, two novel components, the cytokinin oxidase/dehydrogenase and the cytokinin adenosine kinase-related activities have been shown.

Using a similar approach, we have continued in our studies of numerous aspects of *N*-glucosyltransferase pathways that are supposed to be involved in irreversible inactivation of cytokinins. The products of this pathway, cytokinin *N*-glucosides, biosynthesized by specific glucosyltransferases encoded by *UGT76C1* and *UGT76C2* genes were found ubiquitous in seed plants in contrast to their mostly relatively low levels or total absence in non-vascular plants and ferns (Záveská Drábková et al., PLOS One 12, e0187331, 2015; Žižková et al., Annals of Botany 119, 151-166, 2017). In contradiction to a generally accepted hypothesis that *N*- glucosylation inactivates cytokinins irreversibly, we have shown cytokinin *N*-glucosides to be subject to metabolic conversions that differ between *N*-glucosides of N^6-(Δ^2- isopentenyl)adenine and *trans*-zeatin in *Arabidopsis* (Hošek et al., New Phytologist, 10.1111/nph.16310, 2019). This work represents the first large-scale study of CK metabolism in planta that reveals directionality and kinetics of yet uncharacterized CK metabolic pathways. We constructed a mathematical model that provides estimates of the metabolic conversion rates, which support the qualitative observations. Our findings fill in fundamental gaps in the current understanding of CK metabolism in Arabidopsis, in particular, hydrolysis of CK N-glucosides was shown for the first time.

We have identified and characterized two tomato CK biosynthetic genes, SlIPT3 and SlIPT4, and reported their different spatio-temporal expression patterns and *in vitro* enzymatic activity of their products during tomato plant development and in response to salinity. Based on these data, we have proposed a hypothetical scheme of SlIPT3 and SlIPT4 action during the early salt stress (Žižková et al., BMC Plant Biology 15, 85, 2015).

Our effort to understand the developmental role of cytokinin metabolism produced many outputs, the most important being focused on developmental switch points and reaction to the environment. The long-lasting focus of the laboratory on cytokinins has been extended with the emphasis on the parallel analysis of whole pant hormonome, which allows us to address numerous phytohormonal cross-talks.

First, in collaboration with the laboratory at Université Catholique de Louvain, Belgium, we have significantly contributed to the understanding of molecular mechanisms engaged in hormonal control of plant development and plant responses to salinity. Using cultured tomato

(*Solanum lycopersicum*), we have demonstrated the involvement of phytohormones in these processes and specified the roles of *SlIPT3* and *SlIPT4* genes coding for CK biosynthesis (Žižková et al., BMC Plant Biology 15, 85, 2015). We show that both SlIPT3 and SlIPT4 enzymes are functional and enhance the tolerance of plants to higher salinity. Our results contribute to the understanding of CK regulation at the molecular level and provide a potentially useful tool to obtain and improve high-quality stress-tolerant crops in agriculture. We have also contributed to the identification and functional characterization of the tomato (*Solanum lycopersicum*) transcription factors SlDREB2 (Hichri et al., Plant Cell and Environment 39, 62- 79, 2016) and SlWRKY3 (Hichri et al., Frontiers in Plant Science 8, 1343, 2017), demonstrating their important roles in the control of tomato plant development and salinity response. SlDREB2 was identified here for the first time as a salt stress-regulated transcription factor. Its overexpression in Arabidopsis and tomato imparted plant tolerance to salinity, mainly due to regulation of synthesis of protecting molecules, phytohormone contents and down-stream genes involved in stress response, osmoregulation and hormone biosynthesis/signalling. The involvement of SlWRKY3 in growth, photosynthesis and salt stress response of tomato plants was found to correlate with changes in the levels of phytohormones, especially salicylic acid and jasmonates. In general, our contribution to this multidisciplinary approach revealed SlWRKY3 as an essential regulator of salinity tolerance in tomato.

Second, in collaboration with Luxembourg and Belgian laboratories, our detailed analysis of phytohormones during the transition from primary to secondary growth in the hemp (*Cannabis sativa* L.) hypocotyls revealed that cytokinins are tightly linked with secondary growth and bast fibre development. Cytokinin-N- and O-glucosides were involved in these processes at later stages of plant development when their levels were decreased indicating reduced inactivation of the total CK pool (Behr et al., Frontiers in Plant Science 7, 1733, 2016). In the same system, we have also demonstrated the impact of jasmonic acid on lignification in the hemp hypocotyl (Behr et al., Plant Signaling & Behavior 14, e1592641, 2019).

Third, in the close co-operation with the Laboratory of Biologically Active compounds, we provided the most comprehensive overview of endogenous phytohormone levels during the somatic embryo development and germination in conifers. Some of the phytohormones were analyzed in conifer somatic embryogenesis (SE) for the first time (jasmonates, phenylacetic and salicylic acid, cis-zeatin- and dihydrozeatin-type cytokinins). The changes in endogenous phytohormones in the course of SE indicated a correlation between phytohormone profiles and particular developmental stages (Vondráková et al., Frontiers in Plant Science 9, 1283, 2018). Within this collaboration, we have also reported contrasting phytohormone profiling in relation to the osmotic adjustment under salinity in the cultivated glycophyte tomato *Solanum lycopersicum* and its halophyte wild relative species *Solanum chilense* (Gharbi et al., Plant Science 258, 77-89, 2017).

Lastly, in the collaboration with Biological Research Centre, HAS, Szeged, Hungary, levels of cytokinins have been shown to be up-regulated during autotetraploidization of energy willow *Salix viminalis* (Dudits et al., Plant Physiology 170, 1504-1523, 2016). Our results show for the first time significantly increased amounts of gibberellins, cytokinins, salicylic acid, and jasmonates compared with diploids as well as higher net photosynthetic CO_2 uptake. Polyploidization thus may represent a promising strategy.

Research on the role of phytohormones in the reaction of plants to stress conditions have been focused on the advanced analytics of hormonomes during heat, salt and drought stress, as well as in reaction to nutrient deficiency and during plant defence against biotic stress. Analytical profiling of phytohormones have been systematically correlated with other analyses, including quantitative determination of gene transcription.

Within the field of a heat stress signalling and the role of phytohormones, the main attention was paid primarily to the role cytokinins. The study of hormonal dynamics during the early heat stress response revealed the crucial role of cytokinins in transient up-regulation of stomata conductance and subsequent stimulation of transpiration, which allows maintaining leaf temperature lower than the environment, at least until defence mechanisms are activated (Dobrá et al., Plant Science 231, 52-61, 2015). Using transformants with induced over-

expression of cytokinin biosynthetic gene, the elevation of cytokinin content has been shown to have a positive effect on leaf proteome in heat stress targeted to roots, while rather a negative effect was observed in case of shoot targeted heat stress (Skalák et al., Journal of Experimental Botany 67, 2861-2873, 2016). This work, performed in collaboration with Department of Molecular Biology and Radiology, Agronomical faculty, Mendel University, Brno, demonstrated that cytokinins have longer-term positive effects on the stress-related proteins, including those regulating cell redox state and photosynthesis.

Mechanisms underlying salt stress tolerance were studied comparing the stress response of glycophyte *Arabidopsis thaliana* and halophyte *Thellungiella salsuginea* (Přerostová et al., Plant Science 264, 188-198, 2017). Enhanced salinity tolerance was associated with higher basal levels of abscisic and jasmonic acid in apices as well as faster and stronger up-regulation of stress-related genes in the whole plant.

As both cytokinin decrease and up-regulation was shown to diminish drought impact, in the co-operation with Research Centre Julich, Germany, we have performed the extensive comparative study to decipher their role. Our results show that cytokinin down-regulation had a positive impact on the retention of water potential, while their increase promoted plant recovery after re-watering (Přerostová et al., Frontiers in Plant Science 9, 655, 2018). This complex phenotyping study evaluated the effect of timing of cytokinin modulation (permanent, before stress, during the stress progression. Our findings may be utilized in the elevation of plant fitness in stress conditions by exogenous cytokinin treatment or by their down-regulation, respectively.

We have also addressed the impact of nutrient deficiency by analyzing organ-specific response to phosphate deficiency in *Arabidopsis thaliana* (Přerostová, Kramná et al., Environmental and Experimental Botany 153, 198-208, 2018). The nutrient deficiency was associated with down-regulation of active cytokinins, especially of *trans*-zeatin type, and gibberellins predominantly in apices and leaves. In roots, up-regulation of *cis*-zeatin was observed. The decisive role of strigolactones was indicated by down-regulation of strigolactone repressors and after exogenous strigolactone application. The involvement of *cis*-zeatin in reaction to phosphate deficiency suggests an essential physiological role of this cytokinin.

Several outputs addressed the role phytohormones in the reaction plants to biotic stressors. Firstly, the role of phytohormone-based defence responses was elucidated at the onset of cyst nematode belowground pathogen *Heterodera schachtii* in Arabidopsis (Kammerhofer et al., New Phytologist 207, 778-789, 2015). In the framework of collaboration with laboratories from University of Natural Resources and Life Sciences, Tulln, Austria, and Bonn University, Germany, we have shown that nematodes trigger profound changes in hormone biosynthetic and signalling pathways. This reaction is in strict dependence on their parasitism stage. Phytohormonal dynamics during plant attack and syncytium formation indicated a possible strategy on how to cope with these widely spread parasites. Secondly, we have contributed to the elucidation of phytohormone responses that accompany the efficient defence of *Brassica napus* plants against *Plasmodiophora brassicae* infection. Comparison of two cultivars differing in their resistance to P. *brassicae* revealed that up-regulation of salicylic acid is associated with enhanced resistance, while stimulation of jasmonic acid pathway with increased plant sensitivity to the pathogen (Přerostová et al., IJMS 19, 4024, 2018). Moreover, these changes were accompanied by faster down-regulation of auxins and cytokinins.

Research activity and characterisation of the main scientific results

Plants and Environment, selected results

Pharmaceuticals

1) An integral part of this ongoing research is evaluation of the fate of xenobiotics in plants at the enzymatic level. We emphasized the environmental importance of plants in the detoxification of veterinary drugs in an extensive review on the transport and biotransformation of veterinary drugs in plants. The risks and consequences of veterinary drug escape into the environment and the potential role of phytoremediation technologies were considered and future perspectives outlined.
Xenobiotic-metabolizing enzymes in plants and their role in uptake and biotransformation of veterinary drugs in the environment. Bártíková, H., Skálová, L., Stuchlíková, L., Vokřál, I., Vaněk, T., Podlipná, R. Drug Metabolism Reviews. 2015, 47(3), 374-387.*
The members of the Laboratory of Plant Biotechnologies conducted an extensive survey of many articles in the field of metabolism of xenobiotics in plants. They described the enzymes of the I. phase of biotransformation as well as the enzymes of the II. phase contributing to veterinary drug biotransformation.
The corresponding author R. Podlipná and the other co-author (TV), who outlined the review structure and wrote its substantial part, are from our lab. PhD. student L. Stuchlíková and 3 other co-authors are members of Faculty of Pharmacy in Hradec Králové, Charles University.

2) Metabolism of widely used drug contaminant ibuprofen at the cellular level was studied in plant model *A. thaliana* cell suspension in vitro using LC/MS HRAM. The intake, extracellular as well as intracellular metabolism followed by incorporation into cell wall was proved. More than 300 metabolites, including oxidation products, conjugates with amino acids and saccharides, were detected. *Maršík, P., Šíša, M., Lacina, O., Moťková, K., Langhansová, L., Rezek, J., Vaněk, T*. Metabolism of ibuprofen in higher plants: A model Arabidopsis thaliana cell suspension culture system. Environmental Pollution. 2017, 220(JAN), 383-392.*
All authors with exception of O. Lacina are from our lab, including first and corresponding.

3) Paper was focused on the study of the influence of fenbendazole (FBZ) on proteome and transcriptome of *Arabidopsis thaliana*. Our results clearly demonstrated that FBZ is taken up into plants and metabolized in 12 different metabolites. The presence of FBZ and its metabolites in *A. thaliana* influenced both gene expression and the protein abundance. Taken together, fenbendazole could affect many physiological and metabolic processes in plants and this anthelmintic represents a risk for ecosystems.
Metabolism of the anthelmintic drug fenbendazole in Arabidopsis thaliana and its effect on transcriptome and proteome. Syslová, E., Landa, P., Stuchlíková Raisová, L., Matoušková, P., Skálová, L., Szotáková, B., Navrátilová, M., Vaněk, T., Podlipná, R. Chemosphere. 2019, 218(MAR), 662-669*
P. Landa. R. Podlipná (corresponding author) made the experimental set-up, evaluated the results and wrote the paper. The PhD. student E. Syslová (Faculty of Pharmacy in Hradec Králové, Charles University) cultivated the plants and performed the experiments (microarrays as well as quantitative real-time PCR analysis).

4) The study mapped concentration of main non-steroidal antiphlogistic, the most popular group of drugs in the Czech Republic, in the various water streams of Elbe river basin during the vegetation period using LC/MS and GCxGC/MS. The study involved 29 sampling sites involving urban agglomerations, rural areas, small water streams and main river flows Elbe and Vltava. Concentrations of majority of the drugs were higher in small local streams than rivers and ibuprofen was found as the most abundant drug.
Non-steroidal anti-inflammatory drugs in the watercourses of Elbe basin in Czech Republic, Maršík, P., Rezek, J., Židková, M., Kramulová, B., Tauchen, J., Vaněk, T . Chemosphere. 2017, 171(MAR), 97-105.*
All authors with exception of J. Tauchen (PhD. student of the Czech University of Life Sciences Prague) are from our lab, as well as the first and corresponding ones.

5) Contaminated soils near inhabited areas might cause the ecology risk, in addition to endangering the health of local human population. This study can contribute toward the development of a cleaning-up solution of brownfield in city Kladno, integrated with bioenergy demand fulfilment. Our research is an important first step toward friendly remediation of such polluted sites.
Preliminary study of phytoremediation of brownfield soil contaminated by PAHs
Petrová, Š., Rezek, J., Soudek, P., Vaněk, T. Science of the Total Environment. 2017, 599-600(DEC 1), 572-580.*
All authors are from our laboratory.

6) Study was focused on accumulation and metabolism of human and veterinary antiparasitic drug praziquantel in suspension and intact plants of *Phragmites australis*. The results obtained in vitro were confirmed in real conditions using constructed wetland. The results showed ability of plants to uptake and metabolize the drug. LC/MS HRAM was used for quantitation of praziquantel dynamics and metabolite identification.
Study of praziquantel phytoremediation and transformation and its removal in constructed wetland. Maršík, P., Podlipná, R., Vaněk, T. Journal of Hazardous Materials. 2017, 323(FEB 5), 394-399.*
All authors are from our laboratory.

Nanoparticles

7) In this study, we tried to uncover the mechanism responsible for toxicity of ZnO nanoparticles (NPs) for plants. We compared the effect of ZnO NPs with ionic Zn^{+} and with non-nanosized ZnO particles on transcriptomic response of *Arabidopsis thaliana* plants. This design allowed us to compare effect of three zinc materials on transcriptome and estimate the effect of particle size and solubility on the overall phyto-toxicity.
The Transcriptomic Response of Arabidopsis thaliana to Zinc Oxide: A Comparison of the Impact of Nanoparticle, Bulk, and Ionic Zinc
Landa, P., Přerostová, S., Petrová, Š., Knirsch, V., Vaňková, R., Vaněk, T. Environmental Science and Technology. 2015, 49(24), 14537-14545.*
The first (P. Landa) and corresponding author (T. Vanek) are from our lab. Plant cultivation and quantitative RT-PCR was ensured by our colleagues Sylva Přerostová, Vojtěch Knirsch, and Radomíra Vaňková from Laboratory of Hormonal Regulations in Plants from our institute.

8) The other from the series of studies focused on the nanoparticle - plant interactions has been aimed to characterization of the effect of ZnO nanoparticles (NPs) on phytohormone pools *Arabidopsis thaliana*. The phytohormones regulate multiple physiological processes, including plant growth and development. Therefore, we determined levels of five main phytohormones (cytokinins, auxins, abscisic acid, salicylic acid and jasmonic acid as well as

their metabolites) in apices, leaves and roots of plants exposed to a wide range of ZnO NP concentrations.
ZnO nanoparticle effects on hormonal pools in Arabidopsis thaliana
Vaňková, R., Landa, P., Podlipná, R., Dobrev, P., Přerostová, S., Langhansová, L., Gaudinová, A., Moťková, K., Knirsch, V., Vaněk, T. Science of the Total Environment. 2017, 593(SEP 1), 535-542.*
Our group designed the experiments and participated in the manuscript preparation. T. Vanek from our lab is the corresponding author. We collaborated with colleagues from Laboratory of Hormonal Regulations in Plants from our institute, who are specialists in plant hormone analysis.

9) In this study we focused on the elucidation of the mechanism responsible for toxicity of CuO nanoparticles (NPs) for plants. We used similar design as in ZnO NP study (The Transcriptomic Response of Arabidopsis thaliana to Zinc Oxide: A Comparison of the Impact of Nanoparticle, Bulk, and Ionic Zinc). We compared the behavior of CuO NPs and non-nanosize CuO particles (both bulk and ionic form) in the cultivation medium and in contact with plant roots. Detail transcriptomic analysis of Arabidopsis responses to various copper materials was performed.
Transcriptomic Response of Arabidopsis thaliana Exposed to CuO Nanoparticles, Bulk Material, and Ionic Copper. Landa, P., Dytrych, P., Přerostová, S., Petrová, Š., Vaňková, R., Vaněk, T. Environmental Science and Technology. 2017, 51(18), 10814-10824.*
This study was conducted in our lab (transcriptomic analysis and investigation of particle solubility). The first and corresponding author (P. Landa) is from our lab. Quantitative real-time PCR was measured by Sylva Přerostová and Radomíra Vaňková from Laboratory of Hormonal Regulations in Plants. Pavel Dytrych (Department of Catalysis and Reaction Engineering, Institute of Chemical Process Fundamentals of the CAS) acquired pictures from transmission electron microscope.

10) In this study we demonstrated that isotopically-labelled nanoparticles (NPs) in combination with single particle ICP-MS provide a useful tool for study of NP uptake by plants. We described differences in solubility of silver NPs, ZnO NPs and CuO NPs in cultivation medium as well as their accumulation and translocation in model plant *Arabidopsis thaliana*.
Synthesis and characterization of isotopically-labeled silver, copper and zinc oxide nanoparticles for tracing studies in plants
Nath, J., Dror, I., Landa, P., Vaněk, T., Kaplan-Ashiri, I., Berkowitz, M. L. Environmental Pollution. 2018, 242(NOV), 1827-1837.
This paper is one of the results of our common Czech-Israeli project. We participated in the experimental design. Our crucial role was plant cultivation and application of NPs to cultivation media. Further, we collected and processed cultivation media and plant samples. We were also actively involved in manuscript preparation.

Plant stress

11) The paper contributed to understanding of the mechanism of metal uptake by plants, which may be utilized in practical applications, such as phytoremediation or biofortification. The goal of the research was to determine the effect of the exogenous polyamine application on the metal uptake. Our results showed that the consumption of these enriched vegetables could be a suitable way to solve the problem of a deficiency of essential micronutrients in the diet. This topic is of global relevance.
Improving crop tolerance to heavy metal stress by polyamine application
Soudek, P., Ursu, M., Petrová, Š., Vaněk, T. Food Chemistry. 2016, 213(DEC 15), 223-229.*
Members of the team of the Laboratory of Plant Biotechnology established the experimental set-up, performed the experiments and wrote the paper. Marina Ursu, (student of the

University of Chemical Technology) contributed to the experimental data, working on her Master thesis.

12) In this study we investigated transcriptomic response of tobacco plants to the presence of thorium in cultivation medium. Thorium effects were previously studied only in the case of microbes. Our study can be useful for estimation of potential ecological risks, biotechnologies and remediation of polluted areas.
Thorium impact on tobacco root transcriptome
Mazari, K., Landa, P., Přerostová, S., Müller, K., Vaňková, R., Soudek, P., Vaněk, T. Journal of Hazardous Materials. 2017, 325(MAR 5), 163-169.
This study was done at our Institute. The corresponding author (T. Vanek) is from our lab. The first author (K. Mazari) was student of the Czech University of Life Sciences Prague. We cooperated with Sylva Přerostová and Radomíra Vaňková (real-time qPCR) and Karel Müller (annotation of tobacco transcriptome) from Laboratory of Hormonal Regulations in Plants.

13) Study was focused on the transcriptomic response of *Arabidopsis* plants to two pharmaceutically important drugs naproxen and praziquantel. We demonstrated different effects of diverse classes of pharmaceuticals (naproxen is non-steroidal anti-inflammatory drug, while praziquantel is anthelmintic medicament). Both compounds represent rather common environmental contaminants. Potential risks and possible candidates in drug metabolism were suggested.
Transcriptomic response of Arabidopsis thaliana roots to naproxen and praziquantel
Landa, P., Přerostová, S., Langhansová, L., Maršík, P., Vaňková, R., Vaněk, T.*. Ecotoxicology and Enviromental Safety. 2018, 166(DEC 30), 301-310.*
Experimental design, most of the experiments as well as manuscript preparation was done in our lab. Therefore, the first and corresponding authors are from our team. Colleagues Přerostová S. and Vaňková R. from Laboratory of Hormonal Regulations in Plant did quantitative real-time PCR.

14) The threat of thorium contamination in the environment is becoming a current topic because of its possible use in nuclear power plants. Therefore, thorium effects are currently intensively investigated. Our research could provide important insights into understanding the mechanism of thorium uptake into the plants. This is crucial in relation to the increased risk of thorium exposure of the human population, which could be a substantial problem for human health.
Thorium as an environment stressor for growth of Nicotiana glutinosa plants
Soudek, P., Hrdinová, A., Rodriguez V, I. M., Lhotáková, Z., Mihaljevič, M., Petrová, Š., Kofroňová, M., Moťková, K., Albrechtová, J., Vaněk, T.. Environmental and Experimental Botany. 2019, 164(AUG), 84-100*
Half of authors are members of the team of the Laboratory of Plant Biotechnology (including the first and corresponding author). A. Hrdinová (student of PřF UK) contributed to the results, working on her Master thesis in our lab.

Plant metabolites, selected results

15) In this study, we compared content of phenolic compounds in wines produced in Georgia (traditional country of viticulture and winemaking) with wines produced in Central and Western Europe. Georgian producers cultivate local varieties and use traditional methods during winemaking process (fermentation with skins, seeds, and bunch stems in clay vessel called kvevri). This study extents considerably only scarce information about chemistry and biological activity Georgian wines.
In vitro antioxidant activity and phenolic composition of Georgian, Central and West European wines

Tauchen, J., Maršík, P., Kvasnicová, M., Maghradze, D., Kokoška, L., Vaněk, T., Landa, Přemysl*. Journal of Food Composition and Analysis. 2015, 41(AUG 2015), 113-121.*
Corresponding authors are P. Landa and T. Vanek from our team. The first author Jan Tauchen was PhD. student from Czech University of Life Sciences Prague (CULS) working on his thesis in our lab. Optimization of antioxidant assays was done in cooperation with Professor L. Kokoska from CULS. Very kind and irreplaceable colleague was David Maghradze (Institute of Horticulture, Viticulture and Oenology, Agricultural University of Georgia) delivering wine samples.

16) This article is a comprehensive review focused on chemical reactions and biological activities of tetracyclic diterpenoids. Publication represents a literature survey from the year 2000 till 2015. It is a part of international cooperation network, published in a special issue of Current Pharmaceutical Design journal, which is devoted to natural product diversity in research of new pharmaceutical leads.
Synthesis of Tetracyclic Diterpenoids with Pharmacologic Relevance
Šíša, M., Vaněk, T. Current Pharmaceutical Design. 2016, 22(12), 1767-1807.*
This complex review was fully elaborated in our research group.

17) This review summarizes current knowledge about in vitro, in vivo and clinical activities of natural stilbenoids, which are popular nutraceuticals for the prevention of cancer and other age-related diseases. Special attention is paid to their metabolism and relevance of the published data. Also, methods to increase their availability and efficacy are suggested. As nowadays people tent to turn to natural compounds and folk medicine, the topic of bioactive nutraceuticals is highly relevant.
Anti-inflammatory activity of natural stilbenoids: A review
Dvořáková, M., Landa, P. Pharmacological Research. 2017, 124(OCT), 126-145.*
Both authors are from the Laboratory of Plant Biotechnologies.

18) The topic of this paper is synthesis and biological activity of strigolactone mimics based on triazolide scaffold. The mimics markedly induced root elongation and lateral root formation in Arabidopsis thaliana, which may have positive effect on plant growth and product yield when applied to agricultural crops. In addition, these mimics are prepared using a concise three-step reaction procedure, which notably simplifies their synthesis when compared to natural strigolactones.
Triazolide Strigolactone Mimics Influence Root Development in Arabidopsis
Dvořáková, M., Soudek, P., Vaněk, T. Journal of Natural Products. 2017, 80(5), 1318-1327.*
All the authors are from the Laboratory of Plant Biotechnologies.

19) In this paper, simple two-step synthesis of new type of strigolactone mimics based on resorcinyl scaffold is presented. These mimics were highly stable even at alkaline pH and able to induce seed germination of parasitic plants *Striga hermonthica* and *Phelipanche ramosa* at low concentrations, EC_{50} ~ 2 × 10^{-7} M (*Striga*) and EC_{50} ~ 2 × 10^{-9} M (*Phelipanche*). These results, namely simple synthesis and high stability, make these compounds an exciting target for their utilization as suicidal germinators.
Resorcinol-Type Strigolactone Mimics as Potent Germinators of the Parasitic Plants Striga hermonthica and Phelipanche ramosa
Dvořáková, M., Hýlová, L., Soudek, P., Retzer, K., Spíchal, L., Vaněk, T. Journal of Natural Products. 2018, 81(11), 2321-2328.*
The first and corresponding authors are from our lab. Three co-authors from the Palacky University in Olomouc contributed to the paper.

20) In this paper, the synthesis of new series of strigolactone mimics based on triazolide scaffold is presented. These mimics were stable at acidic as well as basic environment and effectively induced seed germination of parasitic plant *Phelipanche ramosa* with EC_{50} as low

as 5.2 × 10^{-10} M. Such mimics may therefore be potentially used to control the occurrence of broomrape parasitic weed in fields.
Triazolide strigolactone mimics as potent selective germinators of parasitic plant Phelipanche ramose. Dvořáková, M., Hýlová, A., Soudek, P., Petrová, Š., Spíchal, L., Vaněk, T. Pest Management Science. 2019, 75(7), 2049-2056.*
The first and corresponding authors are from our lab. Four authors from the Laboratory of Plant Biotechnologies have been involved. Marcela Dvorakova designed and synthesized the compounds, Petr Soudek measured the biological data on Arabidopsis, Sarka Petrova did the stability studies on HPLC/UV, and Tomas Vanek contributed to the writing of the paper. The biological data on the parasitic plants were collected in Palacky University in Olomouc by Adela Hylova and Lukas Spichal.

List of selected papers mentioned in text in alfabethical order

Bártíková et al, *Drug Metabolism Reviews*. **2015**, ID 448675

Dvořáková et al, *Journal of Natural Products.* **2017**, ID 476611

Dvořákova et al, *Journal of Natural Products.* **2018**, ID 497794

Dvořáková et al, *Pest Management Science*. **2019**, ID 506098

Dvořáková et al, *Pharmacological Research.* **2017**, ID 480368

Landa, P at al. *Environmental Science and Technology*. **2015,** ID 454866

Landa et al, *Ecotoxicology and Enviromental Safety*. **2018**, ID 496183

Landa et al, *Environmental Science and Technology.* **2017**, ID 479928

Maršík et al, *Chemosphere*. **2017,** ID 475711

Maršík et al, *Environmental Pollution*. **2017**, ID 481319

Maršík et al, *Journal of Hazardous Materials*. **2017**, ID 481320

Mazari et al, *Journal of Hazardous Materials*. **2017**, ID 473850

Nath et al, *Environmental Pollutio*n. **2018**, ID 495431

Petrová et al, *Chemosphere*. **2017**, ID 476517

Šíša et al, *Current Pharmaceutical Design*. **2016**, ID 460035

Soudek et al, *Environmental and Experimental Botany.* **2019**, ID 507494

Soudek et al, *Food Chemistry.* **2016**, ID 463451

Syslová et al, *Chemosphere*. **2019**, ID 501890

Tauchen et al, *Journal of Food Composition and Analysis.* **2015,** ID 447148

Vaňková et al*, Science of the Total Environment*. **2017**, ID 476522

Research for practice

Our comprehensive results in the area of plant utilization in environment protection (phytoremediation) enabled us to extend our efforts to semi-real and real conditions. The aim was to contribute to solving environmental problems generally, not only in the Czech Republic.
Other results are connected with the synthesis of natural products analogues.
The Technology Agency funded two of our projects directed to practical tasks. The first was an evaluation of the fate of plastic materials defined as biodegradable in real conditions (Bioplast). The results confirmed, unfortunately, the many caveats about these materials, which are in fact biodegraded very slowly and release toxic chemicals into the environment.

Our results will be utilized by the Ministry of Environment for the development of environmental policy as well as by the company ECO-COM, which is responsible for the collection and separation of waste materials in the Czech Republic.
In the frame of the second project, “Bioclean”, we have demonstrated the ability of constructed wetlands to clean agricultural waste waters and remove not only “standard” contamination but also the residues of veterinary and other pharmaceuticals. We have confirmed the possibility of utilizing this full-scale system not only for decontamination but also for recycling of water, which can be used directly at the farm for irrigation, with high economic impact.
Our laboratory also initiated, submitted and was finally was funded by the Czech Technology Agency large project “Support for the process of commercializing the results of research and development at the Institute of Experimental Botany AS CR v.v.i.”.
The general problem with water shortage led us to utilize phytotechnology method to cope with it. Our project “Utilization of R&D results of the Institute of Experimental Botany AS CR v.v.i.“ was supported by from EU structural Funds and the Prague municipality and started on 1. 1. 2017.

Selected practical results

Patents

306653
Paclitaxel derivatives, the method of production and the use
Dvořáková, Marcela, Vaněk, Tomáš, Tarkowská, Danuše. 2017. ASEP (ID 484275)

ZA2017/08339
Primin derivatives, method of preparation thereof and use thereof
Landa, Přemysl, Šíša, Miroslav, Vaněk, Tomáš, Rárová, Lucie. 2018. ASEP (ID 499587)

308139
Strigolactone derivatives for controlling parasitic plants seed germination. Dvořáková, Marcela, Soudek, Petr, Vaněk, Tomáš. 2019. ASEP (ID 520733)

Other results

Certified technology
Biotechnology of waste waters cleaning in plant systems
Hnátková, T., Syrovátková, O., Vaněk, Tomáš. 2015. ASEP (ID 450803)

Utility model
A module for vertical aeroponic cultivation of plants
Soudek, Petr, Vaněk, Tomáš. 2017 ASEP (ID 499797)

A module for vertical aeroponic cultivation of plants
Soudek, Petr, Vaněk, Tomáš. 2018. ASEP (ID 499697)

A modular system for capture, decontamination and use of rainfall water
Petrová, Šárka, Vaněk, Tomáš 2018. ASEP (ID 499798)

Licence agreement
“Utilization of module for vertical aeroponic cultivation of plants”
signed with Poweregia s.r.o., company number_28896050, 18.12. 2018

“Utilization of modular system for capture, decontamination and use of rainfall water”
signed with Poweregia s.r.o., company number_28896050, 18.12. 2018

Proof-of-concept sample
New anti-inflammatory drugs based on natural substances
Šíša, Miroslav, Landa, Přemysl, Vaněk, Tomáš. 2018. ASEP (ID 517349)

Software
Complementary software application “IonCalc” for data processing of MS analysis outputs
Maršík, Petr 2019. ASEP (ID 520417)

Research activity and characterisation of the main scientific results

In the years 2015-2019, we extended our previous activities and our research was focused mainly on several aspects of pollen development, pollen communication with female tissues and genome organization. Since our research represents a continuous effort, here we sometimes refer also to our results and activities before and after the evaluation period.

1) Regulation of Arabidopsis pollen development

1.1) Screen and functional analyses of male gametophytic transcription factors

Male gametophyte development leading to the formation of a mature pollen grain is precisely controlled at various levels, including transcriptional, post-transcriptional and post-translational, during its whole progression. The pollen development thus represents a fragile and vital phase of plant ontogenesis and pollen was among the first singular plant tissues thoroughly characterized at the transcriptomic level [1]. Arabidopsis pollen developmental transcriptome has been published over 15 years ago revealing the uniqueness of pollen transcriptome and the dynamics of early and late successive global gene expression programs [2]. The above transcriptomics datasets were, together with other results used in our well-cited review describing pollen development [3]. In another, invited review in prestigious journal Annual Review in Plant Biology, 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—developmental phase leading to the differentiation of the male germline and the formation of a mature pollen grain, and a functional or progamic phase, beginning with the landing of the grains on the stigma and ending by double fertilization. We highlighted recent advances in complex regulatory mechanisms involved, including transcriptional, post-transcriptional regulation and transcript storage, intracellular metabolic signalling, pollen cell wall structure and synthesis, protein secretion, and phased cell-cell communication within the reproductive tissues [4].

In the evaluation period, this first field of interest has been supported by six projects (2x CSF – P305/12/2611 (Honys), 17-23183S (Honys); 3x MEYS – LD13049 (Honys), LTAIN19030 (Honys), LTC20028 (Honys); 1x MEYS/EFRR - CZ.02.1.01/0.0/0.0/16_019/0000738 (Honys)) and on top of their outcomes it resulted in two defended bachelor theses (Kočová, Nedvědová) and one defended doctoral thesis (Gibalová).

We applied the Agilent 44K tobacco gene chip to conduct the first comprehensive **developmental transcriptomic analysis of the tobacco male gametophyte** representing the first plant species shedding bicellular pollen [5]. This article was fully prepared and written in our laboratory. This transcriptomic dataset presented a benchmark for future functional studies using developing pollen as a model. Comparison with tobacco pollen tube proteome even revealed that most of these transcripts were not translated (joint effort with Z. Zdráhal, CEITEC MU Brno, manuscript in preparation) that highlighted them as the likely candidates for paternal complement to post-fertilization events (Kulichová – ongoing doctoral study). As pollen tube growth and competition of pollen tubes in female pistil can be viewed as a race of the fittest, there is an apparent evolutionary trend among higher plants to store large material reserves and nutrients during pollen maturation. This supply ensures that after pollen germination, the pollen tube utilizes its resource predominantly for its rapid elongation in the female pistil. Previous transcriptomic data from Arabidopsis showed

massive expression of genes encoding proteins forming both ribosomal subunits that were accumulated in developing pollen, whereas their expression was not detectable in growing pollen tubes [2]. We observed a similar phenomenon in less advanced bicellular tobacco pollen [5].

In the follow-up projects, we focused on the identification of **pollen-expressed transcription factors** (TF) involved in the regulation of male gametophyte development. They were identified by thorough screening of 74 T-DNA insertion lines representing 49 genes of 21 TF families active in either early or late pollen development. 29 screened lines showed strong phenotypic changes (i.e., ≥ 25% aberrant pollen) including four lines that produced a remarkably high proportion (70-100%) of disturbed pollen. Our results served as a basal information resource for future functional characterization of specific TFs in male gametophyte development [6]. This phenotype screen was enabled by the optimization of our previously published protocol for large-scale separation of developing spores [7]. Of these transcription factors, we concentrated on the members of two families – bZIP and MYB. On top of this, we extended our perspective by studying pollen development and function under stress conditions reflecting the current need of the wider community [8].

We further functionally analysed the **regulatory network of bZIP transcription factors** in our long-term collaboration with D. Twell, University of Leicester, UK. That extended our previous study of AtbZIP34 [9] by the inclusion of its interactors AtbZIP18, AtbZIP52 [10]; (Gibalová 2016, PhD thesis). This work reported the interaction network of six bZIP TFs expressed in Arabidopsis thaliana pollen and highlighted the potential functional role for AtbZIP18 in pollen. AtbZIP18 was shown to interact with three other pollen-expressed bZIP TFs—AtbZIP34, AtbZIP52, and AtbZIP61 in yeast two-hybrid assays. AtbZIP18 transcripts are highly expressed in pollen and localize in the nucleus and cytoplasm/ER. The phenotypic analysis of a T-DNA knockout allele showed reduced transmission through the male gametophyte. Some of the phenotype defects in *atbzip18* pollen were similar to those seen at higher frequency in the T-DNA knockout of the interacting partner, AtbZIP34. To gain deeper insight into the regulatory role of AtbZIP18, we analysed *atbzip18* pollen microarray data. Our results point towards a potential repressive role for AtbZIP18 and its functional redundancy with AtbZIP34 in pollen. This study was fully designed and mostly executed in our lab [10]; (Gibalová 2016, PhD thesis). Ongoing analyses of the fate of bZIP TFs under stress treatment revealed extremely interesting relocalization of bZIP18 and bZIP52 TFs from the cytoplasm to the nucleus following the exposure to higher temperatures (Steinbachová, Wiese et al. 2020, *in prep*).

During male gametophyte development, the asymmetric mitotic division of an undetermined unicellular microspore segregates these two cell lineages. To explore genetic regulation underlying this process, we screened for pollen cell patterning mutants and isolated the heterozygous *myb81-1* mutant that sheds ~50 % abnormal pollen. Typically, myb81-1 microspores failed to undergo pollen mitosis I and arrested at polarized stage with a single central vacuole. Although most myb81-1 microspores degenerated without division, a small fraction divided at later stages and failed to acquire correct cell fates. We showed that myb81-1 phenotypes resulted from impaired function of the **GAMYB transcription factor MYB81**. The MYB81 promoter showed microspore-specific activity and a MYB81-RFP fusion protein was only expressed in a narrow window prior to pollen mitosis I. Ectopic expression of MYB81 driven by various promoters severely impaired vegetative or reproductive development, reflecting the strict microspore-specific control of MYB81. Our data demonstrated that MYB81 has a key role in the developmental progression of microspores, enabling formation of the two male cell lineages that are essential for sexual reproduction in Arabidopsis. Our role on this project was to provide and analyze transcriptomic data [11].

Rooted in place, plants are faced with the challenge of responding to unfavourable conditions on-site. One such condition, heat stress, contributes massively to crop losses globally. With heatwaves predicted to increase, it is vital to generate crops tolerant to not only one, but several stresses, which will ensure that global food security is protected. A better **understanding of the molecular mechanisms** that underlie the **stress response in pollen** will be a significant step towards developing effective breeding strategies for high and stable production in crop plants. While most studies have focused on the vegetative phase of plant growth to understand stress tolerance, it is the reproductive phase that requires more attention, being more sensitive to elevated temperatures. Every phase of reproductive development is affected by environmental challenges, including pollen and ovule development, gametogenesis, pollen-stigma interactions, pollen tube growth, male-female cross-talk, fertilization, and embryo development. In our recent work, we summarized how pollen is affected by heat stress and the molecular mechanisms employed during the stress period, as revealed by multiomics experiments. This work has been done equally in our lab and the collaborating group prof. W. Weckwerth (University of Vienna, Austria). Single-cell multiomics here encompasses a great set of tools that enable researchers to map individual cell stages, deepening our understanding of regeneration mechanisms, cell fate regulation, and plant cell totipotency. [12]; (Kočová 2018, bachelor thesis; Nedvědová 2020, bachelor thesis).

1.2) The role of NAC proteins in pollen development

Our studies of pollen development were not concentrated solely on transcription factors. We also performed a functional analysis of **components of the NAC complex** (nascent polypeptide-associated complex). In the 2015-2019 period, this topic has been supported by three projects (CSF – 19-01723S (Fíla) and 2xMEYS – LTC18043 (Fíla), LTC20050 (Fíla)) and on top of their outcomes it resulted in one defended diploma thesis (Klodová).

Arabidopsis thaliana genome encodes five homologues of NACα, and two genes for NACβ. A phenotype screen of the respective T-DNA lines revealed the absence of pollen phenotype defects in single mutants that was likely caused by a large extent of functional redundancy within the NAC family. The NACβ T-DNA insertion lines were selected for a more detailed functional analyses including the newly generated double homozygous mutant *nacβ1nacβ2*). The *nacβ1nacβ2* plants showed several apparent phenotypic traits: (1) delayed development for 10–14 days, (2) higher-than-normal number of flower organs, and (3) limited seed set in significantly shorter siliques. Finally, *nacβ1nacβ2* pollen germinated inefficiently and resulting pollen tubes showed growth defects both *in vitro* and *in vivo*. These functional defects were accompanied by poor pollen tube attraction and targeting defects.

The combined transciptomics and proteomics analyses of *nacβ1nacβ2* and Col-0 wild type flower buds revealed 15 upregulated and 98 downregulated genes/proteins with several similar trends between the transcriptome and the proteome. Our analysis also proposed the role of NACs in regulation of photosynthetic apparatus. These data were published recently [13] and comprised part of one defended diploma thesis (Klodová 2019, diploma thesis). The same PhD student (Klodová) still works on her thesis falling into this field.

1.3) Evolutionary history of the pollen specific callose synthases

In this evaluation period, we initiated several new directions of our research where we want to shed light to pollen development in the evolutionary context. First attempt in this field was connected with callose synthase playing an important role in angiosperms in many developmental processes and responses to biotic and abiotic

stresses. Five from twelve **callose synthase family members** previously identified in *Arabidopsis thaliana* are active in pollen. We focused on these five CalS subfamilies and found that proteins expressed in pollen evolved twice. CalS9/10 and CalS11/12 formed well defined groups, whereas pollen-specific CalS5 was found within subfamilies that mostly did not express in mature pollen vegetative cell, although were found in sperm cells. Expression of five out of seven mature pollen-expressed CalS genes was affected by mutations in *bzip* transcription factors. Only three subfamilies, CalS5, CalS10, and CalS11, however, formed monophyletic, mostly conserved lineages. The pairs CalS9/CalS10, CalS11/CalS12 and CalS3 may have diverged after angiosperms diversified from lycophytes and bryophytes. Our analysis of fully sequenced plant proteins identified new evolutionary lineages of callose synthase subfamilies and has established a basis for understanding their functional evolution in terrestrial plants [14].

1.4) Pollen developmental hormonomics

There is increasing number of studies pointing at the **role of plant hormones in the male gametophyte development**. Interestingly, comprehensive studies describing the simultaneous activity and mutual interconnection of individual classes of plant hormones were not performed so far. In relation with *Nicotiana tabacum* pollen ontogeny studied in detail in our lab, we simultaneously quantified metabolic forms from the classes of „growth-related“ hormones (cytokinins, auxins, etc.) and “stress-related” hormones (ABA, salicylic acid, jasmonic acid, etc.). Bioactive cytokinins (CKs) are the most abundantly cytokinin class throughout all tobacco pollen developmental stages ranging from 39% to 65%. They were followed by CK N-glucosides, CK phosphates and very low abundant pool of CK O-glucosides. Similarly, the content of auxin (IAA) metabolites varied during pollen ontogeny. While total amount of IAA gradually decreased up to the BC stage, the levels of IAA-Asp increased at the same time. Later on, between BC and MPG stages, a high pool of IAA was measured again. Variable proportion of OxIAA was apparent in individual tobacco pollen stages. ABA was the most dominant metabolite representing >90% of total pool from five detected ABA metabolites. Other ABA derivatives (DPA, PA, ABA-GE and 9OH-ABA) occurred in minor but varying amounts. Taken together, the composition of endogenous CK, IAA and ABA derivatives dynamically changed throughout six tobacco pollen stages suggesting their important role in pollen growth and development (Záveská Drábková et al. 2020, *in prep.*). This project is supported by ongoing CSF grant 19-02699S (Záveská Drábková).

2) The role of post-transcriptional regulatory levels on pollen development and function

2.1) RNA storage in the male gametophyte and pollen translatomics

Since the 2015 evaluation, our laboratory has continued its activities on the newly initiated research on pollen translatomics and made significant advances that has created not only PhD programs but also funding opportunities. In the 2015-2019 period, this field of interest has been supported by four projects (3x CSF – 15-16050S (Honys), 18-02448S (Honys) and 17-23203S (Hafidh); 1x MEYS – LD13049 (Honys)) and on top of their outcomes it resulted in two defended bachelor theses (Darivčak, Linhart).

It has been well established that both transcription and translation play an important role in global and specific gene expression patterns during pollen maturation. On the contrary, germination of many pollen species has been shown to be largely independent of transcription but vitally dependent on translation of stored mRNAs (Darivčak 2015, bachelor thesis, Linhart 2015, bachelor thesis). Our initial discovery

and publication of the **EPP RNA storage** granules (Honys et al. 2009, J Proteome Res) as **large ribonucleoprotein particles** responsible for **mRNA storage** in tobacco pollen, proceeded with adoption of a new methodology that has resulted in a better refinement of the characteristics of the mRNA storage granules. Since 2015, we have applied microarray and LC-MS/MS to analyse the transcriptome and proteome covering three cytoplasmic sub-fractions containing mRNAs at different translational status and to demonstrate their developmental dynamics – 1) actively translated transcripts associated with **polysomes** (PS - termed **translatome**), 2) pollen mRNAs bound to pollen **stored ribonucleoprotein particles** (stored mRNPs/free mRNPs - termed **mRNPome**) and 3) long-term stored transcripts on **EPP granules** (EPPs - termed **sequestrome)**. This comprehensive analysis led to the isolation of genes whose transcripts enter the EPP storage compartment during pollen maturation associated with “ready-to-translate” complete translation initiation machinery and later shift to actively translating polysomes upon pollen hydration and activation of pollen tube growth [15]. Furthermore, using the new adopted method on polysomes profiling, we have refined the identity of EPP granules as staled monosomes associated with repressed transcripts in the male gametophyte [15]. In collaboration with C. Bousquet-Antonelli (INRA Perpignan, France), we have described the functional complex of stored mRNAs with LARP-family RNA-binding proteins [16]. This topic was also covered in the previously mentioned fast cited review covering advances in the male gametophyte and comparative aspects of translational regulation in tip growing cell types [3].

2.2) Regulation of translation during pollen development and progamic phase

We extended our research of mRNA storage in pollen to research of the translation regulation associated with pollen activation and pollen tube growth. Regulation of translation represents a critical step in the regulation of gene expression. In plants, the translation regulation plays an important role at all stages of development including gametophytes, and during stress responses. It functions as a fast and flexible tool which not only modulates the global translation rate but also controls the production of specific proteins. The above described translatomic study [15] led to the identification of numerous proteins and non-coding RNAs differentially associated with sequestered and translationally active mRNAs. In the 2015-2019 period, this topic has been supported by five projects (3x CSF – 15-16050S (Honys), 18-02448S (Honys) and 17-23203S (Hafidh); 2x MEYS – LD13049 (Honys), LTC18034 (Michailidis)) and on top of their outcomes it resulted in three defended bachelor theses (Hromadová, Klodová, Raabe), four defended diploma theses (Náprstková, Linhart, Kočová, Raabe).

Of these, we selected two groups of proteins for detailed functional studies, 1) the large complex of eukaryotic translation initiation factor 3 (eIF3) and 2) ALBA family proteins.

Regulation of translation is mostly focused on the initiation phase. There, one of essential initiation factors is the large multisubunit protein complex of eukaryotic translation initiation factor 3 (eIF3). In all eukaryotes, the general eIF3 function is to scaffold the formation of the translation initiation complex and to enhance the accuracy of scanning mechanism for start codon selection. Over the past decades, additional eIF3 functions were described as necessary for development in various eukaryotic organisms, including plants. The importance of the eIF3 complex lies not only at the global level of initiation event, but also in the precise translation regulation of specific transcripts. The role of eIF3 was reviewed by Raabe et al. [17] which was written fully in our lab.

The function of several eIF3 subunits is being studied within three ongoing PhD schemes on genetic and biochemical characterization of translation initiation subunits, eIF3A (Raabe), eIF3E (Kumar), eIF3M (Kahrizi) and also as a postdoctoral research topic, eIF3C (Michailidis).

ALBA family proteins are defined by the presence of ALBA (acetylation lowers binding affinity) domain. They dimerize and bind DNA and RNA in a temperature-dependent manner. The *Arabidopsis* genome contains six *ALBA* genes with close homology. All ALBA proteins show reasonable expression signal during male gametophyte development and are associated with pollen mRNA storage complexes. In the last 5 years, we have been investigating the role of ALBA proteins in mRNA storage and activation, not only under standard conditions but also following stress treatment. We demonstrated the subcellular localization and co-localization not only within the ALBA family but also with various markers of individual types of mRNA-containing granules. We also showed the interaction map of the ALBA family (Náprstková 2016, diploma thesis; Kočová 2020, diploma thesis). The same PhD student (Náprstková) still works on her thesis falling into this field.

Finally, we have made outstanding findings in a close collaboration with prof. C. Bousquet-Antonelli (INRA Perpignan, France) on the characterization of La associated RNA binding protein (LARP) and showed that a member of a LARP family protein is required for pollen tube guidance by directly binding 5UTR of galactolipids encoding mRNAs and regulate their gradual translational activation and distribution during pollen tube extension [16]. This collaboration is ongoing to characterise other members of the La-related family proteins. We are also planning a bilateral grant application to further fund this collaboration.

2.3) Tobacco pollen developmental phosphoproteomics

We followed our interest in the regulation of pollen development and activation in particular, initiated in the previous evaluation period, also in the last five years. In the 2015-2019 period, this topic has been supported by three projects (CSF – 19-01723S (Fíla) and 2xMEYS – LTC18043 (Fíla), LTC20050 (Fíla)) and on top of their outcomes it resulted in the defended doctoral thesis (Fíla).

Rapid changes of protein phosphorylation play a crucial role in the regulation of many cellular processes. Angiosperm mature pollen is composed of a strongly dehydrated cytoplasm surrounded by a resistant, tough cell wall in order to protect the carried genetic information from any suboptimal environmental conditions. Upon landing on the papillary cells of the stigma, the pollen grain starts to re-hydrate, its metabolic activity is initiated, and later the pollen tube growth through the selected pollen aperture starts. These changes from tobacco mature pollen to *in vitro* 30-min activated pollen represent a dynamic process accompanied by phosphorylation changes of the existing proteins.

In collaboration with H.-P. Mock's (IPK Gatersleben, Germany) and R. Zahedi's (ISAS Dortmund, Germany) groups, we finely performed the phosphoproteomic analysis of three time points - mature pollen, 5-min and 30-min-activated pollen. The majority of differentially phosphorylated proteins fell into GO categories clearly connected to pollen activation with the re-initiation of transcription and translation. The quantitative data highlighted the regulatory trends; we showed that several phosphopeptides representing the same phosphoprotein underwent different regulation, which pinpointed the complexity and dynamics of protein phosphorylation at the initiation of the progamic phase. Collectively, we showed the first phosphoproteomics data on activated pollen where the position of the respective

phosphorylation sites was clearly demonstrated. [18] (Fíla 2016, doctoral thesis). Furthermore, the applied protocol was published as a book chapter [19]. Of the identified differentially phosphorylated candidate proteins, NAC complex subunits were selected for detailed characterization (see Task 1.2).

3) Progamic phase and male-female crosstalk

3.1) Male contribution to the male-female dialog

Since our discovery of mRNA storage in the male gametophyte, we hypothesised that some of the stored mRNAs at pollen maturity could encode for secreted proteins required for male-female signalling during pollen tube guidance. In the 2015-2019 period, this topic has been supported by six projects (2x CSF – 15-16050S (Honys), 17-23203S (Hafidh); 4x MEYS – LD13049 (Honys), 7AMB16AT036 (Honys), LTAUSA18115 (Honys) and LTAIN19030 (Honys)).

To understand the spectrum of translational regulation and mRNA storage, we studied **pollen tube secretomics** as a "bottom-up" approach to link with our sequestrome transcriptome [15]. Several ovule-secreted peptides have been identified that guide the pollen tubes towards the ovule for fertilization. Nevertheless, there are no exact findings on how these signals are perceived by the pollen tube. In collaboration with M. Johnson, Brown University, USA and R. Palanivelu, Univ. of Arizona, USA, and 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 using our improvised SIV **(semi-*in vivo*) technique**, SIV-PS (SIV pollen tube secretome) [20-22]. The main finding from the analysis was that pollen tube secretome comprised vastly of non-classical type of secreted proteins that likely promote pollen tube guidance towards the ovule. Intriguingly, we discovered that TCTP1, a **non-classically-secreted protein hijacked the classical secretory pathway** and co-localized with nanovesicles exosome marker Ole-e-1. Moreover, the analysis revealed that primarily proteins of ≤30kDa (60%) of which 40% were ≤20kDa dominated the pollen tube secretome. They included Plant defensin subfamily, Cysteine-rich, LORELEI-like GPI-anchored 3 (LLG3), Thionin-like protein, RNAses, lipid transfer proteins (LTPs), pollen Ole-e-allergen, arabinogalactants, pectinases and invertases. The pollen tube **secretomics** has uncovered **novel pistil-dependent pollen tube-secreted proteins** critical for establishing **male-female signalling** interaction network for successful sperm cells delivery and fertilization and as means to overcome interspecies pre-zygotic barriers [23].

In collaboration with prof. T. Dresselhaus group (Regensburg University, Germany), we have applied our secretomics expertise to study the evolutionary conservation of pollen tube secreted proteins and their secretion pathways in ancient Angiosperm *Amborella trichopoda,* a monocot *(Zea mays)* and comparison with dicot *N. tabacum*. Our findings suggest that only a recent dicot likely have adopted a dominant unconventional secretion, whereas ancient profile was balanced between the two secretion pathways. A manuscript highlighting our primary findings has been submitted [24].

As a continuation on pollen secretomics, we have comprehensively characterised TCTP1 as a male-specific factor promoting pollen tube guidance and we have created a master thesis topic to verify TCTP1 protein interaction and production of recombinant protein (Pitoňak, ongoing MSc. study). Using a combination of public database and our findings on pollen secretomics, we have also created a PhD program investigating pollen secreted GPI-anchored proteins to establish their contribution in pollen tube guidance (Pieters, ongoing Ph.D. study). As an extension on the role of

GPI-anchored proteins on pollen tube guidance and reception, we have ongoing collaboration (LTAUSA18115) with prof. R. Palanivelu, Univ. of Arizona, USA, on the role of female-specific LORELEI protein, a GPI-anchored protein, responsible for pollen tube reception, and how LORELEI might be responsible for interspecies pre-hybridization barrier.

3.2) The role of pre-mRNA splicing in male-female crosstalk and cell fate

A survey of the stored transcripts at pollen maturity (see topic 2.1) also led to the identification of **mRNA splicing subunit, PRP8A and PRP8B**, as potential candidate genes for late pollen tube function. We employed genetics, biochemical, next generation sequencing and live cell imaging to establish that PRP8A and PRP8B co-ordinately function as master regulators of genes essential for pollen tube guidance. In the female gametophyte, we discovered that among others, PRP8A/8B control MYB98 transcription factor that activate transcription and secretion of LUREs family proteins essential for pollen tube attraction. We also established that *prp8a;8b* double mutant pollen tubes are incompetent in navigating towards ovules. Our findings have recently been published [25] (Pitoňak 2020, bachelor thesis) and will be a part of one doctoral thesis Kulichová – ongoing doctoral study, currently on maternity leave). In the period 2015-2019, this topic has been supported by the CSF projects 17-23203S (Hafidh) and on top of its outcome it resulted in one defended bachelor thesis (Pitoňak).

3.3) Viroid transmission through pollen tubes

Some viroids—single-stranded, non-coding, circular RNA parasites of plants—are not transmissible through pollen to seeds and to next generation. Our transcriptomic expertise was utilized in the collaborative project with J. Matoušek (Biological Center, Czech Academy of Sciences, České Budějovice) and G. Steger (Heinrich-Heine-Universität Düsseldorf, Germany) on hop genomic and viroid transmission analyses (ongoing CSF grant 19-02699S, Matoušek/Honys/Steger). Within this project, we analyzed the cause for the elimination of apple fruit crinkle viroid (AFCVd) and citrus bark cracking viroid (CBCVd) from male gametophyte cells of *Nicotiana tabacum* by RNA deep sequencing and molecular methods using infected and transformed tobacco pollen tissues at different developmental stages. AFCVd was not transferable from pollen to seeds in reciprocal pollinations, due to a complete viroid eradication during the last steps of pollen development and fertilization. In pollen, the viroid replication pathway proceeds with detectable replication intermediates, but is dramatically depressed in comparison to leaves. Specific and unspecific viroid degradation with some preference for (−) chains occurred in pollen, as detected by analysis of viroid-derived small RNAs, by quantification of viroid levels and by detection of viroid degradation products forming “comets” on Northern blots. The decrease of viroid levels during pollen development correlated with mRNA accumulation of several RNA-degrading factors, such as AGO5 nuclease, DICER-like and TUDOR S-like nuclease. In addition, the functional status of pollen, as a tissue with high ribosome content, could play a role during suppression of AFCVd replication involving transcription factors IIIA and ribosomal protein L5. Our role was the isolation of tobacco pollen developmental stages and subsequent transcriptomic an analysis [26, 27].

4) DNA stability and chromosome dynamics

4.1) DNA stability

In the evaluation period, this third field of interest has been supported by the CSF project 16-01137S (Angelis) and the MEYS project LD13006 (Angelis) and EU IRES 612587 (Angelis). On top of their outcomes it resulted in one defended master thesis (Vágnerová) and one doctoral thesis (Holá).

The Group of DNA Repair developed and keeps using the microscopic based electrophoretic analysis of single cell genomic DNA damage – "**Comet assay**" in plants with modifications enabling **detection of specific DNA lesions** as single (SSB) and double (DSB) strand breaks, DNA-DNA and DNA-protein crosslinks and specific base modifications as oxidative damage or formation of UV photoproducts CPDs. This methodological advantage of direct measurement of DNA damage enabled to established picture of overall two phase DSB repair kinetic with extremely rapid first phase.

We analyzed DSB repair kinetics of single and double mutants with a comet assay and provided the crucial data for explanation of sequential action of both alleles of RAD21 and for eventual switch to another DSB repair pathway in their absence. To ascertain the mechanism of procession of uneven DSB ends we investigated possible role of POLΛ in partial filling of gaps in cooperation with T. Furukawa and A.B. Britt, UC Davis [28]. Similarly, we confirmed the role of RAD51 in the repair of DSB induced by genotoxins with various modes of action [29]. Our contribution was the execution of the single-cell comet assays as well as in the project showing that amifostine, protecting normal cells from DNA damage induction by ionizing radiation or chemotherapeutics, supports DSB repair in γ-irradiated normal NHDF fibroblasts but alters it in MCF7 carcinoma cells. Our results helped to demonstrate that amifostine may make cancer cells suffer from DSB repair alteration [30]. Another use of the comet assay supplemented a recent article showing that the transient induction of DNA strand breaks (SSB, DSB or both) in the moss *Physcomitrella patens* can trigger the reprogramming of differentiated leaf cells into stem cells without cell death. Our results indicated that DNA strand breaks, usually considered to pose a severe threat to cells, triggered cellular reprogramming towards stem cells via the activity of ATR and STEMINs [31].

We also characterized the responses of the green algae *Klebsormidium* and *Zygnema* and of *P. patens* to induction of DSBs by bleomycin, to DNA base alkylation by MMS and to formation of CPDs after UV irradiation. These genotoxic agents with well-characterized modes of action were used to probe the vulnerability of these species to the induction and removal of DNA damage. We demonstrated that the *Klebsormidium* species and *Physcomitrella* show a similar sensitivity toward the induction of DNA lesions and of DSB repair. In contrast *Zygnema* species are less sensitive to the induction of DNA damage and exercise a high rate of DSB repair. Nevertheless, contrary to fewer lesions in DNA, *Zygnema* is more sensitive to genotoxic treatment than *Klebsormidium* and *Physcomitrella* [32].

We explored this *Physcomitrella*-based combined approach to further study the effect of **UV irradiation** [33] and collected extensive mutagenesis data in various *Physcomitrella* repair background lines induced by genotoxins potentially representing various environmental stresses (Vágnerová 2015, master thesis; Holá 2015, doctoral thesis). This methodology, experience and database background was also explored for targeting environmental stresses like drought and salinity, which are on molecular level conveyed as the burst of oxygen reactive species (ROS) and moss as representant of one of the first plants invading land is the right model [34].

4.2) The role of telomerase and telomerase-associated proteins in telomere maintenance

Finally, in collaboration with J. Fajkus, Z. Zdráhal (CEITEC MU, Brno) and E. Sýkorová (Institute of Biophysics CAS, Brno) we have investigated the role of plant **telomerase and telomerase-associated proteins** in telomeres maintenance in Physcomitrella, Algae, Arabidopsis and tobacco. In the 2015-2019 period, this topic has been supported by two CSF projects (13-06943S (Honys), 18-07027S (Hafidh)).

The life cycle of telomerase involves dynamic and complex interactions between proteins within multiple macromolecular networks. Elucidation of these associations is a key to understanding the regulation of telomerase under diverse physiological and pathological conditions from telomerase biogenesis, through telomere recruitment and elongation, to its non-canonical activities outside of telomeres. We used tandem affinity purification coupled to mass spectrometry to build an interactome of the telomerase catalytic subunit AtTERT (telomerase reverse transcriptase) N-terminal domain, using *Arabidopsis thaliana* suspension cultures. Bioinformatic analyses revealed that interaction partners of AtTERT have a range of molecular functions, a subset of which is specific to the network around its N-terminus. In total, our results provide an insight into the composition and architecture of the plant telomerase complex and this will aid in delineating molecular mechanisms of telomerase functions [35]. The above LS-MS/MS identification of candidate proteins putatively interacting with TAP-tagged TERT has led to the identification of candidate **Armadillo** proteins [36, 37] that not only showed the likely interaction with TERT but also interesting expression profile especially in the male gametophyte. Their role is being investigated. We also identified and phylogenetically analysed the telomere-associated RuvBL protein family in plants showing two distinct groups RuvBL1 and RuvBL2 based on the similarity of sequences and branch length. Our detailed phylogeny proved that RuvBL proteins are evolutionarily conserved in land plants and implied plausible functional conservation of the RuvBL proteins [38].

We found in Physcomitrella that telomere phenotypes are absent and DSB repair kinetics is not affected in mutants for DSB factors involved in non-homologous end joining (NHEJ). This is compliant with the overall dominance of homologous recombination over NHEJ pathways in the moss, contrary to the inverse situation in flowering plants [39]. On a similar matter, we analyzed the transcription of TERT variants in correlation with telomerase activity in tobacco tissues. A specific pattern of TERT transcripts was found in samples of tobacco pollen with the TERT_Cs variant clearly dominating particularly at the early stage of pollen development. Detailed analysis of TERT_C variants representation in functionally distinct fractions of pollen transcriptome revealed their prevalence in large ribonucleoprotein particles encompassing translationally silent mRNA. Histones of the TERT_C chromatin were decorated pre- dominantly with the euchromatin-specific epigenetic modification in both telomerase-positive and telomerase- negative tobacco tissues. Nicotiana species have again proved to be appropriate and useful model plants in telomere biology studies [40]. In all these projects, our responsibility was the male gametophytic angle.

Research activity and characterization of the main scientific results

Since its establishment, the **Laboratory of Signal Transduction** (LST) has been focused upon phospholipid signalling. At the beginning we focused on phosphatidylinositol-specific phospholipase C (PI-PLC) and the putative receptor for inositol-1,4,5-trisphosphate (IP3R) (IP3 is the product of PI-PLC activity). Interestingly, the existence and role of IP3R in plants still remains a subject of considerable debate. In 2002, Jan Martinec published together with Gunther Scherer, leader of a team at the University of Hannover in Germany, an article dealing for the first time with plant phosphatidylcholine- hydrolysing phospholipase C, today known as non-specific phospholipase C (NPC) (Scherer *et al.* (2002). Since that time, the Laboratory of Signal Transduction has been more focused on investigating the role of NPC in plant development and stress responses.

Non-specific phospholipase C (NPC) catalyses the hydrolysis of phosphatidylcholine (PC) to generate phosphocholine and diacylglycerol (DAG). NPC has a long tradition in animal signal transduction (where it is called PC-PLC) to generate DAG as a second messenger. Until 2005, however, there was no information as to the role of NPC in plants at gene level. In 2005, Nakamura *et al.* published a paper characterizing the *Arabidopsis* NPC family. During the preceding evaluation period (2009 -2014) we published three research articles, one review and one book chapter dealing with the role of NPCs in plants (Kocourková *et al.*, 2011; Pejchar *et al.*, 2010; Pejchar *et al.*, 2013; Pokotylo *et al.*, 2013; Wimalasekera *et al.*, 2010).

During the current evaluation period, we continue exploring the physiological role of plant (mainly Arabidopsis) NPCs. Two articles were published, extending the original findings from 2010 (Pejchar *et al.*, 2010), reporting upon the role of NPC in response to aluminium toxicity. Aluminium ions (Al) have been recognized as constituting a major toxic factor for crop production in acidic soils, and therefore aluminium's interaction with plants is widely studied. Nevertheless, the exact molecular mechanism and time sequence of individual changes occurring upon Al exposure remains under investigation. In the first of the articles (Pejchar *et al.*, 2015), we focused on the non-specific phospholipase C4 (NPC4). We examined the impact of Al on the expression, activity, and function of NPC4. The growth of tobacco pollen tubes was rapidly arrested by Al, and it was partially rescued by the overexpression of AtNPC4 while *Arabidopsis npc4* knockout lines were found to be more sensitive to Al stress during long-term exposure to Al under low phosphate level conditions. Our observations suggest that NPC4 plays a role in both early and long-term responses to Al stress. In the second article (Pejchar and Martinec, 2015), we hypothesize that the activity of NPC is affected by Al-induced changes in plasma membrane's physical properties.

Furthermore, we focused on NPC1 and NPC2, the isoforms of NPCs, which have not yet been characterized experimentally. We showed that the NPC1 possesses phospholipase C activity and is localized intracellularly to the secretory pathway compartments. Moreover, we found that the knockout T-DNA insertion line NPC1 (*npc1*) basal thermotolerance was impaired compared with the wild- type (WT); *npc1* exhibited significant decreases in survival rate and chlorophyll content on the seventh day after heat stress (HS). Conversely, plants overexpressing NPC1 (NPC1-OE) were more resistant to HScompared with WT.These findings indicate that NPC1 is involved in the plant response to heat (Krčková *et al.*, 2015).

In Krčková *et al.* (2018), Arabidopsis NPC2 was cloned and heterologously

expressed in *Escherichia coli*. The protein possessed phospholipase C activity, being able to hydrolyse phosphatidylcholine to diacylglycerol. GFP-labelled NPC2 was predominantly localized to the Golgi apparatus. The level of NPC2 transcript was rapidly altered during plant immune responses and correlated with the activation of multiple layers of the plant defence system. Transcription of *NPC2* decreased substantially after plant infiltration with *Pseudomonas syringae*, flagellin peptide flg22 and salicylic acid treatments and expression of the effector molecule AvrRpm1. The decrease in *NPC2* transcript levels correlated with a reduction of NPC2 enzyme activity. Additionally, NPC2- overexpressing mutants showed higher reactive oxygen species production triggered by flg22. The results suggest that NPC2 is part of Arabidopsis response mechanism to *P. syringae* attack.
These articles increased our contribution to global knowledge of the role of the NPC protein family. In total, 29 articles dealing with NPCs were published. Members of the evaluated team contributed to nine of them.

In addition to the NPC study, we were participating in a joint research project of the Laboratory of Pathological Plant Physiology and the team from the University of Chemistry and Technology, Prague dealing with the role of flotillins stress responses and development. First, we published a review on plant flotillins (Daněk *et al.*, 2016). The flotillin protein family are well-known membrane domain-forming proteins that are underexplored in plants. FLOT1 was reported to interact with phospholipase Dβ, and that is why we began to be interested in flotillins. Hence, we screened knockout flotillin mutants for any phenotype alteration in response to stresses (Kroumanová *et al.*, 2019). To reveal flotillin mode of action, we identified FLOT2-interacting partners in Arabidopsis plasma membrane (Junková *et al.*, 2018). Finally, we showed that the cell wall contributes to the stability of plasma membrane nanodomain organization of Arabidopsis FLOT2 and HYPERSENSITIVE INDUCED REACTION1 proteins (Daněk *et al.*, 2020).

Another joint project with the Laboratory of Pathological Plant Physiology and the University of Chemistry and Technology, Prague is focused on the impact of noble metal nanoparticles. The main aim of this study was to evaluate potential hazardousness of this little studied group of metal nanoparticles for the environment. Round-shape gold nanoparticles had a direct effect on root development of *in vitro* grown Arabidopsis plants (Siegel *et al.,* 2018). The indirect effect of nanoparticles on plants was investigated via modification of the root microbiome following application of silver nanoparticles to soil. Current results indicate dramatic changes in the composition of individual microbial species in soil treated with nanoparticles.

Close collaboration with the Laboratory of Pathological Plant Physiology (LPPP) and the team from the University of Chemistry and Technology, Prague led by Olga Valentova (UCT) can be demonstrated by the following numbers. Out of our 15 publications, seven were created in collaboration with LPPP and eight in collaboration with UCT. Within our institute we also collaborated with the Laboratory of Cell Biology (Pejchar *et al.*, 2015).

Laboratory of Pathological Plant Physiology is mainly engaged in plant-microbe interactions study. Both manipulation of the plant immune system by a pathogen and the related signalling pathways were studied in a neglected pathosystem *Brassica napus – Leptosphaeria maculans*, studied by the laboratory for about 15 years. *L. maculans* is a biotrophic pathogen; this offers the opportunity to reveal defence mechanisms implicated in pathogen transition from the biotrophic to the necrotrophic stage. Numerous effectors have been reported to date; however, their function during their interaction with *B. napus* remains unclear. Methodological

approaches at disposal for the study of this ascomycete pathogen are limited due to the fact that *L. maculans* is not pathogenic for Arabidopsis. Within the evaluated period the laboratory published findings on the function of the effector AvrLm4-7 (Nováková *et al.*, 2016a) which manipulates defence-related signalling by salicylic acid and ethylene and affects the production of hydrogen peroxide as one of important reactive oxygen species. Attention was also paid to phytohormones, since as indicated by previous research of the laboratory, *L. maculans* produces a wide spectrum of phytohormones *in vitro* and, as supposed, also *in planta*. The main concern was devoted to cytokinins and auxins synthesized by this pathogen. Changes induced in the cytokinin profile in *L. maculans* colonized tissues, as well as biosynthetic pathways of cytokinins in this fungus were described (Trdá *et al.*, 2017). Using functional genomics, enzymatic and feeding assays it was shown that *L. maculans* contains a functional isopentenyltransferase involved in cZ production, adenosine kinase involved in phosphorylation of cytokinin ribosides to nucleotides, and cytokinin-degradation enzyme cytokinin oxidase/dehydrogenase. In addition, the crucial role of adenosine kinase for *L. maculans* fitness and virulence was demonstrated. Besides signalling molecules, protein secretome of this pathogen was also investigated (Nováková *et al.*, 2016b), which could provide information on molecules important in the *B. napus – L. maculans* interaction. Mass spectrometry analysis revealed predominantly the enzymes involved in the degradation of plant cell wall polysaccharides. These enzymes and their products could serve as elicitors recognized by plant activating immune responses in *B. napus.* Current knowledge of MAMP and DAMP in grapevine and the perception of pathogenic and beneficial bacteria by pattern recognition receptors were reviewed in collaboration with French colleagues from the University of Burgundy (Bourgogne) (Heloir *et al.*, 2019; Trdá *et al.*, 2015).

The focus of the laboratory on signalling is documented by review and research papers studying salicylic acid (SA) signalling (Janda and Ruelland, 2015; Pluhařová *et al.*, 2019) and research papers concerning the role of phospholipid signalling in plant defence (Janda *et al.*, 2015a; Janda *et al.*, 2015b; Krčková *et al.*, 2018; Kalachova *et al.*, 2019). Most of these results were published in close collaboration with the Laboratory of Signal Transduction. A recent paper Pluhařová *et al.* (2019) provides a useful tool to researchers involved in SA signalling studies. It presents a collection of Arabidopsis mutants deficient in SA biosynthesis or perception.

Phospholipid signalling in biotic interactions represents one of the traditional topics of the laboratory. During the evaluated period, a study demonstrating the interconnection between SA and phospholipid signalling was published (Janda *et al.*, 2015a). An inhibitor of phospholipase D (PLD), n- butanol, affects transcription of SA-regulated *PR1* gene and decreases NPR1 translocation to the nucleus in the presence of SA. This study proposes that PA produced by PLD is involved in the SA signalling pathway. Other interesting findings are reported on relationships between the actin cytoskeleton, phospholipid signalling and plant immunity. Prolonged exposure of plant tissues to actin- disrupting drugs (latrunculin and cytochalasin) causes immune-like responses in plants, especially an increase of SA content and resistance to subsequent infection (Leontovyčová *et al.*, 2019). This study pointed out that actin depolymerisation triggers both SA-dependent and SA-independent defence demonstrated by callose deposition via callose synthase PMR4. As actin cytoskeleton is closely related to membrane trafficking, the effect of latB on mutant plants invalidated in phosphatidylinositol 4-kinase beta1 and beta2 (PI4K beta 1 beta 2) was assayed. Deficiency in PI4K beta 1 beta 2 enhanced latB- triggered actin filaments depolymerisation. However, it did not lead to a stronger callose deposition or SA

biosynthesis in response to latB (Kalachova *et al.*, 2019).

One of the topics related to plant defence and signalling represents induced plant resistance against pathogens. Investigated were both elicitors originating from microorganisms (Nováková *et al.*, 2016) and other natural sources (Jindřichová *et al.*, 2018). Among them, saponins as plant-originating compounds were studied in more detail (Trdá *et al.*, 2019). This study showed both antifungal properties of aescin and the resistance-inducing activity of SA. Current knowledge of bio-based resistance inducers was compiled in a well-cited (WOS 66) review (Burketová *et al.*, 2015). This field of research provides us also with a possibility to collaborate with the field of applied research and to apply for grants of the Technology Agency of the Czech Republic and Ministry of Agriculture. Outcomes of this collaboration devoted to resistance-inducing preparations exploitable for the protection of economically important crops were submitted to the Office of Industrial Property of the Czech Republic in the form of patents (granted in 2016 – CZ305975 and CZ305950; granted in 2019 – CZ308002) and utility models (registered in 2019 – CZ33912 and CZ33723), which enable us to legally protect our research.

The laboratory collaborates with other teams within the institute as well as with universities. The closest collaboration continues with the Laboratory of Signal Transduction documented by 8 joined publications mostly concerning phospholipid signalling and, recently, also noble metal nanoparticles (Janda *et al.*, 2019; Junková *et al.*, 2018; Siegel *et al.*, 2018; Kalachová *et al.*, 2019; Kroumanová *et al.*, 2019). Research on phytohormone production by *L. maculans* and hormonal signalling was performed in collaboration with the Laboratory of Hormonal Regulations in Plants (Trdá *et al.*, 2017; Trdá *et al.*, 2019), and the Laboratory of Cell Biology (Pečenková *et al.*, 2017). Important long-lasting collaboration takes place with the University of Chemistry and Technology Prague (UCT) on phospholipid signalling, with the laboratory of Prof. Valentová. In the framework of joint projects, involving UCT students, a number of publications emerged (Burketová *et al.*, 2015; Janda *et al.*, 2015a; Janda *et al.*, 2015b; Nováková *et al.* 2016; Kalachova *et al.*, 2019; Kroumanová *et al.*, 2019; Leontovyčová *et al.*, 2019; Pluhařová *et al.*, 2019). The laboratory also collaborates with the Charles University, Prague (Kalachova *et al.*, 2019; Leontovyčová *et al.*, 2019; Pluhařová *et al.*, 2019).

The majority of findings published by the **Laboratory of Biologically Active Compounds** (LBAC) can be assigned to three fields: 1) Studies of somatic embryogenesis in conifers; 2) Studies of endogenous polyamines, phenolic compounds and phytohormones; and 3) Studies of abiotic stresses. Our published papers often address an intersection of these three areas.

The main research interest of the LBAC is the metabolism and physiological functions of growth regulators, polyamines and phenolic compounds in plants. We study the role of these biologically active compounds in plant development and in the response of plants to abiotic stress. We use both whole plants as well as cell cultures as model systems in our investigations. The process of somatic embryogenesis of conifers represents our main theme; our aim is to study the regulation of somatic embryo development, the role of phytohormones in somatic embryogenesis and the effect of stress factors on embryo development.

Somatic embryogenesis (SE) is characterized as a developmental process where somatic cells, under suitable induction conditions, undergo restructuring through the embryogenic pathway to generate embryogenic cells and consequently the whole plant. The key substances controlling the whole process are

phytohormones.

In our paper (Vondráková *et al.,* 2018) we provided a detailed analysis of the spectrum of endogenous phytohormones including auxins, cytokinins, the abscisic acid, jasmonates and the salicylic acid over the course of SE in Norway spruce. The results revealed that the concentrations of particular phytohormone classes varied substantially between proliferation, maturation, desiccation and germination. Auxins reached their concentration maxima in the early maturation stage, suggesting their role in embryo polarization; ABA showed a maximum in the late maturation stage. To our knowledge, we have presented for the first time in conifer SE both the evidence for the involvement of the non-indole auxin phenylacetic acid, cis-zeatin and dihydrozeatin-type cytokinins, or patterns of jasmonates and salicylic acid. The presented results provide so far the most comprehensive overview of plant hormone levels in embryos throughout the whole process of conifer SE.

Twenty years of experience concerning plant hormones also resulted in a review on the role of phytohormones in conifer SE, where we focused on seven main group of phytohormones, including the effect of crosstalks between phytohormones and plant growth regulators in terms of highly coordinated interactions within phytohormone signalling pathways. We considered the main mechanism of regulation in plant/embryo development as revealed by studies on zygotic as well as somatic embryos (Vondráková *et al.,* 2016).

Although phytohormones remain the main regulators of SE, other substances (e.g. polyamines) are also active in the process. The study of the role of polyamines in somatic embryo development and during stress reactions of embryogenic cultures and mature embryos was supported by two COST projects (LD130051 and LD130050). We specified the effect of putrescine treatment during maturation and proliferation of spruce embryogenic culture and we described the effect of elevated putrescin levels on somatic embryo development. Exogenous putrescin induced intensive changes in embryo structures (Vondráková *et al.,* 2015).

Polyamines are primarily known as the key substances in plant response to stress factors. Higher polyamine levels of fully developed embryos had positive effects on their ability to tolerate UV-B irradiation compared with responses of early embryos. Accumulation of polyamines thus served as a protective mechanism (Cvikrová *et al.,* 2016). Elevated levels of spermine and spermidine and an increase in total phenolics as a consequence of irradiation with UV-B indicated their involvement in the stress response. UV-B irradiation evoked striking polyphenolic accumulation in specialized idioblastic cells localized beneath the epidermis of the somatic embryo hypocotyl and cotyledons (Eliášová *et al.,* 2017). A detailed anatomic and microscopic study of somatic embryos after UV-B irradiation was then published (Eliášová *et al.,* 2018). In the study, advanced techniques of microscopy were used: double fluorescent staining for confocal laser-scanning microscopy and, for the very first time, the modified technique of environmental scanning electron microscopy AQUASEM II (collaboration with colleagues from the Institute of Scientific Instruments).

Long-term cooperation with INRA, France (lab. of Prof. Lelu-Walter) resulted in two papers on somatic embryogenesis of conifers (our model, Norway spruce, was replaced with Douglas fir) (Lelu- Walter *et al.,* 2018; Gautier *et al.,* 2019). This very complex study used transcriptomic and proteomic approaches, biochemistry, histology and anatomy. Our laboratory took part mainly in histology and anatomy, in the proteomic study, in analyses of plant hormones and tissue cultures. A major part of both papers was elaborated in our labs during the study stay of Florian Gautier at IEB, Prague.

Experience with somatic embryos was utilized during the project QI102A256, in the study of mechanisms of dormancy in beech zygotic embryos (Eliášová *et al.,* 2015). Some scientists of our laboratory are often invited into cooperation due to their knowledge of anatomy and microscopy (Štorchová *et al.,* 2015, 2019; Vítámvás *et al.,* 2019).

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Research activity and characterisation of the main scientific results

The following research activities are ordered chronologically

1) At the beginning of the period we have published a paper in “Plant methods” (Janda et al, Plant methods, 2015). The experiment described in the article arised from the necessity to replace the existing fluorescent light sources in our growth chambers with LED-based light source to save energy and eliminate the problem of excessive heat. We have contacted several companies from which one local supplier of industrial illumination provided us with LED tubes with various spectral properties. We (as the laboratory of virology) got the responsibility to perform the testing. We have used the expertise of the laboratory of phytopathology and stress physiology and performed growth and pathogen challenge experiments under both fluorescent and LED lights. The only material costs were publishing fees. The credit for the experiments and for writing goes about 50:50 share among laboratory of Virology (Moravec) and phytopathology (Janda). (Growth and stress response in Arabidopsis thaliana, Nicotiana benthamiana, Glycine max, Solanum tuberosum and Brassica napus cultivated under polychromatic LEDs, output 2)

2) During the early stages of our work on the soybean glycosylation project, it becomes clear that our ability to create DNA constructs for seed-specific expression in legumes far exceeds our ability to test them (via generation of transgenic plants). Thus some method of how to test the novel seed-specific regulatory elements in a transient way was urgently needed. In the beginning, we have used the in-vitro method of Santarém et al 1998. The method is based on the sterilization of immature cotyledons, their sonication with agrobacterium, and in vitro cultivation for several days. Unfortunately, the method is rather demanding on sterile work skills and only allows a low throughput. We have realized that the transient expression (and even sonication) can be realized more easily in planta with the help of a scalpel blade and parafilm wrap. The strength of expression based on GUS measurement was at least an order of magnitude higher than what we have obtained with the in-vitro protocol. The results were presented at an international conference. Unfortunately, this did not fruit into final publication yet, since the key person responsible for the project left the lab prematurely. We hope to include the obtained data in the manuscript that describes a novel dual GUS assay (see below).

3) Once we had the ability to test the functionality of our genetic constructs in seeds transiently, we started to look for ways how to assess the performance of regulatory elements (promoters, 5’-3’ NTRs, terminators, etc) in a quantitative way. For this, each assay needed some internal standard to filter out the differences in transformation efficiency between individual seeds. Although the in planta protocol performed better than the in-vitro method, it was not sufficiently efficient to use fluorescent markers for this purpose. The gold standard for such an assay would be dual luciferase assay, which is extremely sensitive, however also technically challenging and expensive. We have designed and tested a completely new assay, which is based on the measurement of GUS activity. We took advantage of the thermostable mutant GUS-TR (Xiong et al , Plos ONE, 2011) which was developed through in vitro evolution. This mutant can withstand 10 minutes at 70 C, while the wtGUS from E.coli is completely deactivated by such treatment. Since the working enzyme is a homotetrameric protein, we also needed to prevent the assembly of heterotetramers consisting of both wt and thermostable subunits, since these heteromers are not thermostable. We have sequestered the TR mutant to both chloroplast and nucleus and showed that this can be used as a sensitive, low background assay to measure expression in plant cells. The advantage is also the simple processing of samples because the samples for

enzyme kinetics are processed once, the sample is split into two halves, one is heat-deactivated and then they are measured using the same settings and chemistry on the 96-well plate.

4) As we have progressed in the soybean glycosylation project, it became clear, that while the GoldenBraid system offers simplicity and versatility, it also has serious limitations for the construction of large multi-component DNA structures. For example, the final construct for the biosynthesis and utilization of sialic acid and silencing of plant-specific glycosylation contained 9 genes and 3 matrix attachment regions (MAR). Similarly, many of the constructs that we used for the transient or permanent expression of antibodies contained 3-5 transcription units. And since the GoldenBraid system uses an infinite loop based on binary assembly, it would require multiple cloning steps with multiple intermediate plasmids requiring isolation and verification. Thus we have designed an additional extended set of GoldenBraid compatible plasmids that are fully compatible with the original standard but allow simultaneous assembly of 5 transcription units into one of the destination plasmids. This allowed us to create longer constructs for metabolic engineering, gene editing, and also replicative Geminivirus vectors. This work has been undertaken in cooperation with the laboratory of professor Ed Rybicki at the University of Cape Town, South Africa. Two Master students from UCT visited our lab for 2 weeks in 2016 and created the core set of Geminiviral replicons. Our part was the original idea and later the quantification of expression in leaves and plant cell packs. This work resulted in two recent publications (Poborilova et al, Plant cell reports 2020, Dusek et al, Frontiers in plant biology, 2020), which were designed, executed, written and submitted during the evaluated period, however, they were accepted for publication 2020.

5) The generation of transgenic legumes (IEB – soybean, Agritec – Pea) was eventually not successful. However, we have generated several transgenic lines of *N, benthamiana* that are characterized and used as a platform to express recombinant antibodies in our current projects.

6) Our second project aimed to develop a LAMP-based assay for the detection of economically important viruses of cereals and potatoes. We have carried this research in cooperation with the Czech crop research institute, Prague (characterized isolates of WDV, BYDV, and infected plant material) and Potato research institute (characterized isolates of PVX, PVY, and PLRV viruses). We have designed and optimized LAMP primers for the detection of these viruses. The project resulted in the issue of 6 utility models and one national patent on expression vectors based on defective TMV virus.

7) Since summer 2018 we participate in the large institutional project Exbio. The project aims to study the crosstalk and synergies between stress caused by climate change and biotic stressors, including viruses. To this end we have redesigned full-length infectious clones of several plant viruses – Bean Yellow Dwarf Virus, Tobacco mosaic virus strains U1 and Cg8 (for efficient infection of *A. thaliana*), Potato virus X, Tobacco Rattle Virus, and Apple Latent Spherical Virus (ALSV) into GoldenBraid. The conversion into the GB standard allows a much simpler reverse genetic system of these viruses, the deletion or replacement of a gene in the viral genome is simple and straightforward. It also allows medium to high throughput cloning of inserts for VIGS (Virus-induced gene silencing) into multiple plant viruses at the same time. The article describing this work with the PVX virus has been resubmitted after minor revision to Molecular biotechnology.
In the last two years, we have frequently used the ALSV virus. We have obtained the original clone from dr Ishikawa. The advantage of the virus is it extremely broad host

range and the fact, that the infection is symptomless in most plant species. For the initiation of infection, it is necessary to combine 3 Agrobacterium strains carrying RNA1, RNA2 and silencing suppressor P19 and agroinfiltrate *N. benthamiana* at first. *N. benthamiana* is probably the only plant species in which the agroinfiltration is sufficiently efficient to deliver the T-DNA from 3 bacterial strains together into one plant cell and thus to initiate productive viral replication and infection. The infectious sap is then used to mechanically infect other plant species. The modularity of the GB system allowed us to assemble unchanged plasmid with the ALSV RNA1 cDNA (Kan) and GB-plasmid carrying both recombinant RNA 2 and silencing suppressor P19 (Spec) into one Agrobacterium strain. This system shows an advantage in the direct initiation of infection using Agrobacterium in other plant species.

8) As a result of cooperation with Dr. Fischer, Faculty of Natural Sciences, Charles University, Prague we have designed and cloned a system for repeated gene editing in BY-2 cells. BY-2 cells are a very popular model system to study plant physiology and genetics. However, the use of modern CRISPR/Cas9 gene editing is somewhat limited by the number of selectable markers that work with the system (currently Kanamycin and Hygromycin). Thus repeated rounds of gene editing and transgene insertions are not possible. We have created two versions of Cas9 cassette that can be efficiently self-excised after the addition of either methoxyfenozide (VGE system) or estradiol (XVE) system. The manuscript is being prepared. Laboratory of Virology participation was the original idea, creation of the necessary parts, and creation of various versions of the inducible system. Dr. Fischer lab performed the BY-2 experiments.

9) Focus of the stress physiology group (Dr. Wilhelmova) was the effect of drought-induced stress on plant physiology. The group has extensive cooperation with the Faculty of Natural Sciences, Charles University, Prague and with the 1Department of Population Ecology, Institute of Botany. The work resulted in 4 scientific publications.

Research activity and characterisation of the main scientific results

1. **The Laboratory of Plant Reproduction** followed two research avenues, the investigation of flowering and CMS.

1.1 The genetic background of flowering in the species of *Chenopodium*

The transition from vegetative to generative phase is the most essential commitment in the plant life. It affects the reproduction success and also the crop yield. Plants sense environmental and endogenous conditions e.g. day length, temperature, and age. The signals are integrated by essential regulatory genes, which are capable to induce flowering. The most investigated plant models are *Arabidopsis thaliana* and rice, much less is known about other angiosperm species.

The floral induction in the family Chenopodiaceae is controlled by two paralogous genes *FTL1* and *FTL2* (*FLOWERING LOCUS T-like),* which were first discovered in *Chenopodium rubrum* by our lab (Cháb et al. 2008, Planta 228:929-940). The *FTL1* gene activates flowering, whereas *FTL2* homolog *BvFT1* inhibits flowering in sugar beet (Pin et al. 2010, Science 330:1397-1400), or has so far unknown function (Cháb et al. 2008, Planta 228:929-940). The detailed analysis of the *C. rubrum* transcriptome revealed the third paralog *FTL3*, which was expressed mainly in seeds and during germination, and therefore could not participate in the regulation of flowering (Drabešová et al. 2016, G3 6:3065-3076). It diverged from the ancestor of *FTL1* and *FTL2* before its duplication, and was found also in spinach and sugar beet. The detailed knowledge of the *Chenopodium FTL* sequences made possible to search for the *FTL* genes in the complete genome of the important crop *C. quinoa* (Jarvis et al. 2017, Nature 542:307-312). The identification of *C. quinoa FTL*s is complicated by its tetraploid genome containing two homeolog copies of each *FTL* gene and also by more recent duplications of the *FTL2* and *FTL3* genes. The duplication of the *FTL2* gene, which occurred in the *Chenopodium* species but not in sugar beet, was accompanied by the acquisition of a novel exon by one copy (Drabešová et al. 2016, G3 6:3065-3076), which changed the otherwise highly conserved structure of *FTL* genes consisting of four exons and three introns.

We studied the role of *FTLs* in *Chenopodium ficifolium*, a close relative of the ancestor of *C. quinoa*. *C. ficifolium* is a diploid and harbors a half of the *FTL* genes compared with *C. quinoa*. The second advantage is the availability of two contrasting ecotypes of *C. ficifolium*, which differ in response to photoperiod, being induced by a long day or by a short day. We obtained these ecotypes collected in the field by the collaborating lab of Bohumil Mandák (Institute of Botany CAS and Czech University of Life Sciences). We found that all three *FTLs* (*FTL1*, *FTL2-1*, *FTL2-2*) exhibited rhythmic expression with much higher amplitudes under short days. The same expression pattern was observed also in the *C. ficifolium* ecotype, which flowered earlier under long days without concomitant activation of the *FTL* genes (Štorchová et al. 2019, Planta 250:2111-2125). Floral induction without upregulation of *FTL* is very unusual in the plant kingdom. We have therefore performed comprehensive experiments and sampled RNAseq data in several time points in the course of floral induction in long-day *C. ficifolium* with aim to find the gene, which promotes flowering. Our findings are important for the research of flowering in *C. quinoa*, which is closely related to *C. ficifolium.*

We contributed to the clarification of the origin of *C. quinoa*. We used the *FTL* introns as phylogenetic markers and found the donor of the subgenome B, a species closely related to *C. ficifolium* and *Chenopodium suecicum*, which now occur in Europe, but might have live in America in the past (Štorchová et al. 2015, Genetic Resources and Crop Evolution 62:913-925). We applied the same markers in the comprehensive analysis of Euro-Asian representatives of the *C. album* aggregate, taxonomically intricate group of species (Mandák et al. 2018, Molecular Phylogenetics and Evolution 129, 189-201). This study was performed in collaboration with Bohumil Mandák, our lab designed the primers and consulted complex manual editing of intron alignments. We are now continuing the collaboration with the Mandák aiming to reconstruct evolutionary history of the American representatives of the *C. album* aggregate.

We constructed *de novo* reference transcriptomes of *C. rubrum* and *C. ficifolium*. We are using advanced transcriptomic tools, e.g identification of differentially expressed genes, gene expression profiling, or GO enrichment to discover the candidate genes possibly involved in the regulation of flowering. However, overexpression or knockout of the respective genes is necessary to bring conclusive evidence about gene function. This goal is complicated by the absence of transformation protocol for *Chenopodium*, which is recalcitrant to *Agrobacterium* transformation. We have therfore started to develop Virus induced gene silencing (VIGS) method in *C. rubrum*, *C. ficifolium* and *C. quinoa*, in collaboration with the Laboratory of Virology of IEB CAS (Tomáš Moravec). We achieved the silencing of the marker gene encoding phytoene desaturase (PDS) confirmed both by RT PCR and by yellow spots in uninfected leaves of all three species. We are performing the infection of *Chenopodium* plants with the constructs containing the selected flowering-related genes. The possibility to provide evidence about gene function will significantly accelerate our research of flowering regulation in *Chenopodium*, including the important crop *C. quinoa*.

1.2 Mitochondrial genomes and transcriptomes of *Silene vulgaris* in the context of cytoplasmic male sterility (CMS)

Unlike small, compact and gene-dense mitochondrial genomes of animals, mitochondrial genomes of flowering plants are large, often multipartite, with large intergenic regions and highly rearranged. The genus *Silene* contains the species with the largest known plant mitogenomes (*Silene noctiflora* and *Silene conica*, 7 and 11 Mb, respectively) and as well as the species with extremely rearraneged mitogenome (*Silene vulgaris*). Frequent intramolecular recombinations are associated with DNA repair of double strand breaks in plant mitochondria. Recombinations preferentially occur across the repeats in intergenic regions, but they may also affect coding sequences, sometimes giving rise to chimeric genes composed of the portions of other genes. Chimeric genes may code for the proteins which harm mitochondrial respiratory function. The process profoundly affected by impaired mitochondrial function is the production of pollen. The expression of the specific chimeric mitochondrial genes leads to the generation of male sterile individuals, which is called cytoplasmic male sterilty (CMS). Nuclear fertility restorer (*Rf*) genes may inhibit CMS gene expression and restore pollen production in male fertile (hermaphrodiic) individuals. CMS is widely used in crops for the production of hybrid seed, much less is known about CMS in wild populations. One of the best known models for CMS in the reproduction system gynodioecy is *S. vulgaris.*

Our lab identified the first CMS candidate in *S. vulgaris* (Štorchová et al. 2012, PloS One 7:e30401). This gene called *Bobt* was located in the mitochondrial genome of *S. vulgaris* collected in Krasnoyarsk, Russia (haplotype KRA). We assembled the complete mitogenome of *S. vulgaris* KRA and compared comprehensive mitochondrial transcriptomes between female and hermaphroditic plants with aim to find possible additional candidate CMS genes (Štorchová et al.2018, BMC Genomics 19:1-17). We confirmed the gene *Bob*t as the only region more highly expressed in the females than in hermaphrodites, consistently with its role as the CMS determinant. The *Bobt* gene was co-transcribed with the *Cytochrome b* (*cob)* gene in some genomic configurations, which may constrain the inhibition of *Bobt* transcription and fertility restoration. Homologous recombination moved the *cob gene* out of the control of the *Bobt* promoter, which facilitated suppression of *Bobt* and the restoration of male fertility. Our findings document the impact of mitochondrial genomic rearrangements on the expression of essential mitochondrial genes. This role of recombination process in plant mitochodria was not yet evaluated.

We also analyzed the physical structure of small circular chromosomes lackng coding capacity, which are present in all five *S. vulgaris* completely sequenced mitogenomes. We found that they existed in several oligomeric forms - as open circles, linear or supercoiled DNA molecules resembling bacterial plasmids (Štorchová et al. 2018, BMC Genomics 19:874). However, their carry no sequence homology with known mitochondrial plasmids. Our results are in line with the studies describing very complex physical structure of plant mitogenomes.

CMS is often but not always associated with mitochondrial chimeric genes. We analyzed the transcriptomes in females and hermaphrodites of *S. vulgaris* KOV with the mitogenome lacking any chimeric open reading frame (ORF). We found only one genomic region that was highly overexpressed and differentially edited in females relative to hermaphrodites. This region contained no ORF and represented the first described non-coding RNA associated with CMS, although we do not know whether it is the cause or rather the consequence of CMS. We also estimated the order of editing and splicing across the entire mitogenome of *S. vulgaris* KOV. Most transcripts were edited prior to splicing, but splicing preceded editing in three positions, where editing recognition motifs were created by splicing (Stone et al. 2017, Journal of Experimental Botany 68:1599-1612).

We applied our experience gained in the transcriptomic studies of *S. vulgaris* on the analysis of the mitochondrial transcriptomes of *Silene noctiflora*, the species with extremely large mitogenome of 7 Mb. We participated in the study led by Dan B. Sloan (Colorado State University), which found that the chromosomes of the mitogenome of *S. noctiflora* without coding capacity were actively transcribed (Wu et al. 2015, BMC Genomics 16:938). We summarized the methods and protocols suitable for the comprehensive transcriptomic analyses of plant mitochondria in the review (Stone and Štorchová 2015, Molecular Genetics and Genomics 290:1-9).

Mitochondrial transcriptomes are frequently studied in flowering plants with CMS. Much less is knowne about plastid transcriptomes. We used the same RNAseq data, which were analyzed for mitochondrial transcriptomes, in the construction of plastid transcriptomes in female and hermaphrodite individuals of *S. vulgaris.* We found no significant differences between the two genders (Krüger et al. 2019, BMC Plant Biology 19:568). This observation is surprising, because our current analysis of the cytoplasmic transcriptomes found numerous

genes highly differentilaly expressed between females and hermaphrodites, many of them encoding proteins targeted to plastids.

Miloslav Juříček, the member of The Station of Apple Breeding for Disease Resistance, participated in the last transcriptomic study of *S. vulgaris*. We developped a number of bioinformatic pipelines that were implemented in the course of this research. With a sligth modifications, these pipelines are now used for the investigation of the transcriptomes of apple cultivars.

2. The Station of Apple Breeding for Disease Resistance

is engaged in both oriented and applied research.

2.1 Oriented research

The oriented research follows the investigation of mechanism of *Rvi6* monogenic scab resistance as well as search for suitable markers for polygenic scab tolerance.

2.1.1 Investigating *Rvi6* mediated apple scab resistance

Very little information is known about the mechanism of action of resistance genes and other genes during *V. inaequalis* infection. The information mostly comes from the observation of differences in Rvi6-resistant and susceptible plants during infection. In both cases, the fungus was able to penetrate the cuticle and form the primary stroma in the youngest leaves. Even more scarse information on the possible mechanism of action of the *Rvi6* gene has been obtained from cloning and sequencing of the *Rvi6* gene in the past.

The use of RNA-Seq technology is therefore a logical approach to the characterization of the gene network involved in *Venturia* infection in resistant and susceptible plants. We chose *M. domestica* cv. ´Ametyst´ carrying *Rvi6* resistance gene for our experiments. The seedlings were challenged with two isolates of *V. inaequalis* conidia – isolate "Rin", that cannot overcome the *Rvi6* mediated resistance, and isolate "Rola", that is able to break such resistance. Performed RNA-Seq differential expression analyses revealed number of genes participating in the various processes that are recruited in order to battle *Venturia* infection.

The most studied defense of a plant organism against the invasion of pathogens – chemical defense was the most apparent. The activity of genes encoding PR-proteins (PR-10 such as Mal d proteins, LRR kinase/lipase, thaumatin-like, defensin, chitinase, endochitinase, *β*-glucanase, oxalate oxidase or protease inhibitor) was increased after infection with "Rin" isolate versus control. This indicates an association with the antifungal activity of the ´Ametyst´ variety through various biochemical pathways. Structural defense against pathogens also plays a role (eceriferum, lignin phenylalanine ammonia lyase or cinnamyl-alcohol NADPH dehydrogenase differences (Serrano et al. 2014, Front. Plant Sci 5:274). "Rin" has also influenced reduction of the expression of genes involved in the biosynthesis of hormonal precursors, e.g., downregulation of genes for amino acid transmembrane transporters.

- We have found great difference in gene expression between plants challenged with "Rin" vs "Rola". Noticeable is the enormous increase in *GDSL esterase/lipase* gene expression after

infection in "Rola" infected plants. It has recently been reported that constitutive expression of GDSL esteras/lipase isoforms in rice has reduced immunity and increased susceptibility to infection by pathogens (Gao et al. 2017, PLoS Pathog 13:11). Other interesting increase of activity was observed in major latex protein 423, defective in induced resistance 1 (DIR1), HOTHEAD-like protein or SODIUM POTASSIUM ROOT DEFECTIVE 2-like, etc. On the opposite end, the most down-regulated gene expression was found for LRR receptor-like serine threonine kinase. This disease resistance gene (R-gene) is involved in the detection of various pathogens, including bacteria, viruses, fungi, nematodes, insects and oomycetes. A number of extensive reviews have been conducted confirming the importance of this gene in defense against pathogens. The up or down-regulated expression of these genes were confirmed by qPCR and they are the main candidates for future research in the mechanism of *Rvi6* mediated resistance.

2.1.2 Research and development of genetic markers for polygenic resistance to scab

The recent implementation of a new NGS technology called genome-by-sequencing (GBS) into the GWAS process allows the search for molecular markers as well as genetic linkage analysis or genomic selection in these organisms. Restriction enzyme digestion in GBS is used to reduce the complexity of the genome, so that this method can be applied to very large genomes as well. The final reading of the restriction fragments allows comparison of variants even if the reference genome is not available). We prefer GBS to so far commonly used single sequence repeats (SSR) markers, which sometimes do no not reach sfficinet density and lack segregation in some genera.

GBS is therefore our choice for the implementation of SNP markers to distinguish polygenic sources of resistance to scab (i.g. in cv ´Julia´).

Unfortunately, these works depend on the vegetative stage of the apple tree, i.e. they can only be done once a year, and they are also dependent on the weather. We did the crossing two times already, but all potential hybrids froze. In 2020 we were more successful, our recently harvested apples (´Ametyst´ x ´Julia´ and vice versa) and got more than 200 seeds that are ready for further experiments.

2.2 Applied Research

The station has focused its efforts on breeding of apple trees with complex resistance to the most significant diseases in combination with good economic characteristics, especially optimal vigorous growth, high and regular productivity, attractive fruit appearance, good taste and long storability. In total, several dozens of vegetative, generative, morphological-pomological, analytical and organoleptic traits and characteristics, or optionally also determination of consumer preferences and market value are assessed, evaluated and verified. The most promising varieties undergo advanced testing at particular sites of potential business partners (nurseries, growers, sales organizations, research institutes or marketing companies) under terms and conditions of concluded testing agreements. In case of positive results of tested variety and confirmation of business partner´s interest in obtaining license rights, the breeder applies for national or supra-national plant variety rights (PVR) of the variety. After obtaining of PVR for the variety, the breeder grants the business

partner license rights to propagate and sell trees (or optionally fruits) of the variety under terms and conditions of concluded license agreement. The inventors of the varieties are employees of the Institute and the latter is the owner of the varieties.

2.2.1 Resistance to diseases

The most significant apple tree disease is a scab (*Venturia inaequalis*), causing necrotic spots on leaves and cork-like, often cracking scabs on fruits, especially in humid localities (Carisse and Bernier 2002, Mycol Res 106:1455). Such damaged fruit can no longer be profitably used for dessert apple purposes, but only for processing at usually very low selling prices, which means a significant economic loss for growers. In practice, a scab protection represents dozens of sprays during vegetation time, which is organizationally and financially very demanding and place an excessive load on the environment (Ellis et al. 1998, Plant Dis 82:428). The vast majority of current marketable resistant apple varieties bears scab resistance on monogenic basis type Rvi6, derived from wild apple species *Malus floribunda*. However, this type of resistance is often not sufficiently stable enough at many localities and vintages. Selected new scab races can break through the resistance, resulting in above mentioned symptoms on leaves and fruits. In such cases, originally resistant varieties are characterized by a tolerance (lower degree of resistance) or even sensitivity to scab. Therefore, IEB apple breeding program strive to find a solution consisting of so-called “pyramiding” of multiple monogenic resistance sources in one variety. In addition to resistance of type *Rvi6*, apple varieties carrying resistance in genes *Rvi2*, *Rvi4* or *Rvi17* are used for crossing of new varieties. As a second solution, old regional apple varieties or their next generations with scab resistance on polygenic basis are used in IEB crossings. This type of resistance has shown less favourable conditions for a pathogen so far, manifested by more durable resistance (Kellerhals and Duffy 2006, 12th Ecofruit Conf Proc, 157). Representatives of legally protected, commercially available IEB apple varieties with polygenic scab resistance are for instance ´Admiral´, ´Allegro´, ´Mira´, ´Barby´ or ´Minerva´.

Another harmful apple disease is powdery mildew (*Podosphaera leucotricha*), occurring particularly in dry localities. The main symptoms are whitish coating on leaves and at the ends of shoots. Powdery mildew does not directly affect fruits, but its occurrence is sometimes secondarily accompanied by an increase of skin russeting on fruits. Therefore, IEB apple newselections are subjects to strict selection during testing under experimental field conditions for several years.

The third, but even more serious on worldwide scale, is a quarantine bacterial disease fireblight (*Erwinia amylovora*; Baumgartner et al. 2015, Plant Mol Biol Rep 33:1573). It is manifested by wilting and dying of flowers, burning of leaves and fruits, drying and necrosis of vegetative shoots and branches. Consequences of fireblight are very destructive, often ending in death of the entire tree. The fireblight is fairly rare in the Czech Republic. However, it causes a significant economic loss in many world famous growing areas like Washington State (USA), South Tyrol (Italy) or Lake Constance (Germany). Chosen IEB newselections or varieties in an advance testing stage, showing the perspective of application in praxis, are therefore tested by method of artificial shoot inoculation by pathogen in the international research cooperation with Agroscope Wädensvil (Switzerland) or with Julius Kühn-Institut (Germany).

2.2.2 Modernization of the Station Strizovice IEB, enabling new breeding technologies

One of the most significant events in the evaluated period was modernization of the Střížovice workplace in years 2016-2017, which was professionally followed by a change of breeding technology. The purpose of modernization was speeding up and refining of the selection process of new varieties with the greatest perspective of practical use. The long-term shortcoming of IEB workplace in Střížovice was mainly an absence of a cold storage for fruits and a heated, fully adjustable greenhouse. The Czech Academy of Sciences provided a generous subsidy of 8,4 million Czech crowns for the modernization of the station. For the resulting approximately 15 million Czech crowns, it was managed to build a new greenhouse and cooling hall, as well as reconstruct existing buildings, including a new wastewater treatment plant.

A modern greenhouse with a possibility of automatic regulation of temperature, light, irrigation and air humidity speeds up the selecting process of newselections with perspective of permanent resistance. New seedlings are then grown and artificially infected by Venturia in highly managed conditions of greenhouse. After a few weeks, it is possible to evaluate effects of pathogen infection, or possible defense mechanisms of seedlings with required genetically induced resistance, and select only the most resistant individuals. The resulting seedlings reduction corresponds to an approximate selection of 300 most resistant plants out of about 3 000 sown seedlings. These are consequently grown in technical isolate and subsequently grafted on dwarfed rootstocks to bring the reproductive stage closer. The efficiency of new breeding technology thus lies not only in pre-selection of the most resistant newselections before they are planted in the field, but also in achieving of a rapid entry into fruit productivity, allowing the first evaluation of fruit characteristics several years earlier than previous system did.

Furthermore, the station has a new cooling hall with regulated temperature and ventilation, including boxes equipped with ULO technology (Ultra Low Oxygen). The ULO technology slows down a post-harvest ripening of fruits not only by regulated temperature, but also by reducing of oxygen content and by increasing of carbon dioxide concentration in the atmosphere. The new equipment will enable to precisely evaluate fruit storability of individual newselections or varieties. At the same time, it is possible to keep fruits of selected varieties in an excellent quality practically by next harvest for the purposes of their presentation and promotion, which is necessary for finding of a suitable business partner, ensuring their introduction to market.

The modernization of the workplace has very intensively continued even in following years. In 2019, a gradual replacement of an old training system for high density planting system of slender spindles was started. In addition, the support system of columns has been built with regard to future possibility of supplementing the tree support system with nets, protecting the orchard against the possible hail storm, which often cause a complete damage.

This year, it was managed to buy a neighbouring plot of land in Střížovice with an area of 1,31 ha, which IEB had in a long-term lease. This eliminated the risk of a non-renewal or premature termination of the lease agreement, to which it would be practically impossible to react quickly and fully when growing of apple trees (as a long-term plant culture). The total growing area of the experimental orchard in IEB personal ownership has thus more than doubled to 2,6 ha. In spring 2020, two stationary frost protection devices (Agrofrost,

Frostguard Revolution R20) were purchased, protecting the experimental orchard against possible damage to tree flowers by occurrence of late spring frosts. Another risk in the evaluated period, manifested significantly not only in Střížovice, but atleastin middle Europe, was lack of precipitation or their adverse distribution. In recent years, the torrential rains, from which the water cannot be sufficiently captured and used by plants, became more common. As a reaction to this fact, it was managed to build a 120 m deep well as a long-term sufficient source of underground water, a 100 m^3 retention tank into which water is pumped, and by the end of the year, a drip irrigation is going to be completed in individual rows of the orchard.

2.2.3 Applied research results:

IEB Station of apple breeding to disease resistance is highly productive on a long-term basis, concerning the legal protection of intellectual property of newly bred apple varieties in the Czech Republic and especially abroad.

The significance of results is amplified by the fact that individual varieties are applied for legal protection only after confirmation of interest of relevant business partner to propagate, sell trees or trade fruits of the variety under license terms and conditions.

The **unique know-how** of IEB is **to obtain and make available legal protection** of individual varieties in EU with zero or minimal associated costs. This consists of appointing the relevant license partner as a procedural representative of the variety, with an obligation to pay application and administrative fees for legal protection of the variety in EU. The property rights of IEB for the variety remain unaffected.

In period 2015 – 2019, the Station achieved **49 results of applied research (Z-variety)**:

Note: all concerned licensed varieties are traded on market

- ***Breeding certificate of granting plant variety rights in the Czech Republic:***
  (Central Institute for Supervising and Testing in Agriculture, ÚKZÚZ)
  - 16 apple varieties of IEB
- ***National plant variety certificate in Switzerland:***
  (Bundesamt für Landwirtschaft, BLW)
  - 6 apple varieties of IEB
- ***Community Plant Variety Rights in European Union:***
  (Community Plant Variety Office, CPVO)
  - 15 apple varieties of IEB
- ***United States Plant Patent:***
  (United States and Patent Trademark Office, USPTO)
  - 9 apple varieties of IEB
- ***Plant Patent in Ukraine:***
  (Ministry of Agrarian Policy and Food)
  - 2 apple varieties of IEB
- ***National breeding certificate in Georgia:***
  (National Intellectual Property Centre of Georgia, SAKPATENTI)
  - 1 apple variety of IEB
- ***National breeding certificate in Morocco:***
  (Office National de Sécurité Sanitaire des produits Alimentaires)
  - *1* apple variety of *IEB*

- ***National breeding certificate in South Africa:***
  (Department of Agriculture, Forestry & Fisheries)
  - *1* apple variety of *IEB*

The professional curiosity:

In its annual report for 2017, the European Community Plant Variety Office (CPVO) listed the 15 most active applicants for plant variety rights in individual agricultural sectors. Thanks to the extraordinary performance of the Station of apple breeding, the IEB of the Czech Academy of Sciences, v. v. i. submitted seven applications for plant variety rights of new apple varieties, which resulted in 7^{th} – 9^{th} place among applicants from all over the world in fruit sector. IEB thus confirmed the good name of Czech research and the excellent result from 2016, when IEB placed as 5^{th} – 8^{th}.

2.2.4 The implementation of applied results in practice in years 2015 - 2019

A high ability of application of new IEB apple varieties in practice demonstrate:

- **24 concluded license agreements** (16 Czech and 8 international)
- **5,9 million of sold trees** of IEB apple varieties (worldwide)
- **44,5 mil CZK of a license income** (ca. 1,71 mil €)

Examples of successfully commercialized IEB apple varieties, which were granted by plant variety rights or a registration in evaluated years 2015 -2019:

Topaz and its red mutation **Red Topaz**

It is the most cultivated scab resistant apple variety in the world and at the same time, the most cultivated variety grown in organic growing conditions, planted in Europe on an area of approximately 2 000 ha (Guerra 2018). License rights for the propagation and sale of the variety have already been granted to almost 20 business partners for different territories over the world. In years 2015 – 2019, more than 2 million trees were sold under a license mainly in Europe, but also elsewhere like for example in New Zealand. The variety is very often used worldwide as a valuable genetic source for further breeding.

UEB 32642

The variety is known under the trademark Opal® and is characterized by a bright yellow skin with crunchy flesh and aromatic honey sweet flavour. Growing of variety Opal® is suitable mainly to warm, vineyard areas with additional irrigation. Opal® was introduced into the market according to a worldwide marketing concept managed by companies Webfruit GmbH, Germany and Varieties International, USA. The variety is very popular, especially in the USA, and it has been registered in more than 50 countries in the world. The variety is protected by Plant Patent in the USA, by Community Plant Variety Rights in EU and lately, it has been applied for plant protection in several countries like Australia, Brazil, Chile, South Africa, Canada, Morocco, Mexico or New Zealand. More than 800 000 trees were sold in the evaluated period. In total, more than 2,5 million trees of Opal® have been sold worldwide, which corresponds to plantings in area of approximately 850 ha.

Charitable purposes: In 2018, the Endowment fund of Jaroslav Tupý was established in the Czech Republic in honour of the main Opal® breeder, who deceased in 2016. The fund supports mainly young scientists in the beginning of their professional carrier. In the USA, Opal® is grown at Broetje Orchards – the farm of Mr. Ralph Broetje in Washington State. Its fruits are exclusively sold by company FirstFruits Marketing, which dedicates a part of their total income to charity purposes. For example, they donated 150,000 $ to the charity in 2015. The Vista Hermosa Foundation, which was established by Ralph and Cheryl Broetje, supports an education and development of youth, building of community, leadership

development, small farmer training and also resource development in East Africa, India, Haiti and Mexico.

Selected web links:
http://www.opalapples.com/
https://opal-apple.com/
https://cs-cz.facebook.com/OpalBrand/
https://twitter.com/opalapple?lang=cs
http://www.vistahermosafoundation.org/
http://nfjt.ueb.cas.cz/?lang=cs

Bonita

The variety name Bonita comes from Portuguese and means “pretty” or “beautiful”, which represents an outstandingly attractive appearance of brightly red fruits. The variety is characterized by a high and regular fruit productivity, good tree growth and fruit qualities, including a long storability. Bonita is protected in EU, the USA, Switzerland and South Africa. The variety is commercially applied on the basis of an exclusive license agreement with Konsortium südtiroler Baumschuler (KSB), Italy, signed in 2016. This agreement allows controlling of trademark, marketing, growing, propagation and sales of trees and fruits. In the evaluated period, it was sold more than 1 million of Bonita trees in Italy, South Africa, Austria, Switzerland, France, the Netherlands, Slovenia, Hungary and the Czech Republic. Royalties were contractually agreed to pay not only from sold trees, but also from sold fruits (from yields). It is expected that Bonita will be commercially applied on the basis of a global marketing concept in cooperation with companies KSB from Italy, Varieties International from the USA and Webfruit from Germany.

UEB 6581

Very advanced is a license application of the variety named UEB 6581, which will be traded under trademark with a suitable fancy name. License rights for the propagation and sale of trees and trade with fruits were granted to KSB. In cooperation with Italian sales organization Melinda, which brings together 4 000 growers, KSB planned a spectacular goal to plant the variety on the remarkable area of 200 ha in Italian region of Trentino. Although the variety was not introduced to the market until 2018, more than 180 000 trees have already been sold under license in two seasons. Fruits of UEB 6581 are characterized by an exceptionally sweet flavour, reminding tones of tropical fruit.