
ČESKÁ
SPOLEČNOST
EXPERIMENTÁLNÍ
BIOLOGIE ROSTLIN

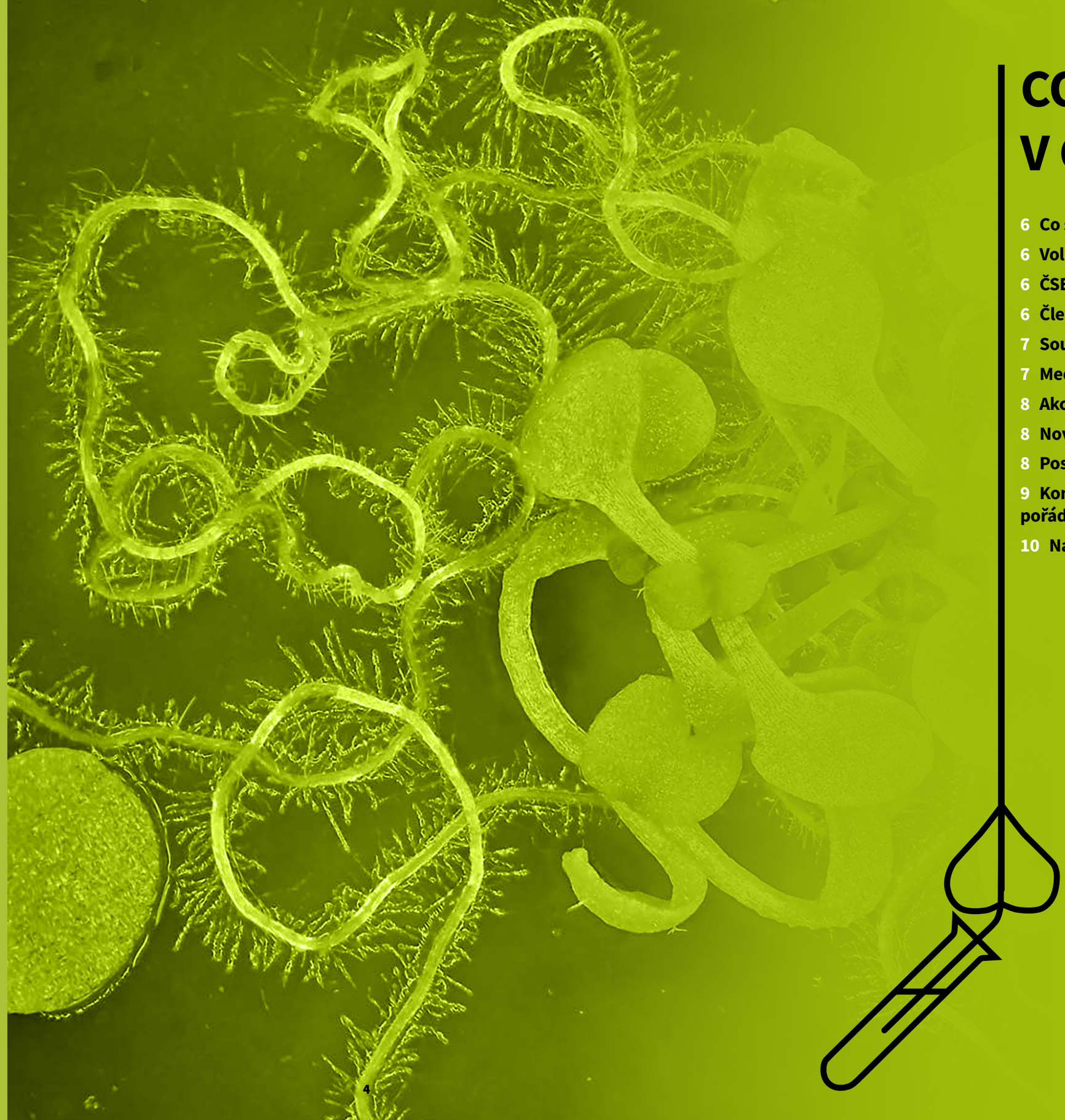
Editorial

Vydání bulletinu tentokrát proběhlo dříve, než je obvyklé. Důvodem je konání dlouho očekávané mezinárodní konference „Metodické dny“ neboli Methods in Plant Sciences 2023. Naše společnost se významně spolupodílí na organizaci této konference, a navíc v čele vědeckého a organizačního výboru stojí předseda ČSEBR Martin Janda, což nás velmi těší. Konference se bude konat v druhé polovině září v Srní, v srdci Národního parku Šumava. Můžete se těšit nejenom na nabitý vědecký program konference, ale také na krásnou přírodu. Tentokrát vyjde Bulletin již v září. Obsah Bulletinu bude mít klasické členění, na které jste zvyklí, a navíc součástí tohoto čísla bude soubor abstraktů ke konferenci „Metodických dní“.

Kromě této akce, která proběhne v letošním roce, se můžete těšit na informace o dalších vědeckých setkáních, která jsou naplánovaná na období 2024–2025. V tomto čísle se můžete také dovědět, kdo získal Cenu ČSEBR 2023 nebo jaká kniha o rostlinách by vám neměla uniknout či jaká univerzita slaví 450 let od svého založení.

Jana Šedivá,
šéfredaktorka Bulletinu ČSEBR v září 2023

OBSAH



CO SE UDÁLO V ČSEBR

Co se událo v ČSEBR v roce 2023

Jsem moc rád, že se nám opět daří vydávat Bulletin ČSEBR každoročně. Ani v uplynulém období se toho v ČSEBR neudálo málo. Poprvé zasedl nově zvolený výbor ČSEBR a zvolil si své předsednictvo, máme novou reprezentantku v rámci celoevropské společnosti FESPB (Federation of European Societies of Plant Biology), uspořádali jsme soutěž o Cenu ČSEBR a podpořili jsme několik velmi zajímavých akcí. Navíc nás čeká událost, která se nekonala již čtyři roky, a sice plenární schůze ČSEBR. O tom všem se více dozvíte dále v této části Bulletinu.

Martin Janda,
předseda ČSEBR, Přf JU České Budějovice

Volba předsedy a místopředsedů výboru ČSEBR

Podle Stanov ČSEBR: „*Výbor volí ze svého středu předsedu, místopředsedu či dva místopředsedy*“ jsme si na první schůzi nově zvoleného Výboru ČSEBR, konané (hybridně) na Ústavu experimentální botaniky AV ČR v Praze dne 11. ledna 2023 zvolili vedení ČSEBR na období od 1. 1. 2023 do dalších voleb ČSEBR, předpokládané v r. 2025. Jediným kandidátem na předsedu/předsedkyni výboru jsem byl já (Martin Janda) a obdržel jsem jednomyslnou podporu všech 11 přítomných členů Výboru ČSEBR. Kandidáti na místopředsedy byli dva, Jana Albrechtová a Peter Váczi, a též byli zvoleni jednomyslně všemi přítomnými členy Výboru. Oběma svým kolegům bych chtěl tímto poděkovat, že mají chuť a vůli pracovat pro ČSEBR jako členové předsednictva.

Pro volební období od 1. 1. 2023 byl tedy jako předseda Výboru ČSEBR zvolen Martin Janda a místopředsedy se stali Jana Albrechtová z Univerzity Karlovy v Praze a Peter Váczi z Masarykovy univerzity v Brně.

Martin Janda

ČSEBR má datovou schránku

Stejně jako jiné spolky jsme byli nuceni zřídit si datovou schránku. Musím říci, že z funkce předsedy na tuto povinnost nežehrám. Ba naopak. Veškerou komunikaci s Radou vědeckých společností, se soudem kvůli zapsání nového výboru do rejstříku nebo daně mohu v klidu komunikovat z “tepla domova” Českých Budějovic. Za mě určitě změna k lepšímu. ID naší datové schránky je “Česká společnost experimentální biologie rostlin, z.s. (b9jvp7i)”

DATOVÉ SCHRÁNKY

Martin Janda

Členská plenární schůze ČSEBR

Po čtyřech letech se opět uskuteční prezenční plenární schůze naší společnosti. Naposledy se tak stalo v rámci konference Plant Biology CS 2019, která se konala v Českých Budějovicích. Tentokrát bude plenární schůze součástí konference „Methods in Plant Sciences 2023“, která se koná v Srní a vlastní schůze se uskuteční v úterý 26. září od 18:30. Na schůzi bude možno se připojit i online přes Zoom. Odkaz na Zoom bude rozeslán e-mailem celé členské základně ČSEBR.

Na schůzi budou představeny především aktivity naší společnosti včetně hospodaření.

Martin Janda

ČESKÁ SPOLEČNOST EXPERIMENTÁLNÍ BIOLOGIE ROSTLIN

Soutěž o cenu ČSEBR 2023

Ve Výboru ČSEBR bylo koncem února 2023 rozhodnuto opět vyhlásit soutěž o Cenu ČSEBR 2023. Tato soutěž zároveň slouží k výběru vhodného kandidáta za ČSEBR na FESPB Award 2023. Cena FESPB byla udělena na kongresu Plant Biology Europe 2023: https://europlantbiology2023.org/.

Organizátorem soutěže byl za Výbor ČSEBR, předseda Martin Janda. Soutěž byla vyhlášena v Newsletteru ČSEBR 2023/01 (rozeslán 24. února 2023). Přihlášky do soutěže ČSEBR bylo třeba zaslat do 21. března 2023. Přihláška měla strukturu jako pro FESPB Award. Přihlásili se čtyři kandidáti: **Tomáš Figura** (Univerzita Karlova); **Jana Koller** (Ostravská univerzita); **Hana Leontovyčová** (Ústav experimentální botaniky AV ČR); **Adéla Přibylová** (Univerzita Karlova). Poté probíhalo hodnocení kandidátů. Devítičlennou komisi pro hodnocení kandidátů tvořili: Lubomír Adamec (Botanický ústav AV ČR); Martin Janda (Jihočeská univerzita); Tetiana Kalachova (Ústav experimentální botaniky AV ČR); Marek Klemš (Mendelova univerzita); Jiří Kubásek (Jihočeská univerzita); Jana Oklešťková (Univerzita Palackého); Aleš Pěnčík (Univerzita Palackého); Olga Valentová (VŠCHT v Praze); Viktor Žárský (Univerzita Karlova). Tímto ještě jednou moc děkuji všem členům komise, že si na hodnocení kandidátů našli čas. Každý člen komise udělal pořadí. Hodnotila se kvalita CV (získaná ocenění, získaná stipendia, zahraniční stáže, počet zvaných přednášek, atd.), publikační aktivita (počet a kvalita, prvoautorství) a detailní představení výsledků výzkumu kandidáta (kvalita předloženého popisu výzkumu, plány do budoucna). I když všichni kandidáti (kandidát a tři kandidátky) byli velmi kvalitní, tak nakonec hlasování komise dopadlo jednoznačně z hlediska vítěze, jímž se stal Tomáš Figura, který se u osmi z devíti hodnotitelů umístil na prvním místě a u jednoho na místě druhém (celkem tedy 10 bodů – čím méně bodů tím lepší umístění). Soutěž o druhé až čtvrté místo se rozhodovala o jediný bod. Na druhém místě nakonec skončila Jana Koller, která obdržela 22 bodů. Na třetím místě byla Hana Leotovyčová s 23 body a na místě čtvrtém se ziskem 24 bodů skončila Adéla Přibylová.

Tomáš Figura se tedy stal nominantem ČSEBR do soutěže o FESPB Award 2023. S udělením Ceny ČSEBR 2023 jsme vyčkávali, neboť naše pravidla říkají, že Cenu ČSEBR získá kandidát na FESPB Award v případě, že FESPB Award nezíská. Pokud ji získá, pak Cenu ČSEBR získá druhý v pořadí soutěže ČSEBR o nominaci na FESPB Award; tedy v tomto případě Jana Koller.

Tomáš Figura bohužel cenu FESB nezískal, byl čtvrtý v pořadí **a tak mu byla udělena Cena ČSEBR** ve výši 25 000 Kč, dále mu byla nabídnuta zvaná přednáška v rámci konference 17th Student Days in Plant Biology CS 2023 (https://csebr.cz/plantmethods2023/) a pobyt na této konferenci zdarma. Aby i ostatní kandidátky cítily, že si vážíme jejich úsilí a práce vynaložené k zapojení do soutěže, tak všem byl odpuštěn poplatek za roční členství v ČSEBR a rovněž jako Tomáši Figurovi, jim byla nabídnuta přednáška v rámci 17th Student Days in Plant Biology CS 2023 a pobyt na této konferenci zdarma. Potěšilo mě, že všichni nabídku přednášet na studentské konferenci přijali.

Martin Janda

ČESKÁ SPOLEČNOST EXPERIMENTÁLNÍ BIOLOGIE ROSTLIN

Medailonek laureáta ceny ČSEBR 2023

Tomáš Figura vystudoval magisterské studium biologie na Katedře experimentální biologie rostlin PřF UK v r. 2016, na které navázal doktorským studiem Anatomie a fyziologie rostlin na stejné katedře a v Muséum national d‘Histoire naturelle v Paříži pod vedením Dr. J. Ponerta a prof. M-A Selosse na téma Interakce inicíálně mykoheterotrofních rostlin s prostředím. Rovněž vystudoval inženýrský obor Ochrana přírody na České zemědělské univerzitě v r. 2017 pod vedením prof. B. Mandáka. V současnosti pracuje jako post-doc na oddělení mykorhizních symbióz v BÚ AVČR a letos nastupuje na post-doc v holandském Naturalis Biodiversity Center v Leidenu pod vedením Dr. V.S.F.T. Merckx.

ORCID: 0000-0001-5714-7810, Google scholar

Tomáš Figura

Akce podpořené ČSEBR

V letošním roce ČSEBR finančně podpořila:

» Minisymposium: "Hot Topics and Advances in Plant Cell Biology" konané na Ústavu experimentální botaniky (3. 5. 2023), které se uskutečnilo ku příležitosti 65. narozenin Viktora Žárského. Hlavním organizátorem akce byl Martin Potocký (z ÚEB).

» Konference "Botanik a politik Bohumil Němec v kontextu", která se konala na PřF UK (7.–8. 6. 2023) a uskutečnila si ku příležitosti výročí 150 let od narození prof. Němce. Jedním z hlavních organizátorů byl Viktor Žárský.

» Konference "The Czech Plant Nuclear Workshop 2023" konaná Mendelově refektáři v Augustiánském klášteře v Brně (20.–21. 6. 2023) pod záštitou Masarykovy univerzity a ÚEB. Jedním z organizátorů byl i člen výboru ČSEBR Lukáš Fišer.

Více informací o výše uvedených akcích naleznete v sekci Vědecká setkání a akce sponzorované ČSEBR tohoto bulletinu.

» Dvě na sebe navazující konference "17th Student Days in Plant Biology CS 2023" a "Methods in Plant Sciences 2023", obě se uskuteční v Srní po vydání tohoto bulletinu (22.–27. 9. 2023). Součástí tohoto bulletinu jsou knihy abstraktů k těmto konferencím.

Martin Janda

Nový zástupce ČSEBR při FESPB

Je mi ctí, že jsem byla zvolena novým zástupcem ČSEBR při FESPB. S českou vědou jsem spojena v různých rolích od roku 2016. Vše začalo studentskou stáží v rámci programu Erasmus. Poté jsem se do ČR vrátila na postdok pozici a od roku 2023 jsem se stala vědeckým pracovníkem Ústavu experimentální botaniky AV ČR. Celkově jsem ve své vědecké kariéře působila ve čtyřech zemích (Magisterské studium na Ukrajině, Doktorské studium ve Francii a postdoktorandská stáž ve Velké Británii). Současně se snažím věnovat zavádění co nejlepších postupů v propagaci a rozvoji rostlinné biologie v České republice a ČSEBR - na mezinárodním poli. Těším se na nové příležitosti spojit se s nadšenými rostlinnými biology z celé Evropy a posílit spolupráci mezi našimi společnostmi. Také proto, že si myslím, že nejlepší výzkum vzniká, když vědci a komunity spolupracují, plánuji se zapojit a podporovat iniciativy spojující vědce a veřejnost.

Tetiana Kalachova

Federation of European Societies of Plant Biology

Poskytování informací členské základně ČSEBR

ČSEBR informuje (ne)pravidelně své členy o dění a o událostech, které souvisejí s činností a posláním společnosti především v **Newsletteru** (přibližně 5-7x za rok) rozesílaném elektronicky.

Dále má ČSEBR funkční **webové stránky (www.csebr.cz**), které se postupně snažíme vylepšit tak, aby bylo pro členy inspirativní se na naše stránky jednou za čas přijít podívat. Co se nám povedlo díky spolupráci s panem Maliňákem, správcem webu, bylo napojení webových stránek konferencí „Methods in Plant Sciences 2023" a „Student Days in Plant Biology" k našemu webu. Chtěli bychom takto poskytnout službu i pro pořadatele konference Plant Biology CS a také bychom naše webové stránky chtěli využít ke správě databáze členů naší společnosti.

Dlouhodobým problémem stránek byla absence administrátora, který by se o webové stránky ČSEBR staral s patřičným odhodláním. To se, doufám, změní se zapojením kolegyně *Jitky Janové (KEBR, PřF JU)*, která kývla na mou prosbu zkusit být administrátorkou našeho webu. Věřím, že tato změna povede k tomu, že budou naše stránky vnímány jako živé a hodné občasného navštívení.

Jednou ročně vychází především pro členy ČSEBR **Bulletinu** v tištěné a elektronické formě podle zájmu členů. Pro rychlou komunikaci je využívám **email**.

Martin Janda

Kongres Plant Biology Europe 2023 (Hlavní událost pořádaná FESPB)

Ve dnech 3.–6. července 2023 se ve francouzském Marseille konala tradiční konference Plant Biology Europe, která se koná každé dva roky. Tento kongres je hlavní akcí pod hlavičkou FESPB (*Federation of European Societies of Plant Biology*). Tentokrát kongres pořádala Francouzská společnost rostlinné biologie a Institut biologických věd a biotechnologií v Aix-Marseille. Setkání bylo velmi zajímavé. Sešlo se na 400 účastníků z Evropy i z celého světa s expertízami napříč širokým spektrem výzkumu rostlin. Program byl nabitý a vynikající přednášky probíhající v souběžných sekcích způsobily, že bylo docela náročné si vybrat čeho se má člověk zrovna účastnit. Vynikající prezentace špičkové molekulární a buněčné biologie se střídaly s populačně genetickými studiemi planě rostoucích druhů; pokročilé počítačové modelování buněčných procesů bylo spojeno s mikroskopií se superrozlišením, -omikou jednotlivých buněk a sledováním chování jednotlivých částic.

Pozoruhodné je, že velká část prezentovaného výzkumu se spíše odklání od modelové rostliny *Arabidopsis thaliana* a směřuje k plodinám a nemodelovým druhům. To je nyní možné díky rychlému pokroku v metodických přístupech a přináší to nový rozměr do našeho chápání rostlin. Doufejme, že se tím naše poznatky ještě více přiblíží i využití na polích.

Zvláštní sekce byla věnována adaptaci rostlin na klimatické změny. S celkovým nárůstem teplot a zvyšujícími se emisemi CO_2 ze zemědělství a průmyslu je hledání nových řešení, jak zprostředkovat jejich poškození, také žhavým tématem výzkumu v oblasti biologie rostlin. Proběhl kulatý stůl, jemuž předsedal Dr. Heribert Hirt, který představil iniciativu „PlantACT!": cílem této iniciativy je propojit odborníky z oblasti rostlinných věd za účelem řešení klimatických změn. V České republice je PlantACT! iniciativa zastoupena Dr. Ivou Mozgovou (Ústav molekulární biologie rostlin, Biologické centrum, AV ČR).

Celkově byla česká rostlinná biologie a zejména začínající vědci na setkání PBE2023 dobře zastoupeni. Členům ČSEBR byly uděleny čtyři cestovní granty: Hana Leontovyčová (Ústav experimentální botaniky AV ČR), Tereza Kalistová (Jihočeská univerzita), Nikoleta Rubil (Ústav experimentální botaniky AV ČR/Česká zemědělská univerzita) a Miroslav Berka (Mendelova univerzita v Brně). Dvě ceny za nejlepší poster získali také mladí čeští vědci: Hana Leontovyčová (Ústav experimentální botaniky AV ČR) za práci „Fytohormony produkované houbovým rostlinným patogenem jako komunikační prostředek" a Unnikannan Prabhullachandran (CEITEC MU, Brno) za práci „Odhalení procesu termoregulace během vývoje semen u *Brassica napus*".

Tetiana Kalachova

Naše nové knihy

Rostlina jako pevnost

Dale Walters
Oxford university press (2017)
Překlad: Stanislav Mihulka
Nakladatelství Jihočeské univerzity v Českých Budějovicích (2023)

Nakladatelství Jihočeské univerzity v Českých Budějovicích přináší první český překlad knihy s poutavým názvem **Rostlina jako pevnost. Jak se nenechat sežrat** *od britského fytopatologa Dale Walterse. Publikaci z angličtiny do češtiny přeložil Stanislav Mihulka.*

Rostliny představují lákavou kořist plnou živin pro ohromnou armádu patogenů a predátorů. Dokonce se ani nemohou hnout z místa. Jak vůbec mohou přežít? Odpověď nabízí populárně naučná kniha Dale Walterse, která čtenáře seznamuje s nekonečnou válkou mezi rostlinami a jejich protivníky. Walters vykresluje rostliny nikoliv jako bezbranné oběti potravního řetězce, ale jako mistry v přežívání, kteří mají po ruce řadu skvělých triků, díky nimž jsou důstojnými soupeři pro nelítostné mikroskopické patogeny i věčně hladové herbivory. Kdo se bojí, nesmí na louku!

© Nakladatelství Jihočeské Univerzity v Českých Budějovicích

Text převzatý z webových stránek Nakladatelství Jihočeské Univerzity
https://nju.jcu.cz/aktualne/rostlina-jako-pevnost

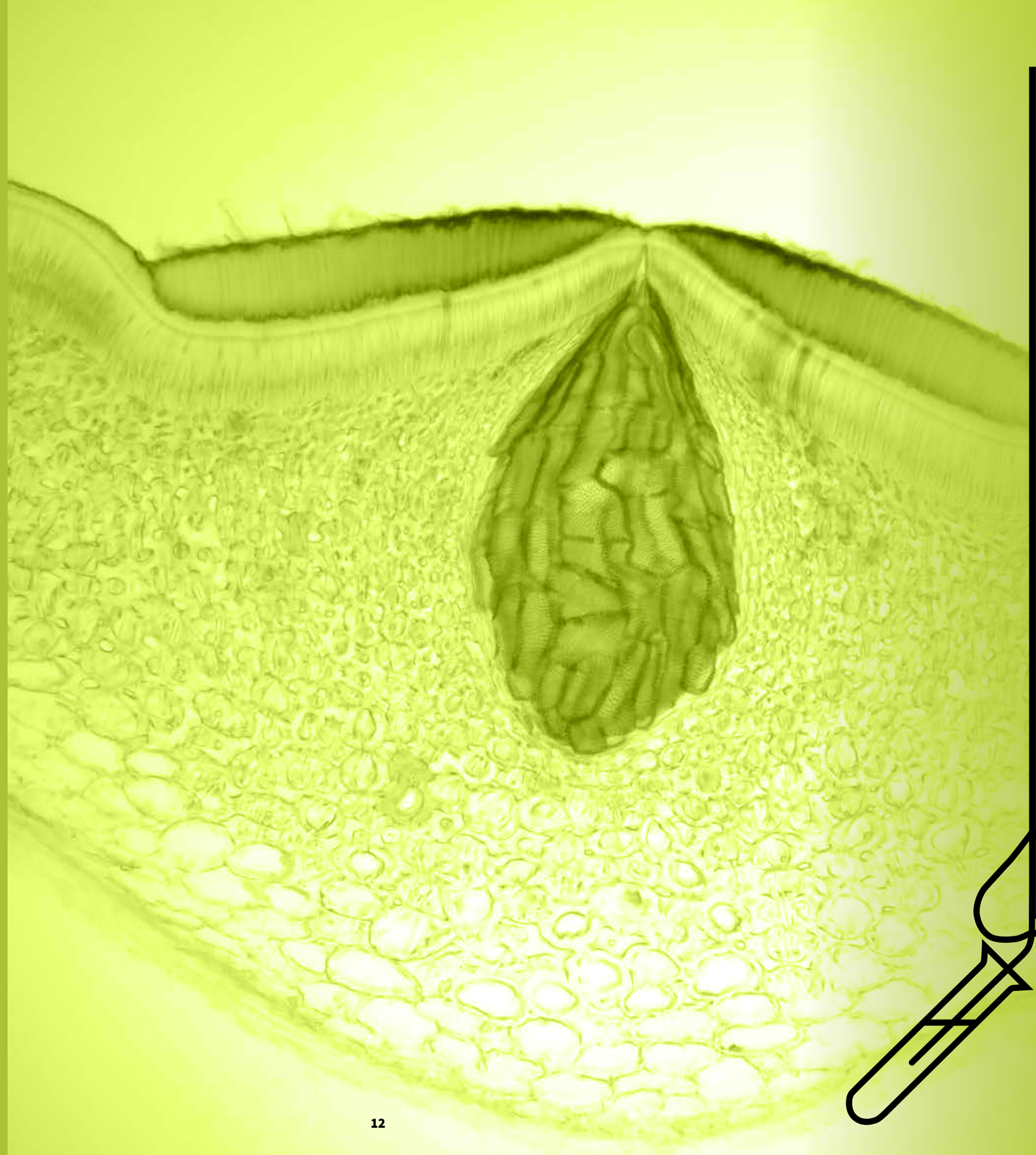
Komentář ke vzniku překladu knihy Rostlina jako pevnost

V roce 2021 jsem začínal s prací na svém novém předmětu „Imunita rostlin“ na Přírodovědecké fakultě, JU v Českých Budějovicích. Na jaře toho roku se ke mně dostala informace, že univerzita vypisuje každoročně soutěž s cílem podpořit mimořádné projekty týkající se výuky a výzkumu na JU. V hlavě se mi okamžitě vyrojila chuť přeložit knihu „Fortress Plant“ od prof. Walterse, kterou jsem využíval k inspiraci při tvorbě předmětu. Současně ve mně převládal pocit, že podobná populárně-naučná literatura napsaná odborníkem v oboru na českém knižním trhu chybí. Kontaktoval jsem Nakladatelství JU, které to zaujalo a vyšli mi vstříc. Naštěstí mi nabídli možnost, že by se překladu zhostil jejich překladatel a já bych byl „jen“ odborným editorem, což byla varianta, která se mi velmi zalíbila, i když mě původně ani nenapadla. Přihlášku jsme podali, peníze získali a tak se překladu chopil, podle mě velmi zdařile, kolega Stanislav Mihulka, který je vystudovaným botanikem a tak mu téma nebylo úplně vzdálené.

Byla to zajímavá zkušenost. Naivně jsem si myslel, že to přece v tom roce musíme dokončit a že na Vánoce roku 2021 budu mít dárky pro rodinu. Poznal jsem kolik práce překlad knihy stojí a co všechno se v jeho průběhu řeší. Nakladatelství nevzalo vydání knihy na lehkou váhu a bylo vidět, že jim na kvalitním zpracování záleží, což podle mě výborně ilustruje grafické zpracování knihy, které je, troufnu si říci, daleko lepší než u originálu. Kniha se na pulty knihkupců dostala v dubnu 2023, jsem přesvědčen, že stojí za přečtení. Což si ostatně můžete ověřit, pokud budete chtít, sami.

Pozn.: Kniha je mimo Nakladatelství JU k dostání též v síti knihkupectví Kosmas.

Martin Janda



CO NÁS ČEKÁ

Co nás čeká v roce 2024 (a 2025)

This part of the Bulletin will be unusual because we decided to write it in English. The reason is simple. This year's Bulletin consist of the Abstract books of two international conference, and a substantial part of the visitors to the conference and readers of the Bulletin/Abstract book will be non-Czech speaking colleagues. Who also represent potential visitors of upcoming events.

The European Plant Cytoskeletal Club - EPCC conference

In 2024, the European Plant Cytoskeletal Club - EPCC conference will be held in Prague for the 9th year. This meeting of plant cytoskeleton scientists was founded in 2015 by Kateřina Schwarzerová and Daniël van Damme due to the absence of such an oriented European conference. The informal, open and low-cost conference makes this two-day meeting a student-friendly environment where student and young scientists can network with each other and with established scientists. The preferential location of the conference venue in the heart of Europe allows easy access by many modes of transport - by car, train or plane from more distant locations. I am glad that only one year was skipped during the Covid period; we refused to cancel the EPCC in the second Covid year and together with Katharina Bürstenbinder we organised EPCC 2021 as a Zoom conference, which had a great attendance of scientists from all over the world. In subsequent years, the conference returned to its normal format. In 2023 Katharina Bürstenbinder was the main organizer and a report on the EPCC 2023 in Halle (Germany) can be found below. The EPCC has become a traditional and successful meeting that fully fulfils its original goal: to create a platform for the discussion of plant cytoskeleton research results in a highly specialized community. I would hereby like to invite all those interested in the plant cytoskeleton from any perspective to the next EPCC which will be held in Prague in the second half of June 2024. We would like to thank our home institutions and sponsors for their support of EPCC. Details of the future conference will be published regularly on the conference website http://kfrserver.natur.cuni.cz/EPCC.

I look forward to seeing you there!

Kateřina Schwarzerová
Department of Experimental Plant Biology, Faculty of Science, Charles University

Introduction of the European Plant Cytoskeletal Club

The cytoskeleton is a fundamental and highly conserved cellular structure with essential roles in a multitude of processes, such as organelle movement, cell movement, and nuclear division. In plants, the cytoskeleton has acquired novel and specific functions in cell division, cell wall formation, mechano-sensing, stress responses and plant-microbe interactions that are essential for development and growth. The European Plant Cytoskeletal Club (EPCC) (http://kfrserver.natur.cuni.cz/EPCC) was established in 2015 by Daniël van Damme and Kateřina Schwarzerová as an annual informal meeting for researchers specializing in cytoskeleton-related topics unique to plants. The motivation for this meeting was the absence of a dedicated conference in Europe focusing on the plant cytoskeleton. European conferences focusing on the eukaryotic cytoskeleton provide limited space for plant research due to the high prevalence of medical aspects of cytoskeletal research.

The goal of the EPCC is to provide a platform for interaction among experts, particularly encouraging the participation of students who can present and discuss their work in a forum of specialists. Low-cost organization and a central location in Europe promote student involvement and accessibility to a wide range of scientists. After a Covid break in 2020, the meeting resumed in 2021, since then co-organized by Kateřina Schwarzerová and Katharina Bürstenbinder. Typically held in Prague, Czech Republic, or a neighboring German city, this year's 8th annual conference took place

Figure 1: Attendees of the 8th EPCC 2023 at the Leibniz Institute of Plant Biochemistry in Halle (Saale).

in Halle (Saale), Germany, on June 28 and 29, 2023, under the main organization by Katharina Bürstenbinder's laboratory at the Leibniz Institute of Plant Biochemistry. The conference attracted more than 60 scientists from eleven European countries, with ~70% being undergraduates, PhD students, and early-career postdoctoral scientists. The event featured thematic lecture sessions, poster flash talks, a poster session, as well as ample networking opportunities during coffee and lunch breaks and the conference dinner. These networking opportunities allowed for valuable exchanges with established senior scientists in the field of plant cytoskeleton research, ultimately contributing to the conference's overall success. In addition, this year's EPCC included a competition for the best student lecture and poster, supported by the journal Cytoskeleton (Wiley) and the German Society of Plant Sciences (DBG), respectively.

The topics presented correspond to current hot issues in plant cytoskeleton research and can be divided into to the following categories:

1. Plant cell division: Several lectures were devoted to investigating the molecular mechanisms and molecules associated with plant cytokinesis, as well as the relationship between the cytoskeletal apparatus and membranes, including its composition and interactions with membrane proteins.
2. Cell wall synthesis and plant growth regulation: The lectures focused on the role of microtubules in secondary cell wall deposition, the control mechanisms ensuring maintenance of cellulose synthesis, and the importance of control mechanisms in preserving microtubular structures in the cortical layer necessary for cell wall synthesis. Several talks have shed light on the role of actin nucleators in cell wall deposition and the interaction between actin and plasma membrane lipids.
3. Molecular motors, the cytoskeleton and their role in cellular organization and stress responses: The lectures covered several specific roles of molecular motors in plants, such as cold sensing, responses to plant hormones, and control of organelle movement. Additionally, the lectures also touched upon the role of the plant cytoskeleton in responding to biotic stress, a topic that is currently under intensive study.
4. Interdisciplinary approaches in plant cytoskeleton research: Several lectures were devoted to physical principles of pathogen penetration into plant cells, mechanical properties of microtubules and bioinformatics methods to analyze cytoskeleton dynamics. These interdisciplinary studies have highlighted the significance of incorporating mathematical and biophysical models in plant cell research. This integration offers great potential in unraveling numerous mechanisms related to the function of the plant cytoskeleton.

The meeting's informal and collaborative atmosphere facilitated lively discussions and the establishment of new connections among attendees. Overall, the EPCC 2023 meeting proved to be a successful event, enriching the European plant cytoskeleton research community, and paving the way for future advancements in the field. The upcoming EPCC conference is set to take place in Prague in June 2024. The organizing committee is excitedly anticipating an even larger gathering of enthusiastic plant cytoskeleton researchers.

Acknowledgements We would like to thank to Leibniz association for core funding, to German Society for Plant Sciences for travel grants and to journal Cytoskeleton (Wiley) for sponsoring the best presentation award. Further we would like to thank to Gina Stamm and Jonas Buhl for excellent support in conference organization. We are grateful to Malte Kölling, Daniela Zehnich and Hendrik Krolle for on-site support during the meeting. We greatly acknowledge the assistance and general support from the IPB.

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Cytokinin forefront research

OLOMOUC, CZECH REPUBLIC
5 – 6 SEPTEMBER 2024
Laboratory of Growth Regulators

Venue: Fort Science, Interactive science centre of Palacký University Olomouc

Ondřej Novák, Markéta Pernisová, Klára Hoyerová

Cytokinins are small but inspiring molecules. We would like to invite you to **CKFR 2024** – an non-formal and friendly meeting for open discussion on our favorite topic. We hope that the third meeting with cytokinins will provide new inspirations, ideas and visions, and connect our small community more closely.

More detailed information will be available on the website: https://lgr.upol.cz/ckfr2023

You are welcome in Olomouc to enjoy the hottest news in cytokinin research.

Plant Biology CS 2025 in Bratislava

Dear friends and lovers of the mysteries of the plant kingdom. Two years in advance, we would like to invite you to the traditional Czech-Slovak conference Plant Biology CS 2025 (formerly known as KEBR conference). The last meeting was held in the "pre-Corona" era in 2019 in České Budějovice. We would like to organize the next meeting in cooperation with the Slovak Botanical Society and the Czech Society of Experimental Plant Biology in the summer of 2025 in the newly renovated premises of the Faculty of Natural Sciences of Comenius University in Bratislava. We are looking forward to meeting you in our University and in the city of Bratislava.

Marek Vaculík
(main organizer of Plant Biology CS 2025)

OBOROVÉ AKTUALITY

Dynamický vápníkový signál zprostředkovává odpověď na chycení kořisti u masožravé rosnatky

Nejnápadnější projevy rostlinné masožravosti v podobě rychlých pohybů pastí najdeme u zástupců čeledi rosnatkovitých (Droseraceae). Nejpočetnějším rodem masožravých rostlin je rosnatka (*Drosera*) s více než 250 známými druhy. Po chycení kořisti v listových pastech s mnohobuněčnými tentakulemi dochází k poměrně pomalému pohybu, ohybu tentakulí, nejčastěji v řádu minut až desítek minut a u většiny druhů také k pomalejšímu prohnutí listové čepele pastí kolem chycené kořisti v řádu hodin, čímž kolem kořisti vznikne jakýsi "vnější žaludek" umožňující účinnější rozklad trávicími enzymy a využití ztrávené kořisti. Molekulární zprostředkování této odpovědi zůstává u rosnatek částečně neznámé, přestože je prokázáno u různých druhů, že dráždění hlaviček tentakulí kořistí mechanicky nebo chemicky vede k vyvolání elektrických signálů, které se rychle šíří podél tentakulí a navodí jejich ohyb i tvorbu obranného hormonu kyseliny jasmonové (JA), která zahájí genovou expresi pro tvorbu trávicích enzymů. Je zajímavé, že jasmonátová regulace byla dosud zjištěna v pastech u všech tří rodů rosnatkovitých (mucholapka /*Dionaea*/, aldrovandka /*Aldrovanda*/ a rosnatka) a u láčkovky (láčkovkovité, Nepenthaceae), ale nebyla prokázána u rodů tučnice (*Pinguicula*) ani bublinatky (*Utricularia*) z čeledi bublinatkovitých (Lentibulariaceae). U nemasožravých rostlin je tvorba JA způsobena elektrickými signály prostřednictvím zvýšení koncentrace iontů Ca^{2+} v cytosolu buněk. Připomeňme, že ionty Ca^{2+} jsou výbornými kandidáty na zprostředkování této obranné odpovědi, protože koncentrace Ca^{2+} v cytosolu je velice nízká (řádově 10^{-7} až 10^{-6} M) a buněčný elektrický membránový potenciál má obvykle hodnotu -100 až -150 mV, takže ionty Ca^{2+} mají obrovskou snahu difundovat z buněčných stěn do buněk podle strmého gradientu elektrochemického potenciálu. Funkce vápníkového signálu byla již nedávno prokázána pro zavření pasti u mucholapky i pro tvorbu JA po mechanickém poranění listu huseníčku italského (*Arabidopsis thaliana*). Shodná tvorba JA v obou těchto reakcích podporuje hypotézu, že využití jasmonátové signalizace kořisti u některých masožravých rostlin se vyvinulo z obranných reakcí rostlin proti hmyzím herbivorům.

Kolektiv v Ústavu pro biologická studia ve městě La Jolla v Kalifornii v USA vedený Carlem Prockem se snažil prokázat, že podobné dynamické změny koncentrace Ca^{2+} v buňkách tentakulí, zprostředkovávající odpovědi na chycení kořisti (tj. mechanické a chemické podněty), probíhají také v listech australské rosnatky *Drosera spatulata*. Ke sledování koncentrace Ca^{2+} v cytosolu buněk tentakulí vytvořili reportérové linie této rosnatky transformované fluorescenčním Ca^{2+} indikátorem GCaMP3. Krmení rostlin živými muškami octomilkami na listy způsobilo výrazné a dynamické zvýšení koncentrace Ca^{2+} v cytosolu buněk tentakulí. Tentakule v kontaktu s muškou vyslaly rychlou vápníkovou vlnu z hlavičky tentakule směrem k její bazi a potom následovala poměrně slabá a pomalá vlna šířící se mimo tentakuli. Vápníkové vlny celkově odezněly do 30 min a časový průběh Ca^{2+} vlny koreloval s průběhem šíření elektrického signálu v tentakulích i v listové čepeli, který je známý z jiných studií u rosnatek. Stejné zvýšení koncentrace Ca^{2+} v tentakulích nastalo i po dráždění hlaviček tentakulí skleněnou kapilárou anebo po nanesení kapičky živného roztoku na tentakule. Naopak nanesení kapičky vápníkového inhibitoru $LaCl_3$ snížilo expresi cílových genů JA i pohyby listové čepele po chemickém dráždění. Tyto výsledky souhrnně vypovídají, že po chycení kořisti jsou změny koncentrace Ca^{2+} v cytosolu buněk tentakulí nezbytné jak pro pohyby tentakulí a listů, tak i pro expresi genů pro tvorbu trávicích enzymů.

Výsledky studie jednoznačně prokázaly přítomnost vývojově starobylé jasmonátové signální dráhy závislé na Ca^{2+} v odpovědi na chycení kořisti u rosnatky a přinášejí další důkaz o fyziologickém vývoji masožravosti z původní obrany rostlin proti herbivorům. Tato dráha přenáší jak mechanické, tak i chemické podněty. Těmito podněty generované elektrické akční potenciály v hlavičkách tentakulí se šíří do bazí tentakulí, a přitom dochází k dynamickému přechodnému zvýšení koncentrace Ca^{2+} v buňkách, což je následně převedeno do zvýšené tvorby JA, která indukuje pohyby tentakulí a listů a spouští i expresi genů kódujících trávicí enzymy. [Proc. Natl. Acad. Sci. USA 2022, 119: e2206433119].

Lubomír Adamec

Genomika turionů okřehku závitky mnohokořenné odhaluje cesty vedoucí k dormanci

Vodní rostliny nejméně z 9 čeledí v subtropickém až subarktickém pásu vytvářejí přezimovací pupeny zvané turiony (*turio* = latinsky prýt). Turiony jsou orgány stonkolistového původu, které vznikají nejčastěji na vzrostných vrcholech prýtů následkem snížení teploty vody a zkracující se délky dne a nebo intenzity světla na konci vegetační sezóny, zpravidla na podzim. Turiony jsou kulovité, protáhlé nebo ploché útvary vzniklé mimořádným zkrácením internodií (mezičlánků) se silně pozměněnými, nejčastěji šupinovitými, zkrácenými listy. Jejich účelem je přežít zimní podmínky v nezamrzlé vodě na dně vodního biotopu, zatímco mateřský prýt se dříve nebo později oddělí a uhyne. Na jaře z nich v teplejší vodě podle druhu buď na hladině nebo u dna vyrůstají rychle nové rostliny, což jim dává fenologický náskok před jednoletými druhy klíčícími ze semen. Turiony jsou dormantní (tj. klidové), zásobní, vegetativní a mírně mrazuvzdorné přezimovací orgány obsahující chlorofyl, které slouží i k šíření těchto druhů na další lokality.

Turiony velmi hojného okřehku závitky mnohokořenné (*Spirodela polyrhiza*, Araceae – áronovité, dříve Lemnaceae – okřehkovité) se od ostatních druhů výrazně odlišují morfologicky i fyziologicky: jsou to ploché, tuhé, kulovité orgány široké 2-3 mm, které vznikají někdy už uprostřed léta hlavně následkem sníženého příjmu fosforu z vnějšího prostředí; vliv poklesu teploty je druhořadý. Mohou obsahovat až 70 % škrobu v sušině jako zásobní látku. Zásluhou zejména doc. K. J. Appenrotha z Univerzity v Jeně v Německu byly v posledních 40 letech v mnoha desítkách studií důkladně popsány procesy vedoucí u závitky k tvorbě turionů i jejich klíčení. Závitka se stala velice výhodným modelem studií tvorby i klíčení turionů zejména pro své malé rozměry a jednoduché pěstování ve sterilní kultuře in vitro i pro možnost srovnání mnoha geografických populací. Model je výhodný i tím, že přidání inhibičního fytohormonu kyseliny abscisové (ABA) do média k listům závitky (tzv. frondům) spolehlivě a rychle do týdne vyvolá tvorbu turionů a že tento proces může být zvrácen přidáním syntetického cytokininu kinetinu, který také sám navozuje klíčení zralých turionů. První transkriptomická studie amerických autorů (Wang a spol. 2014) s využitím tohoto modelu zjistila rozsáhlé změny exprese genů po indukci tvorby turionů závitky přidáním ABA ve srovnání s rostoucími frondy: v turionech celkem 208 genů jevilo více než čtyřikrát zvýšenou expresi ve srovnání s frondy, kdežto 154 genů mělo výrazně sníženou expresi. Studie ukázala, že tvorba turionů u závitky představuje na genové úrovni velmi komplexní vývojovou změnu srovnatelnou např. s kvetením. Nicméně, kvůli metodickým problémům s izolací kvalitní mRNA z turionů (vysoký obsah škrobu a třísloviny) byl vysoký podíl chybně složených, a proto neinterpretovatelných transkriptů.

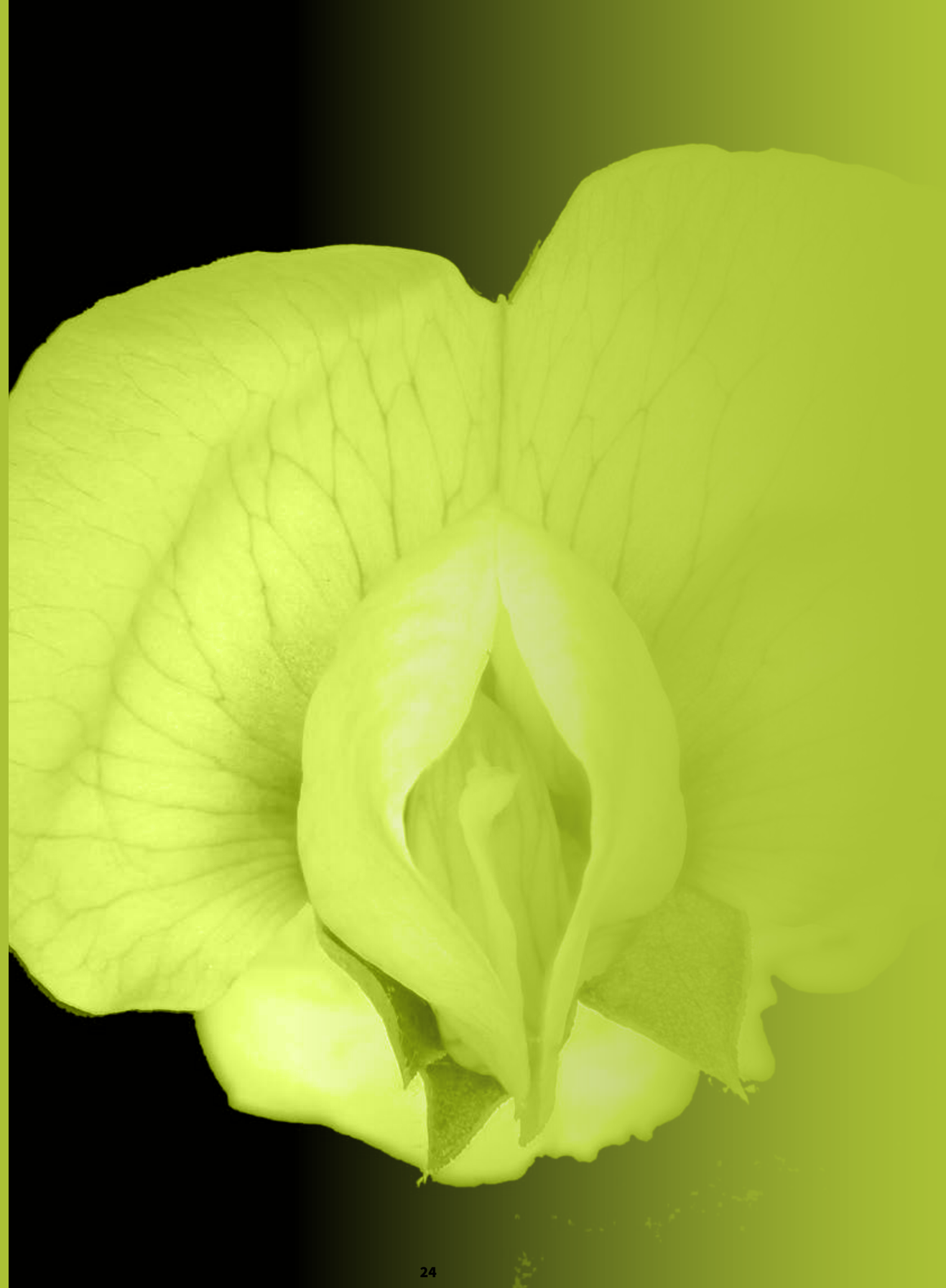
Buntora Pasaribu z Rutgers University v New Jersey v USA se spolupracovníky z různých amerických univerzit navázali na předchozí výzkum transkriptomiky tvorby turionů u závitky s tím rozdílem, že tvorbu turionů vyvolali ekologicky nedostatkem fosfátů v médiu a vypracovali zlepšený protokol izolace kvalitní RNA. U daného genotypu závitky autoři zjistili 17 397 transkriptů ve frondech anebo v turionech, z nichž 14 137 (tj. 81 % všech) se lišilo svou expresí navzájem ve frondech a turionech méně než 4×, tj. většina genů se exprimuje podobně v rostoucích frondech i v dormantních turionech. Geny s výraznou expresí (>8×) v turionech než ve frondech bylo možno rozřadit do 4 hlavních funkčních skupin: odpovědi na stres, sekundární a lipidový metabolismus, obranné odpovědi a vývoj a klíčení semen. Odpověd na stres byla hlavní a nejvýše zastoupenou skupinou silně exprimovaných genů v turionech a zahrnovala různé geny odpovídající na různé abiotické – chemické a fyzikální – podněty. Patří k nim např. geny zprostředkovávající regulační funkci ABA v turionech. Tato zjištění podporují význam regulačních drah zprostředkovaných pomocí ABA pro vývoj a funkce turionů a svědčí i o podobnosti s analogickými procesy dormance a klíčení semen u suchozemských rostlin. Další aktivované geny zprostředkovávají toleranci např. k anoxii, chladu a suchu. Druhá největší skupina silně exprimovaných genů v turionech se podílí na sekundárním metabolismu, syntéze fytohormonů, lipidů a jejich metabolismu. Jak lze očekávat ze zvýšeného obsahu antokyanu v turionech závitky, silně jsou exprimovány geny pro biosyntézu flavonoidů. Zvýšený obsah ABA v turionech je výsledkem až stonásobně vyšší hladiny transkriptů pro enzymy biosyntézy ABA. Výrazně aktivovány jsou také geny pro biosyntézu dvou dalších fytohormonů, kyseliny jasmonové a gibberelové (GA), což naznačuje, že oba fytohormony mají spolu s ABA významné úlohy v biologii turionů, a připomíná to jejich roli při klíčení semen. V této kategorii jsou také silně exprimované geny pro hromadění a katabolismus lipidů. Není překvapením, že byly zjištěny i velmi vysoké hladiny transkriptů pro tvorbu i rozklad škrobu, které se jeví být odpovědné za tvorbu velmi vysokého obsahu škrobu ve zralých turionech i jeho následný rozklad a využití při klíčení a růstu turionů. Třetí skupina silně aktivovaných genů v turionech se účastní obranných reakcí na biotické podněty – bakteriální a houbové patogeny i hmyzí herbivory – a podporuje zvýšenou

odolnost turionů proti těmto parazitům i herbivorům. Čtvrtá skupina silně exprimovaných genů v turionech ve srovnání s rostoucími frondy je dobře známa z vývoje (zrání) a klíčení semen. Některé tyto geny regulují odpověď ABA na zrání a embryogenezi semen, jiné regulují signální dráhy pro ABA a GA při klíčení semen u modelových rostlin, tvorbu slizu v osemení semen nebo dozrávání endospermu. Do této skupiny patří i dvojice silně aktivovaných genů pro enzymy štěpící mastné kyseliny a triglyceridy a zabezpečující klíčícím turionům dostatek respiračních substrátů a tím i energie. Naopak v turionech jako klidových orgánech s nízkou spotřebou energie byla u závitky zjištěna silně snížená hladina transkriptů spojených s dělením buněk, replikací DNA, tvorbou cytoskeletu, dělením mitochondrií a také s transportem iontů (zejména aniontů a nitrátu) a zpevněním buněčných stěn (suberinem). V neposlední řadě byla v turionech závitky zjištěna podstatně nižší exprese všech zástupců dvou jaderných genových rodin zajišťujících fotosyntézu: pro malou subjednotku klíčového enzymu ribuloso-bisfosfát-karboxylázy-oxygenázy (RubisCo) a proteiny světlosběrné antény (LHC). Odráží to známou skutečnost, že fotosyntéza turionů závitky je i v laboratorních podmínkách velmi nízká.

Epigenetická regulace zprostředkovaná vratnou metylací cytosinu v DNA je jedním z mechanismů zahrnutých ve vývojových přechodech u živočichů i rostlin. Sledováním metylace cytosinu v celém genomu závitky autoři potvrdili průkazné zvýšení metylace téměř ve všech částech genomu na přechodu od frondů k turionům. Avšak průkazná korelace mezi odlišně exprimovanými geny a úrovní metylace prokázána nebyla. Charakter metylace v turionech byl opět podobný tomu, který byl zjišťován během dozrávání a nástupu dormance u semen huseníčku (*Arabidopsis*). Soudí se, že metylace pomáhá zajistit, aby genom zůstal inaktivní během období dormance, a také brání možnému poškození DNA v dormantních turionech, které mohou být citlivější v tomto klidovém stádiu, kdy jsou enzymy opravující DNA méně aktivní.

Autoři ve své práci prokázali v dormantních turionech závitky na celogenomové úrovni výrazné změny (zvýšení i snížení) exprese tisíců genů, z nichž mnohé je možno jednoduše spojovat se známými morfologickými nebo biochemickými charakteristikami turionů, a tím je vysvětlit na genové úrovni. Nápadné podobnosti v genových expresích v turionech a semenech podávají důkaz, že klíčové regulace pro dozrávání a klíčení semen byly přesměrovány pro svoji novou funkci ve vývoji a klíčení turionů u vodních rostlin. [New Phytologist (2023): doi: 10.1111/nph.18941].

Lubomír Adamec



CO NOVÉHO V APLIKOVANÉM VÝZKUMU

Konsorcium pod vedením Ústavu experimentální botaniky získalo OP JAK zaměřený na moderní šlechtění plodin

"Prestižní výzkumný projekt, který získal Ústav experimentální botaniky AV ČR, přispěje ke vzniku plodin nové generace"

Ke šlechtění nových, odolných plodin s vyšším výnosem by měl napomoci důležitý projekt Ústavu experimentální botaniky Akademie věd ČR (ÚEB AV ČR) a jeho partnerů, který ve výzvě Špičkový výzkum podpořil Operační program Jan Amos Komenský (OP JAK).

ÚEB AV ČR se bude společně s dalším pracovištěm Akademie věd ČR a třemi českými univerzitami intenzivně věnovat výzkumu, jehož cílem je získat poznatky nutné pro šlechtění plodin nové generace přizpůsobených změně klimatu a vhodných pro udržitelné zemědělství.

Letošní výzvy OP JAK se účastnilo 74 projektů. Projekt ÚEB AV ČR *Nové poznatky pro plodiny nové generace* se umístil na pátém místě a v sekci zemědělských věd byl dokonce nejlépe hodnocený. Podle ředitele ÚEB AV ČR Jana Martince jde o velký úspěch. *„Projektu, který řeší naléhavou potřebu nových plodin přizpůsobených změně klimatu, se účastní osm špičkových vědeckých týmů nejen z našeho ústavu, ale i z Biofyzikálního ústavu AV ČR, Univerzity Karlovy, Masarykovy univerzity a Univerzity Palackého. Za významné považuji, že tyto výzkumné skupiny budou moci navzájem úzce spolupracovat, což nepochybně přinese unikátní výsledky a v konečném důsledku umožní pokrok ve šlechtění,"* uvedl Jan Martinec. Podle Romana Hobzy, vedoucího výzkumné skupiny z Biofyzikálního ústavu AV ČR, která se bude v projektu zabývat problematikou modifikace genomu, je přínosné být součástí interdisciplinárního týmu. *„Věříme, že se nám díky spolupráci s dalšími partnery podaří využít nové biotechnologické metody tak, aby mohly co nejdříve sloužit současnému zemědělství. Bude se jednat zejména o editování dědičné informace, které je základem nových šlechtitelských postupů. Bez cílené modifikace dědičné informace bude téměř nemožné vyšlechtit plodiny s vlastnostmi, které současné odrůdy postrádají,"* dodal Roman Hobza.

Na pětiletý projekt, který startuje v říjnu, obdrží výzkumné konsorcium 435 milionů korun. Jak zdůraznil hlavní řešitel, rostlinný genetik Jaroslav Doležel z ÚEB AV ČR, ve šlechtění odolných rostlin závodí vědci i šlechtitelé s časem. *„Zajištění dostatku potravin pro světovou populaci udržitelným způsobem v době změny klimatu je jednou z největších výzev současnosti. Už nyní zaznamenáváme extrémní výkyvy počasí. Negativní vliv na rostliny se bude postupně zvyšovat, což ohrožuje světovou produkci potravin. Je chvályhodné, že se Evropská unie snaží být progresivní a usiluje o snižování negativního vlivu zemědělství na životní prostředí. Podle neziskového sdružení Euroseeds mohou ale opatření Zelené dohody pro Evropu (European Green Deal) způsobit snížení rostlinné produkce až o 23 procent. Dohoda totiž předpokládá snížení používání chemikálií o 50 procent a hnojiv o 20 procent. Na tyto požadavky je nutné rychle reagovat vyšlechtěním nové generace plodin. Věřím, že k tomu náš projekt významně napomůže,"* vysvětlil olomoucký vědec a vedoucí projektu.

Konsorcium bude podle něj zkoumat rostliny, které mají pro Českou republiku zvláštní význam a pro které jsou k dispozici rozsáhlé genomické zdroje, jako je ječmen (*Hordeum vulgare*), řepka olejka (*Brassica napus*) a hrách (*Pisum sativum*), který dokáže vázat dusík v půdě a je zdrojem bílkovin. *„Kromě toho se budeme věnovat výzkumu rostlin, které jsou vhodné ke studiu konkrétních procesů důležitých pro šlechtění, jako jsou genomové konflikty, způsob rozmnožování a tolerance vůči stresu a interakce s patogenními půdními houbami. Podle potřeby využijeme ve výzkumu i modelovou rostlinu huseníček rolní (Arabidopsis thaliana),"* doplnil Jaroslav Doležel.

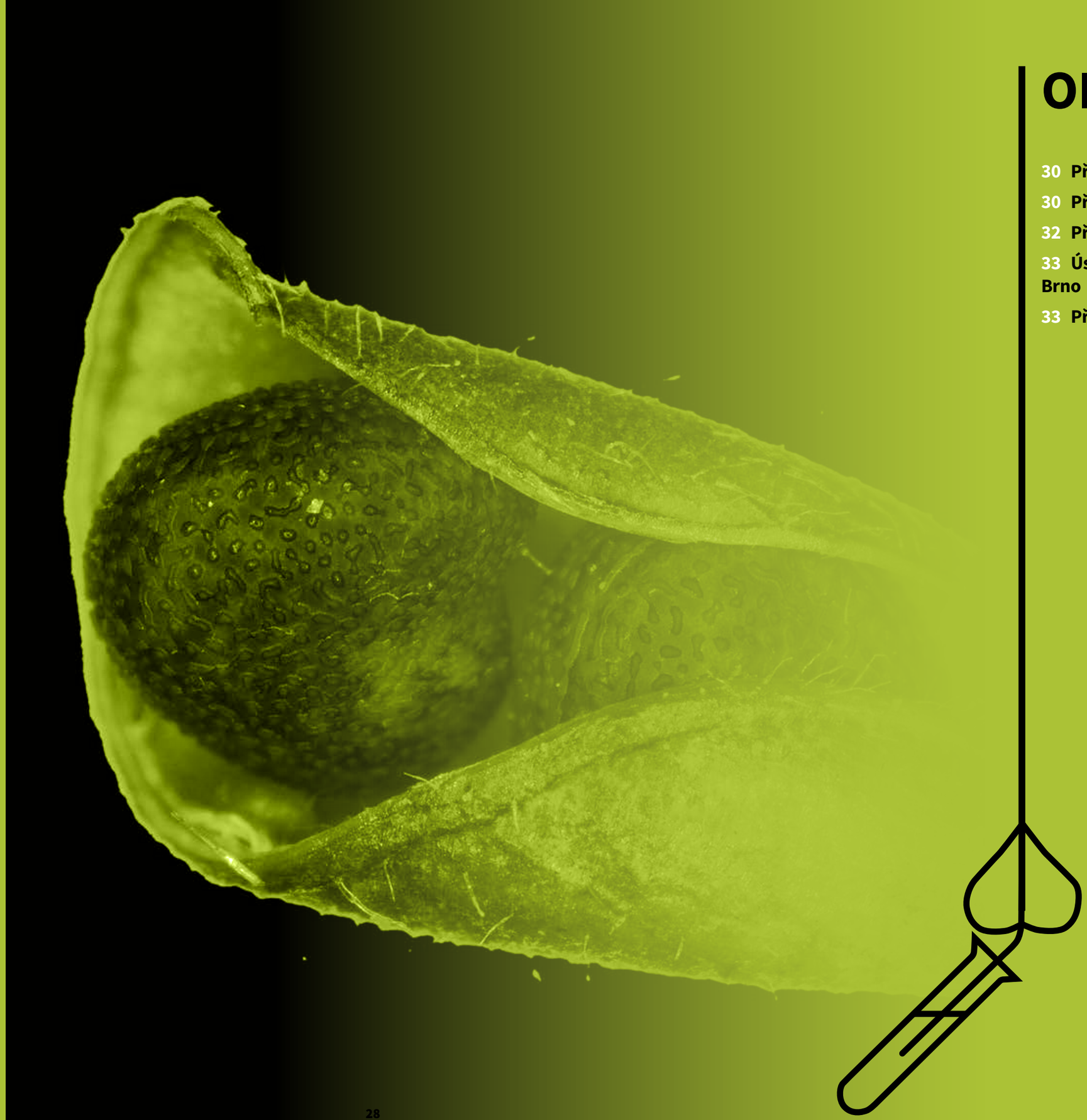
Operační program Jan Amos Komenský vyčlenil na podporu špičkových výzkumných projektů celkem 8 miliard korun. Cílem bylo podpořit excelentní výzkumné týmy, které zvýší zapojení českých výzkumných organizací do sítí mezinárodní spolupráce a v dlouhodobém horizontu přispějí také k posílení konkurenceschopnosti ČR.

Text i foto jsou přebrány z https://aplab.ueb.cas.cz/prestizni-vyzkumny-projekt-ktery-ziskal-ustav-experimentalni-botaniky-av-cr-prispeje-ke-vzniku-plodin-nove-generace/

Za ČSEBR gratuluji konsorciu k zisku grantu!

Martin Janda

Coby člen EU-SAGE vítáme posun v postojích Evropské komise k regulacím týkajících se plodin získaných novými technikami genového inženýrství.



OBHAJOBY

Obhájené disertační práce

Přírodovědecká fakulta Masarykovy univerzity, Brno

Morfologicko-anatomická studie kořenů rostlin rostoucích v prostředí s léčivy

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Nesteroidní protizánětlivá léčiva jsou jedna z nejpoužívanějších skupin humánních a veterinárních léčiv. Vzhledem k nedostatečné efektivitě jejich odstranění z odpadních vod se stávají běžnou součástí znečištění vodního prostředí. Jejich vliv na necílové organismy, rostliny, doposud nebyl zcela prozkoumán. Je známo, že rostliny jsou schopny léčiva přijímat, akumulovat, transportovat a transformovat. Doposud však nebyly dostatečně prostudovány změny, které léčiva u rostlin vyvolávají, včetně toho, zda se rostliny mohou jejich působení alespoň do jisté míry bránit. Cílem práce bylo posoudit změny v morfologii a anatomii kořenů v odezvě na vybraný stresový faktor, léčiva. Z výsledků provedených studií je zřejmé, že naproxen a produkty jeho transformace (především konjugáty s glukózou, malonovou kyselinou a s glutaminem a také jeho metylované formy) způsobily změny v architektuře kořenů. Při všech zatíženích (0,1-0,5-1-10 mg/L) naproxen negativně ovlivnil tvorbu laterálních kořenů, čímž se snížila absorpční plocha kořenového systému. Také bylo zjištěno navýšení podílu xylému ve stélé primárního kořene, které může souviset se snahou navýšit jeho transportní kapacitu. Redukce růstu kořenů a dřívější diferenciace pletiv včetně strukturních bariér (Caspary ho proužky, suberinová lamela) pod vlivem vyššího zatížení naproxenu může souviset i se zvýšením hladin fytohormonů (auxin a kyselina abscisová), indukcí oxidativního stresu, limitací protektivních mechanismů a narušením semipermeability buněčných membrán. Při nižším environmentálním zatížení u rostlin hrachu byly vytvářeny delší a tenčí kořeny, a díky aktivaci protektivních mechanismů (zvýšení antioxidační kapacity, antioxidačních enzymů), včasnějšímu formování Caspary ho proužků a zvýšené hladině kyseliny abscisové byly rostliny schopny se do jisté míry bránit toxickým účinkům léčiva.

Klíčová slova: léčiva, NSAID, naproxen, hrách, kořen, morfologie, anatomie, růstová analýza, oxidativní stres, antioxidační ochrana, Caspary ho proužek, suberinová lamela, auxin, kyselina abscisová

Publikace související s disertační prací:

Svobodníková L, Kummerová M, Zezulka Š, Babula P, Sendecká K (2020) Root response in Pisum sativum under naproxen stress: Morpho-anatomical, cytological, and biochemical traits. Chemosphere 258: 127411. https://doi.org/10.1016/j.chemosphere.2020.127411

Svobodníková L, Kummerová M, Zezulka Š, Martinka M, Klemš M, Čáslavský J (2022) Pea root responses under naproxen stress: changes in the formation of structural barriers in the primary root in context with changes of auxin and abscisic acid levels. Ecotoxicology 32:1-11. https://doi.org/10.1007/s10646-022-02613-8

Přírodovědecká fakulta Univerzity Karlovy, Praha

Buněčné determinanty distribuce auxinových přenašečů PIN v plazmatické membráně

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Asymmetric localization of auxin carriers has always attracted the attention of many scientists around the world. However, to address this topic while focusing on the plasma membrane (PM), requires advanced microscopy techniques and knowledge of biophysics and biology. This doctoral work bridges the two disciplines to contribute to our understanding of the dynamics and distribution of PIN-FORMED auxin carriers from tobacco (NtPINs) within the PM. I have developed a novel correlative light electron microscopy (CLEM) method using total internal reflection fluorescence microscopy (TIRFM) and advanced environmental scanning electron microscopy (A-ESEM). To my knowledge, this is the first effort to achieve a correlation between immunofluorescence and electron microscopy imaging of plant integral PM proteins. As I have shown, individual NtPINs are organized differently within the PM. Dynamic analyses that quantify individual nanodomains' diffusion rates allowed me to show that NtPINs have constraints behavior with different diffusion rates. I have investigated the role of the cell wall and cytoskeleton in the organization and dynamics of NtPINs. Using pharmacological treatments, I have shown that they differentially affect the mobility and organization of NtPINs within the PM. Complete removal of the cell wall shows significant changes in the distribution of the NtPINs nanodomain compared with pharmacological treatments. Finally, I performed a co-IP/MS2 analysis to identify NtPINs interactors. This helped me to summarize the knowledge and propose further scenarios and pathways for NtPINs protein-protein interactions that determine its dynamics and organization within the PM.

Charakterizace faktorů podílejících se na regulaci intracelulární dynamiky vybraných auxinových přenašečů

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Konzutlant / Consulting Supervisor: Dr.nat.techn. Katarzyna Retzer, Ústav experimentální botaniky AV ČR
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Plants are known to adjust the orientation of their organs, shoot and root, to ensure maximal energy generation and nutrient uptake, but also to avoid toxic growth conditions. Directional growth regulation depends on asymmetric plant organ growth and it is crucial to ensure plant survival. It is orchestrated on cellular level in concert with exogenous and intrinsic signals. Even though tropistic growth responses of plants were described by Darwin on macroscopic level already in 1880, now it is necessary to understand molecular mechanisms that underpin efficient modulation of directional plant growth. During my studies I focused on factors that modulate directional root growth regulation. The root is a complex, three-dimensional object, which continuously modifies its shape and growth path. Since the root needs to expand its surface to supply the plant with nutrients and water, it is important to understand how roots cope with changing growth conditions while exploring the soil. If the root cannot manage to grow through soil efficiently, mechanical impedance and lack of resources will also restrict shoot growth as well. Manifold signaling pathways coordinate the complex processes that underpin efficient root growth, including those modulated by phytohormones, sugars, flavonoids and other metabolites. Detailed mechanistic studies of how those signaling cascades are interconnected on subcellular level are still partly missing. Previously published studies showed that the key molecular players, which are responsible for the asymmetric distribution of auxin, often called a morphogen, delimit directional root growth and speed depending on growth conditions, including changing energy supply. On molecular level, auxin distribution is controlled through precise regulation of localization, abundance and activity of auxin carriers. My thesis consists of published articles that demonstrate on one side the importance of molecular regulation of a proteins involved in auxin distribution and thereby modulation of root growth. Furthermore, I showed how the inability to steer directional root growth in mutants with delimited auxin distribution in roots impairs the roots' ability to react to exogenous growth conditions. My research allowed to describe the importance of two highly conserved cysteines in the protein sequence of the auxin transporter PIN-FORMED 2, which determine the protein abundance and subcellular distribution. This results in different root waving pattern, which reflects the difficulty of the root to compensate deviation of root growth that occurs when a root is grown on the surface of agar supplemented growth medium. The intensive study of root growth dynamics further resulted in a better understanding of how cultivation conditions affect orchestration of directional root growth. Therefore, in my follow-up publications I described the relationship between exogenous signals and auxin dependent modulation of directional root growth by observing root growth responses of mutants lacking either a well-studied plasma membrane located auxin importers or exporters.

Přírodovědecká fakulta Univerzity Palackého, Olomouc

Identifikace genů podílejících se na opravách toxických DNA – proteinových vazeb u housenìčku rolního

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Opravné dráhy poškozené DNA jsou zásadní pro udržení stability genomu, a tak i pro životaschopnost buněk živých organismů. Tyto procesy se vyvinuly s cílem působit proti škodlivým lézím, které vznikají v buněčné DNA působením vnějších i vnitřních faktorů. Nejsou-li poškození DNA řádně opravena, představují potenciální zdroj buněčného stresu, mutací nebo, v nejzazším případě, vedou k buněčné smrti. Relativně málo prozkoumané typy lézí představují DNA-proteinové vazby a jejich opravné dráhy. Tato poškození vznikají v situaci, kdy je protein zachycen na DNA a vytvoří s ní stabilní kovalentní vazbu. Vzniklý komplex představuje fyzikální bariéru pro metabolické procesy probíhající na DNA jako je např. replikace či transkripce.

V této práci jsme jako první ukázali, že cytidinový analog zebularin, který je znám hlavně pro své DNA hypometylační účinky, rovněž indukuje v živých organismech stabilní kovalentní vazbu mezi DNA a enzymem odpovědným za DNA metylaci během replikace. Dále jsme pak zamýšleli odhalit molekulární mechanismus opravy zebularinem indukované DNA-proteinové vazby u housenìčku rolního. Pomocí dopředného genetického screenu jsme vyselektovali soubor kandidátních rostlin, u nichž jsme následně identifikovali kandidátní geny zapojené do opravy daného poškození pomocí tzv. mapování sekvenováním. Ze všech zmapovaných kandidátů jsme nakonec vybrali geny kódující podjednotky multiproteinového komplexu, kondenzin II, pro charakterizaci jeho role v kontextu organizace chromatinu a opravy dané léze indukované zebularinem. Přesný mechanismus však nadále zůstává předmětem našeho zkoumání.

Modulace růstu a vývoje rostlin cytokininovými analogy

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Předmětem disertační práce bylo studium aplikace vybraných sloučenin, odvozených od rostlinných hormonů cytokininů, u modelové rostliny Arabidopsis thalina v kombinaci s různými faktory biotického stresu. Především se jednalo o studium rostlin infikovaných Verticillium longisporum po ošetření látkou INCYDE, která působí jako inhibitor cytokininové degradace. V průběhu studia byly analyzovány hladiny genů SAG12, SAG13 a WRKY53, odpovědných v senescenčním programu studovaného organismu z důvodu nastolení brzké senescence patogenem (chloróza, nekróza). Paralelně k tomuto projektu byl studován patosystém Arabidopsis thaliana- Plasmodiophora brassicae v kombinací s ošetřením studovaných rostlin látkou PI-55, která působí jako cytokininový antagonista na úrovni receptorů pro cytokininové vnímání. Záměrem této studie byla komplexní analýza genů cytokininového metabolismu a také analýza fotosyntetických parametrů.

Ústav biologie rostlin, Agronomická fakulta, MENDELU Brno

Fyziologické a anatomické odpovědi rostlin hrachu setého (Pisum sativum L.) na organické polutanty

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Školitel: RNDr. Ing. Marek Klemš, Ph.D.
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Životní prostředí je znečištěno širokým spektrem chemických látek antropogenního původu. Protože rostliny žijí přisedlým způsobem života s velmi omezenou až nulovou možností aktivního pohybu a jsou schopny absorbovat tyto škodliviny z prostředí prostřednictvím kořenů či listů, může přítomnost polutantů v prostředí nepříznivě ovlivnit jejich metabolismus a růst. Tato práce se zabývá aktuálním tématem studia účinků polycyklického aromatického uhlovodíku fluorantenu (FLT) a herbicidu flurochloridonu (FLC) na fotosyntetické dispozice a růst rostlin hrachu (*Pisum sativum* L.). Uvedené výsledky ukazují, že 5 µM FLT a/nebo FLC negativně ovlivňují fotosyntetický systém na všech jeho úrovních, což v konečném důsledku vede až k inhibici růstu ovlivněných rostlin. Poškození způsobená oběma sloučeninami byla pozorována jako pokles fluorescenčních parametrů chlorofylu, v případě FLT byl detekován pokles NPQ, Rfd a QY max. až o 50 % ve srovnání s kontrolou, u variant ošetřených FLC byl tento pokles prakticky až k nulové hodnotě. Zjištěná snížení těchto parametrů, odpovídala rovněž detekovanému poklesu hladin fotosyntetických pigmentů, zvláště pak chlorofylu b a karotenoidů. Poškozením primárních fotosyntetických procesů odpovídala rovněž zvýšená hladina MDA v listech rostlin ošetřených FLC, u kterých byla také pozorována tvorba nefunkčních plastidů. Negativní účinek FLC i FLT se projevil i změnami v anatomické struktuře listů, a sice reorganizaci bifaciální stavby mezofylu. Tato modifikace byla výrazně patrná především u rostlin varianty FLC. V pozdějších růstových fázích byly v listech rostlin ošetřených FLC zjištěny rovněž až čtyřnásobné hladiny ACC, prekurzoru etylénu, ve srovnání s kontrolou. Negativní účinek obou látek se projevil na inhibici růstu kořenů i nadzemních částí rostlin, a také v podobě změny pigmentace listů. Rostliny ošetřené FLT měly chlorózami postiženo asi 60–75 % celkové asimilační plochy. V případě aplikace FLC bylo postiženo až 80 % listové plochy. Obě látky v koncentraci 5 µmol/l mají tedy na rostliny jednoznačně negativní účinek. Přestože se v případě FLT jedná o koncentraci odpovídající nižšímu až střednímu zatížení životního prostředí touto látkou, lze konstatovat, že jeho působení mělo na většinu hodnocených parametrů méně negativní dopad než v případě FLC, u kterého aplikovaná koncentrace odpovídá pouze 0,1% množství běžně aplikovaného v zemědělství.

Přírodovědecká fakulta Ostravské univerzity, Ostrava

Vliv klimatických faktorů na expresi genů podílejících se na produkci fenolových látek a antioxidační ochraně rostlin

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Vedoucí práce: doc. RNDr. Vladimír Špunda, CSc.
Konzultant: Mgr. Jakub Nezval, Ph.D.
Pracoviště: Department of Physics, Faculty of Science, University of Ostrava

Abstrakt
Fenolové sloučeniny jsou důležitou skupinou různorodých sekundárních metabolitů sUV-stínícími a antioxidačními vlastnostmi. Jejich množství i profil se mohou dynamickyměnit v závislosti na okolních podnětech (např. vysoká ozářenost, teplota, koncentrace CO_2). Tyto změny jsou částečně způsobeny změnami enzymatické aktivity proteinů (Duke and Naylor, 1974; Zhan et al., 2020) podílejících se na jejich biosyntéze, ale takéexpresí genů kódujících vlastní biosyntetické enzymy, které jsou schopny vytvářet fenolické sloučeniny, a přispívají tak i k pozorovaným změnám v celkovém obsahu fenolových látek. Na katedře fyziky je jedním z hlavních směrů výzkumu pochopení vlivu fotosynteticky aktivního záření na akumulaci fenolových sloučenin v nepřítomnosti UV radiace a úlohy těchto látek v ochraně rostlin (fotosyntetického aparátu) před oxidativním stresem. Dosavadní výzkum byl založen na analýze fenolových látek a také na odhadu různých funkčních vlastností odhadujících stav asimilačního aparátu pomocí

biochemických a biofyzikálních metod, avšak s tématem regulace genové exprese a molekulárně biologickými analýzami a technikami nebyly na pracovišti zkušenosti. Aby tedy bylo možné provádět komplexnější výzkum zahrnující jak metabolomiku, tak transkriptomiku, bylo nutné zavést, upravit a dále optimalizovat metodu RT-qPCR včetně izolace celkové RNA, ošetření DNAzou a reverzní transkripce na Katedře Fyziky. V předkládané práci shrnuji jak publikovaná, tak dosud nepublikovaná data z experimentů zabývajících se především stimulačními účinky modrého světla na expresi vybraných genů souvisejících s antioxidační ochranou a syntézou fenolových sloučenin. Kromě toho byly provedeny také experimenty zabývající se interaktivními účinky teploty a spektrální kvality jak na expresi genů, tak na akumulaci fenolických sloučenin. Pro další pochopení regulace genů zapojených do biosyntézy fenolových látek jsem začala studovat obecné epigenetické mechanismy řízení genové exprese, včetně struktur tvořících kvadruplexy ve spolupráci s kolegy z Katedry Biologie a Ekologie OU a Biofyzikálním Ústavem Akademie věd České republiky.

Structural and functional plasticity of thylakoid membranes. Role of lipid polymorphism

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Abstract

Our knowledge about the structure and function of thylakoid membranes (TMs) of higher plant chloroplasts is well established, but some important research areas remain to be explored. Albeit the energization of TMs relies on its bilayer organization, about half of the TM lipids are monogalactosyldiacylglycerol (MGDG) molecules, which are non-lamellar (or non-bilayer) lipids. It is known that, in model systems, the activity of the water-soluble photoprotective violaxanthin de-epoxidase (VDE) TM luminal enzyme depends on the presence of a non-lamellar phase. Using 31P-NMR spectroscopy, it has also been shown that isolated fully functional spinach TMs contain, in addition to the lamellar (bilayer) phase, three non-lamellar lipid phases. It was proposed that these non-lamellar phases contribute to the structural plasticity of TMs and safe-guard their high protein-to-lipid ratio; also, tentative assignments were put forward on the origin of the non-lamellar lipid phases. However, numerous questions remained to be answered about the structural and functional roles of the non-lamellar lipid phases in TMs.

In this thesis, I focused on three main problems: (1) general properties of the polymorphic phase behavior of isolated plant TMs, (2) structural entities responsible for the origin of different lipid phases and (3) their physiological significance. To elucidate these questions, we performed 31P-NMR spectroscopy on isolated TMs from spinach, pea, spruce and Arabidopsis thaliana, and granum and stroma sub-chloroplast membrane particles isolated from spinach, as well as isolated plastoglobuli; applied mathematical deconvolution and carried out saturation transfer experiments. The lipid polymorphism of TMs was modulated mainly by varying their physiological state and physico-chemical environments, and by applying lipases and proteinases. The molecular organization and the photochemical activity of TMs were characterized by using optical spectroscopy techniques and biochemical and analytical tools; the ultrastructure of different preparations was portrayed by electron microscopy techniques and small-angle X-ray scattering.

We (1.1) established that the phase behavior of different higher plant TMs largely resemble to each other; (1.2) provided a mathematical deconvolution of the 31P-NMR spectra, which on spinach TMs revealed that only about 37 ± 7 % of the bulk lipid molecules are found in the lamellar phase, and the non-lamellar phases HII and different isotropic phases are represented by 42 ± 10 % and about 15, 3 and 2 %, respectively (n = 14); and (1.3) the spectral distributions, used for the mathematical deconvolution, were confirmed by saturation transfer experiments.

We have shown that (2.1) contribution of plastoglobuli to the lipid polymorphism of isolated TMs is negligible; (2.2) the lipid polymorphisms of isolated granum and stroma TMs are almost identical with that in intact TMs; (2.3) VDE is present in these sub-chloroplast preparations; (2.4) the non-lamellar lipid phases are to be found outside the protein-rich areas which contain the photosynthetic pigment-protein complexes; (2.5) in particular, the isotropic phases are proposed to originate from membrane fusions and junctions as well as from the association of lipid molecules with VDE and/or with other lipocalins, in accordance with earlier tentative assignments, and the HII phase may originate from lipids encapsulating stroma-side proteins or polypeptides.

Regarding the physiological significance and dynamics of the lipid phase behavior of TMs (3.1) we have demonstrated that the temperature- and pH-induced changes are largely reversible: low pH and elevated temperatures have been shown to enhance the isotropic phases and to accelerate the relaxation of the proton-motive force; nevertheless, (3.2) the enhancement of the isotropic phases has turned out to be correlated with the activity of VDE, the low luminal pH-activated enzyme of the xanthophyll cycle; as well as (3.3) with the photoprotective mechanism of non-photochemical quenching (NPQ) of the singlet excited state of chlorophyll a.

These data are interpreted within the frameworks of the Dynamic Exchange Model (DEM) of TMs, which is based on the co-existence of different lipid phases and their dynamic equilibria that had been proposed to respond to changes in the physico-chemical environment of TMs and the physiological processes in the chloroplast.

VĚDECKÁ SETKÁNÍ A AKCE SPONZOROVANÉ ČSEBR

Minisymposium „Hot Topics and Advances in Plant Cell Biology“

Dne 3. 5. 2023 se konalo v Praze minisymposium u příležitosti 65. narozenin profesora Viktora Žárského, dlouholetého vedoucího Laboratoře buněčné biologie Ústavu experimentální botaniky Akademie věd České republiky. Symposium se uskutečnilo v prostorách Ústavu experimentální botaniky (ÚEB), který celou akci zaštítil. Pozvání organizátorů přijali také přední rostlinní buněční biologové z Belgie, Francie, Izraele, Německa a Rakouska. Účast zahraničních vědeckých pracovníků byla podpořena Českou společností experimentální biologie rostlin.

Viktor Žárský s darem od kolegů Jenny Russinové a Daniela van Damma z VIB Gent, kteří měli na minisymposiu příspěvek v podobě přednášky.

Před většinově mladým publikem, které do posledního místa zaplnilo přednáškový sál ÚEB, zazněly prezentace ukazující nejnovější trendy a poznatky výzkumu signálních drah rostlinných fytohormonů brassinosteroidů, auxinu a kyseliny abscisové a jejich rolí v buněčné morfogenezi (Jenny Russinova – VIB, Belgie, Claus Schwechheimer - TUM, Německo, Shaul Yalovsky - Tel Aviv University, Izrael). Buněčné polarity, váčkového transportu a autofagie, dalších vědeckých zájmů jubilanta, se týkaly i přednášky Daniëla Van Damme (VIB, Belgie), Ivana Kulicha (IST, Rakousko), Sébastiena Mongranda (University of Bordeaux, Francie), Fatimy Cvrčkové (Univerzita Karlova, ČR), a Yasina Dagdase (Gregor Mendel Institute, Rakousko). Během přestávek i po příspěvcích měli účastníci možnost seznámit se s kolegy, dopřát si kávu a občerstvení a zároveň diskutovat jednotlivé přednášky. Že vědecké diskuze mohou mít i bouřlivý charakter, ukázala poslední přednáška Františka Balušky (Bonn University, Německo) o konceptu vnímající eukaryotické buňky. Setkání bylo zakončeno závěrečnými poznámkami Viktora Žárského, po kterých následovala všeobecná diskuse, krátká recepce na terase ÚEB a závěrečná večeře. Miniysymposium tak přineslo nejen aktuální poznatky z oboru buněčné biologie, ale rovněž příležitost k setkání a diskusi s předními evropských oborníky v této oblasti biologie rostlin.

Martin Potocký

Komentář účastníka k minisymposiu

Organizátoři nejprve zamýšleli dvoudenní akci, ale myslím, že změna k jednodennímu formátu této akci slušela. Spouštěčem a motivací k minisymposiu bylo životní jubileum významného rostlinného fyziologa Viktora Žárského. Bylo (a vždy je) milé vidět, jak vynikající zahraniční vědci jsou ochotni účastnit se akce k takové příležitosti a přijet díky tomu přednášet k nám do ČR. V tomto případě konkrétně na Ústav experimentální botaniky AV ČR v Praze.

Martin Janda

Botanik a politik Bohumil Němec v kontextu

Konference ke 150. výročí narození
8.–9. června 2023

PhDr. BOHUMIL NĚMEC,
univ. profesor, Praha.

Rozumět vlastní minulosti – to je důležité pro jednotlivce, pospolitosti i vědecké obory. Letošních 150 let od narození profesora Bohumila Němce (1873–1966) bylo nepřehlédnutelným podnětem k uspořádání setkání k jeho odkazu. Katedra experimentální biologie rostlin (zastoupená Viktorem Žárským) a Katedra filosofie a dějin přírodních věd (zastoupená Tomášem Hermannem) Přírodovědecké fakulty UK uspořádaly pod záštitou rektorky Prof. Mileny Králíčkové ve dnech 8. a 9. června 2023 konferenci „Botanik a politik Bohumil Němec v kontextu“. Profesor Němec bezesporu patří nejvýznamnějším českým vědeckým osobnostem 20. století. Stojí nejen na počátku české a slovenské, ale také světové experimentální biologie rostlin zaměřené na studium buněčných základů vývojových a fyziologických procesů. Zároveň mimořádně aktivně vstupoval do akademického a politického veřejného života v nově založeném poválečném Československu. Proto byl první den konference zaměřen na jeho veřejné působení a probíhal česky, během druhého dne pak mladší generace špičkových badatelů pracujících u nás předvedla v angličtině, kam se široká tématika biologie rostlin, otevřená u nás působením profesora Němce, dostala v současnosti. V krátké zprávě není možno obsáhnout všechny příspěvky a proto vybíráme.

Pro většinu účastníků konference (proměnlivě kolem padesáti) byla překvapením a přínosem už úvodní přednáška Doc. Tomáše Hoskovce (Předseda Pražského lingvistického kroužku, přednáší na Filozofické fakultě Jihočeské univerzity) „Věda a zednářství v meziválečném Československu“ ve které předvedl jak zednářství od 18. století také v našich zemích významně a dlouhodobě přispívalo k rozvoji věd, svobodného bádání a svobody projevu. Prof. Němec byl jeho součástí, i když v době svého vrcholícího angažmá na universitě a v politice měl v tomto směru omezené časové možnosti. Prof. František Baluška (Universita v Bonnu) ukázal, jak hnutí rostlinné neurobiologie čerpá z podnětů prof. Němce – založených nejen na jeho práci v rostlinné cytologii (př. návaznost cytoplasmatických provazců v sousedních buňkách přechodné zóny kořene), ale také na jeho osobních kontaktech se zakladatelem rostlinné elektrofyziologie – Jagadishem Chandrou Bosem (1858 – 1937). Jedním z vrcholů prvního dne konference byl příspěvek Dr. Michaely Zemkové (Katedra filosofie a dějin přírodních věd) a Dr. Yvonny Fričové (nakladatelství Titanic) „B.N., zejména Duše rostlin, a Alberto Vojtěch Frič“, ve kterém ukázaly jak Alberto Vojtěch Frič jako nezávislý botanik-samouk, expert na kaktusy kritizoval akademický svět školské botaniky včetně Prof. Němce a zároveň narážel na jeho nepřízeň, která také komplikovala Fričovy publikační aktivity. S prof. Němcem vedl např. polemiku kolem apikální dominance a vzniku několika vzrostných vrcholů po ztrátě původního; v ní proti Němcovi ukázal, že je z hlediska přežití rostliny pochopitelné, že zpočátku se aktivuje několik úžlabních pupenů/vzrostných vrcholů. Přes tyto spory AV Frič uvítal publikaci Němcova spisu „O duši rostlin“ (1937 - ve skutečnosti kniha pojednává o životě rostlin) s nadšením. Prof. Alexandr Lux, také na základě osobních vzpomínek, představil Prof. Němce jako zakladatele slovenské experimentální biologie po druhé světové válce. Vedle učitelského působení na mladé slovenské badatele např. díky podpoře syna Pavla Němce získal pro Botanický ústav Univerzity Komenského v Bratislavě budovu bývalého lihovarského výzkumného ústavu. Prof. Němec byl nejen velmi aktivní popularizátor vědy v časopisech Živa a Vesmír, ale, jak připomněl ve své přednášce Dr. Tomáš Hermann, také zakladatel odborného mezinárodního časopisu v oboru experimentální botaniky „Biologia Plantarum“ (1959).

Druhý den konference zaměřený na současnou experimentální botaniku zahájily příspěvky Dr. Ivana Kulicha (ISTA, Klosterneuburg) a Dr. Matyáše Fendrycha (Katedra experimentální biologie rostlin PřF UK), kteří přímo dále rozvíjejí původní Němcův objev účasti přesýpavého škrobu v kořenové čepičce na gravitropické reakci kořene (1900). Časné fáze reakce se účastní bílkoviny, které jsou přednostně lokalizovány na kontaktních doménách škrobových zrn (vlastně plastidů) a cytoplasmatické membrány, spouštějí celou škálu reakcí (vápníkovou signalizaci, změny dynamiky endocytózy) které jsou součástí rychlé auxinové signalizace závislé na cytoplasmatickém receptoru auxinu AFB1. Dr. Hana Šimková (ÚEB AVČR, Centrum Haná, Olomouc) představila nově získané poznatky o molekulárních detailech 3D uspořádání genomu/chromatinu v jádrech rostlin a Dr. Iva Mozgová (Ústav molekulární biologie rostlin AVČR, České Budějovice) ukázala jak se klíčové represory genové exprese bílkoviny rodiny

polycomb podílejí vývojových regulacích závislých na celkovém metabolismu – zvl. fotosyntéze. Dr. Albert Cairo Calzada (CEITEC, Masarykova univerzita, Brno) na příkladu regulace dynamiky meióze ilustroval současné velmi žhavé téma fázových přechodů bílkovinných komplexů do specifických nemembránových kompartmentů v buňkách eukaryot. V závěrečné detektivní přednášce („How lovely plants became merciless killers“) celé konference Doc. Andrej Pavlovič (Katedra biofyziky, Univerzita Palackého, Olomouc) osvětlil opakované nezávislé (a konvergentní) případy evoluce masožravosti u rostlin a účast jasmonátové signalizace na jejich molekulárních mechanismech.

Všechny příspěvky na konferenci vyvolaly živé diskuse ať už přímo v sále či o přestávkách a večerní recepci nedaleko jeho busty na budově kateder ve Viničné 5. Umožnily velmi plasticky nahlédnout osobnost profesora Bohumila Němce ve vědecké i společenské sféře, ale také aktuální život vědeckých problémů, které před více než sto lety nastolil.

Odkaz na stránky konference s celým programem - https://sites.google.com/natur.cuni.cz/bn150/domovsk%C3%A1-str%C3%A1nka

Komentář účastníka ke konferenci “Bohumil Němec v kontextu”

Účastnil jsem se pouze první den, který nebyl tolik ve znamení experimentální biologie rostlin. Pro nás experimentální biology to byla netradiční akce, neboť první den konference byl ve znamení přednášek a příspěvků týkajících se politické či administrativní a organizační činnosti prof. Němce přednesených především kolegy z jiných vědních disciplín. I když i na náš obor se první den dostalo. Pro mě to bylo oživující a pomohlo mi to vystoupit z naší „experimentálně rostlině-biologické bubliny“. Skvělé bylo, že se organizátorům podařilo přimět k řeči i prof. Luxe ze Slovenska, který prof. Němce, díky svým rodičům, osobně znal. Z mého pohledu je škoda, že se nenaplnila celá aula, protože by si to osobnost profesora Němce zasloužila. Ale i tak, když vezmu v úvahu termín konference (počátek června), byla účast solidní. Jsem moc rád, že ČSEBR na tuto chvályhodnou událost přispěla.

Martin Janda

The 2nd Czech Plant Nucleus Workshop 2023 v Brně

The Czech Plant Nucleus Workshop (CPNW) je událostí, kde se setkávají vědečtí pracovníci a studenti zabývající se dědičnou informací rostlin. Workshop je zaměřen nejen na studium buněčného jádra a chromozomů, ale hlavně je jedinečnou příležitostí pro navázání kontaktů a spolupracím mezi účastníky napříč Českou republikou. Letošní ročník CPNW2023 byl pořádán v místě, které je pro rostlinné biology zvláště důležité, a to v Augustiniánském opatství v Brně v prostorách, kde Johan Gregor Mendel prováděl své pokusy s rostlinami a kde objevil základní zákony dědičnosti. Vědci tak měli ojedinělou příležitost prezentovat a diskutovat své výsledky jak v historických prostorách Mendelova Refektáře, tak v prostorách nově vybudovaného Skleníku, který stojí přímo v místech, kde opat Mendel prováděl své pokusy s hrachem či fuchsiemi.

Vědecký program byl naplánován organizačním výborem tvořeným Petrou Procházkovou Schrumpfovou (Masarykova univerzita, Brno), Alešem Pečinkou (Ústav experimentální botaniky AV ČR, Olomouc), Lukášem Fisherem (Univerzita Karlova, Praha), Evou Dvořák Tomaštíkovou (Ústav experimentální botaniky AV ČR, Olomouc) a Ivou Mozgovou (Biologické centrum AV ČR, České Budějovice). Setkání, stejně jako během předchozího ročníku, který se konal v roce 2021 v Olomouci v Pevnosti poznání, trvalo dva dny. Účastníci měli možnost diskutovat své výsledky nejen během vlastních přednášek, ale také během obědů a několika přestávek na kávu pořádaných v zahradě opatství či plakátové sekce konané v nově otevřeném Skleníku. Mnoho neformálních diskusí proběhlo také během večerního posezení v restauraci U Tomana přímo v centru Brna.

Přednášky byly rozděleny do pěti tematických sekcí

- Údržba konců chromozomů
- Organizace a regulace genomů obilovin
- Funkce jader rostlin a organizace chromatinu
- Jaderné proteiny ve vývoji rostlin
- Oprava poškození DNA

Workshop byl zahájen přednáškou profesora Jiřího Fajkuse (CEITEC, Masarykova univerzita, Brno), který v ní shrnul nejen nejnovější poznatky o koncích chromosomů (telomerách), ale také vyzdvihnul důležitost výzkumu zvláště na rostlinách. Prof. Fajkus zdůraznil význam rostlinné biologie nejen při objevu dědičnosti J. G. Mendelem (1865) ale také popisu samotných buněk (R. Hook, 1665) či chromosomů (K. H. Nägeli, 1842).

Celkově bylo předneseno 33 přednášek a bylo prezentováno 24 posterů. Workshop přilákal široké a různorodé publikum napříč mnoha vědeckými institucemi nejen z České republiky (např. Ústav experimentální botaniky AV ČR, Masarykova univerzita, Univerzita Karlova, Mikrobiologický ústav AV ČR, Biofyzikální ústav AV ČR, Biologické centrum AV ČR), ale i ze zahraničí (Freie Universität Berlin, Berlín, Německo).

Značnou část účastníků tvořili mladí vědci, včetně postgraduálních studentů a postdoktorandů, kteří měli jedinečnou příležitost osobního setkání s mnoha renomovanými a zkušenými vědeckými pracovníky. Tito vědečtí pracovníci hodnotili postery a přednášky postgraduálních studentů a postdoktorandů, přičemž ti nejlepší prezentující byli oceněni cenou. Ocenění za nejlepší přednášku si odnesla Kateřina Kaduchová (Ústav experimentální botaniky AV ČR, Olomouc) a cenu za nejlepší poster obdržela Pavla Novotná (Biofyzikální ústav AV ČR, Brno). Kompletní seznam prezentujících a podrobnosti o workshopu jsou k dispozici na webových stránkách workshopu (https://olomouc.ueb.cas.cz/cpnw). Studentské ceny byly podpořeny finančním oceněním sponzorovaným společností ČSEBR, nicméně dík patří také ostatním sponzorům, bez nichž by akce nebyla realizovatelná ve stejném rozsahu (abecedně: BioTech, EastPort Lifescience, Eppendorf, KRD, Labdeers, LifeM, PSI, Sarstedt, Scientifica, Schoeller Instruments).

Na závěr workshopu měli účastníci možnost prohlídky prostor jak Mendelova muzea, tak prostor knihovny, včelínu, gotické baziliky a dalších prostor, kde J. G. Mendel strávil většinu svého vědeckého života.

Petra Procházková Schrumpfová

“Všechny tři akce jsou skvělé reprezentativní ukázky typů akcí, které jsou naší společností s velkou radostí a hrdostí podporovány.”

Martin Janda

Dalšími akcemi významně podpořenými (v tomto případě spolupořádané) ČSEBR jsou 17th Student Days in Plant Biology CS 2023 a Methods in Plant Sciences 2023. O těchto akcích v této sekci nepíšeme neb abstrakt booky k těmto konferencím najdete ve “druhé polovině” tohoto čísla Bulletinu.

Biologia Plantarum – jak dál?

Při úvahách, jak dál s našim mezinárodním časopisem Biologia Plantarum (BP) je třeba se napřed ohlédnout – abychom neztratili ze zřetele východisko a kořeny. Časopis byl založen skupinou mladých kolegů z Biologického ústavu AVČR (po válce Ústřední ústav biologický) za zásadní ideové podpory prof. Bohumila Němce ještě tři roky před vznikem Ústavu experimentální botaniky (ÚEB) v roce 1959. Prvním a dlouholetým šéfredaktorem byl Dr. Bohdan Slavík. Negativním důvodem vzniku odborných časopisů u nás v období pozdního stalinismu byla skutečnost, že bylo obtížné publikovat v časopisech za železnou oponou a zároveň odborná úroveň naší biologie postižené vlnou lysenkismu byla právě v experimentálních oborech velmi nevyrovnaná. Pozitivním důvodem byl nepochybný rozvoj mnoha oborů experimentální biologie rostlin v Biologickém ústavu na Flemingově náměstí – a vznik samostatného časopisu předznamenal (a možná položil ideové základy) emancipaci oboru experimentální botaniky, která vyústila v založení ÚEB. Jistě také díky mezinárodní autoritě prof. Němce si BP od začátku získala zřetelnou důvěru mezinárodní komunity pracující v oboru, což se projevilo také tím, že od samého začátku byly publikovány články autorů ze všech kontinentů. BP nepochybně zásadně pomáhala internacionalizaci výsledků vědeckého úsilí v experimentální botanice u nás za situace, kdy okolnosti spojené se vznikem BP trvaly ve formě normalizace až do roku 1989. V následujícím období se podmínky radikálně proměnily – nejen, že publikovat v zahraničních časopisech bylo jednodušší, zdravé ale také žádoucí. A to i kvůli scientometrii, která začala podstatně ovlivňovat rozdělování finančních prostředků. Publikace v BP začaly být považovány za méně významné – bylo a je to bráno jako projev neschopnosti prorazit v zahraničí. Tato okolnost vedla k tomu, že BP v následujícím období přestala být v centru pozornosti české i slovenské komunity experimentálních botaniků a s jistou setrvačností plnila úlohu úctyhodného, nicméně méně vlivného, mezinárodního časopisu zvláště v oboru klasické fyziologie rostlin. Pro dnešní úvahy o BP je důležité, že období charakterizované raketovým rozvojem molekulárně biologických přístupů, vznikem stovek nových mezinárodních časopisů a jejich elektronizací, rozvojem scientometrického hodnocení vědecké práce ústí do nejistoty jak dál. Praxe ukázala, že mnoho zvláště nových vydavatelů a časopisů bylo a je účelově zařízeno na zvyšování vlastních IF a citovanosti na úkor kvality publikování a fair play – publikační etiky. BP si do dneška přes značné kolísání IF/citovanosti, a tedy vlivu na mezinárodní experimentální botaniku, zachovala nezpochybnitelné solidní standardy odborného hodnocení zaslaných rukopisů a ediční práce. Má tak smysl usilovat nejen o zachování, ale také oživení BP a jejího odborného vlivu v situaci nejistot silně se transformujícího světa vědeckého publikování. Pro nejbližší období bude úkolem zefektivnit časově zpracování zaslaných rukopisů – mimo jiné také aktivnějším zapojením členů redakční rady (také zavedením editorských a příp. oponentských konzultací k zaslaným rukopisům) a také co možno okamžitým publikováním přijatých autorských verzí rukopisů. V průběhu příštího období by mělo dojít k přirozené obměně a doplnění členů redakční rady. Pro posílení zviditelnění BP, a tedy také její citovanosti, bude třeba proniknout do databáze PubMed – a kroky v tomto směru jsou již podnikány. S tím bude spojena mírná úprava struktury a vizuální stránky a také „scope“ - oblastí bádání v biologii rostlin ve kterých BP publikuje. Jako nový typ článku vedle stávajících žánrů bude zvážen formát „Hypotéza“; – jak upozorňuje Paul Nurse hromadí se ve vědě výsledky a scházejí myšlenky. Klíčovým nástrojem k oživení BP v této fázi bude šíření povědomí zvl. mezi českými, moravskými a slovenskými experimentálními botaniky (ale také ovšem polskými, maďarskými a ukrajinskými …), že máme solidní mezinárodní časopis s dlouhou, vzácnou a úctyhodnou tradicí a že je správné také tento časopis využívat k publikaci výsledků, které vznikají u nás a blízkém okolí. BP by se měla vyhnout snaze uměle zvyšovat svůj IF – ovšem zároveň nadále vedle výzkumných článků by měla vyhledávat možnosti zvl. zvaných (ale ovšem i nabídnutých) review a tematicky zaměřených speciálních čísel. Také z oblastí, které jsou možná v současné době mimo hlavní proud zájmu a při tom mají jasný potenciál do budoucna.

Viktor Žárský, Editor-in-Chief

Cílem ČSEBR je podporovat snahy o oživení časopisu Biologia Plantarum a opětovné zvýšení jeho nejen české, ale i mezinárodní reputace. Časopis vnímáme jako nedílnou a významnou součást ”Česko-Slovenského” experimentální biologie rostlin. Jedním z příspěvků ČSEBR v rámci podpory Biologia Plantarum je iniciování speciálního čísla, které zastřešuje ČSEBR v rámci kterého mají členové ČSEBR možnost publikovat bez poplatku za publikaci. Sběr článků do tohoto čísla stále probíhá.

Martin Janda

PŘIPOMÍNÁME SI

Jan Květ devadesátiletý

V sobotu 26. srpna oslaví/oslavil 90. narozeniny vědecký pracovník, vysokoškolský učitel, veřejný činitel a hlavně moudrý a laskavý člověk RNDr. Jan Květ, CSc., Dr.h.c., řečený Hony. Jeho vědecká, pedagogická i společenská činnost by vydala na celé knihy a ve svém dlouhém a plodném životě pozitivně ovlivnil tisíce lidí u nás i v zahraničí, kteří na něj vzpomínají. Zájemcům o podrobnější bibliografické údaje o Janu Květovi můžeme doporučit např. články napsané k jeho 70. nebo 80. narozeninám (Živa 4/2003 a 4/2013). Co z toho vyzdvihnout v následujících několika odstavcích? Snad to, co při pohledu zpět nejvíce vystupuje do popředí...

Vědeckému výzkumu se Hony věnoval postupně v Botanickém ústavu ČSAV (1962-2002) a Ústavu ekologie krajiny AV ČR (nyní Ústav výzkumu globální změny AV ČR, v.v.i.; 2003-2022). Stál u zrodu vědeckého výzkumu v oborech produkční ekologie rostlin a ekologie mokřadů v Československu. Mimo jiné navrhl i český termín mokřad jako ekvivalent anglického termínu wetland. Společně s Dr. Milenou Rychnovskou a Dr. D. Dykyjovou se zasloužil o to, že český výzkum produkční ekologie a ekologie mokřadů byl zařazen do Mezinárodního biologického programu (IBP, 1965-1974) a později do programu UNESCO Člověk a biosféra (Man and Biosphere). Tak se dostal do světového kontextu, což bylo za minulého politického režimu velmi výjimečné. Stal se tak – také díky svým mimořádným jazykovým schopnostem a lidským vlastnostem – celosvětově respektovanou vědeckou osobností, která bez přehánění spojovala vědce z Východu se Západem. Pokud bychom měli shrnout jeho ekologický výzkum mokřadů do jediné věty, říkala by, že mokřady a mokřadní vegetace mají i v naší zemi velký význam, který je třeba rozumně využít pro zachování přírodních procesů, a že mokřady rozhodně nejsou ztracenou a hospodářsky jalovou oblastí („wetlands are not wastelands“), za niž ji často ještě dnes považují technokraté.

Hony při přebírání Recognition of Excellence, udělené řídícím orgánem Ramsarské úmluvy o mokřadech r. 2008 v Jižní Koreji za celoživotní dílo a podporu vědy o mokřadech a jejich ochrany

Hony publikoval výsledky v mnoha desítkách článků v mezinárodních vědeckých časopisech. Syntézy týmového výzkumu vtělil do knih, v nichž působil jako editor i autor a k autorské spolupráci na nich získal typicky velký počet kolegů, takřka všechny české odborníky, kteří k problematice měli co říci. Některé z těchto knih bereme do ruky tak často, až se pro ně vžily přezdívky „zelená“ (Pond Littoral Ecosystems, 1978), „bílá“ (Freshwater Wetlands and their Sustainable Future, 2002), a „modrá“ kniha (The Production Ecology of Wetlands, 1998). V posledním desetiletí se odborná práce Jana Květa zaměřuje na českého čtenáře. Koncepčně, editorsky i autorsky se podílel na knize Mokřady: Ekologie, ochrana a udržitelné využívání, která získala Hlávkovu cenu za nejlepší odbornou knihu v biologických oborech v roce 2017. Nyní se těšíme na vydání Atlasu vodních a mokřadních rostlin ČR v nakladatelství Academia, který inicioval a připravuje společně s kolegy z Botanického ústavu AV ČR. Za celosvětový přínos k výzkumu mokřadů získal mnohá národní i mezinárodní ocenění a vyznamenání, mj. cenu vědecké společnosti Society of Wetland Scientists (2001, s Dr. Dykyjovou) a cenu Ramsarské úmluvy o mokřadech (v r. 2008). Své zkušenosti i nadhled Jan Květ stále dává k dispozici v národních i mezinárodních vědeckých institucích a společnostech, z nichž jmenujme např. Českou učenou společnost, do níž byl zvolen v r. 2002.

Přestože Hony vychoval mnoho mladých odborníků, za minulého režimu musela tato činnost probíhat nenápadně kvůli jeho náboženskému přesvědčení. Po změně režimu ale využil svých zkušeností a pedagogických schopností a aktivně se spolupodílel na založení Jihočeské univerzity. Stál při založení Biologické, dnes Přírodovědecké fakulty, kde spoluvytvářel studijní kurikulum, studijní obor Biologie ekosystémů a náplň stejnojmenné katedry. Od samého začátku aktivně přednášel a školil studenty všech stupňů studia. Jeho pedagogické aktivity spojené s vědeckou erudicí a lidskými i společenskými postoji již tehdy bohatě splňovaly požadavky na udělení akademického stupně profesor. Přesto profesorem jmenován nebyl. Nebylo to dáno neochotou fakulty profesorské jmenovací řízení zorganizovat. Právě naopak. Zamezila tomu ale existující legislativa a Honyho zásady. Legislativa předepisuje, že akademickému stupni profesor musí předcházet udělení akademického stupně docent. Výjimky nejsou možné. Hony by tak musel vypracovat habilitační práci a projít habilitačním řízením a teprve poté připravit podklady pro jmenovací řízení. To bylo poněkud krkolomné řešení pro člověka s Honyho vědeckou i pedagogickou erudicí. Na uspořádání zrychleného dvoustupňového procesu ve svých více než 60 letech nepřistoupil. Výsledky práce pro něj byly a jsou vždy důležitější než tituly. Jako výraz uznání mu PřF JU udělila čestný doktorát (Doctor honoris causa) v oboru Biologie ekosystémů.

Jan Květ (Hony) při terénním cvičení na Mokrých loukách u Třeboně. Foto K. R. Edwards. Cvičení ke kursu Produkční ekologie na Přírodovědecké fakultě JU v ČB r. 2017. V rukách drží rámeček velikosti základní plochy 0,5 x 0,5 m na odběr nadzemní biomasy porostu

Hony se vždy zajímal o politické dění. Aktivně do něj vstoupil hned po sametové revoluci jako člen Občanského fóra, za něž byl zvolen do České národní rady na období 1990-92. Jako poslanec se zasloužil zejména o tvorbu Zákona ČNR č. 114/1992 Sb. o ochraně přírody a krajiny, jehož progresívní podobu v době jeho přijetí uznale komentovali i zahraniční politici a přírodovědci, a Zákona ČNR č. 244/1992 Sb. o posuzování vlivů na životní prostředí, který byl vůbec prvním tohoto zaměření v české legislativě. Významně se zasadil též o Zákon ČNR 314/1991 Sb., jímž se zřizovalo několik regionálních univerzit včetně jeho „kmenové“ Univerzity Jihočeské. Po ukončení mandátu v ČNR se Jan Květ po mnoho let věnoval jako zastupitel komunální politice v Třeboni a rozvoji přeshraniční spolupráce s Rakouskem. Mnoho let vedl třeboňskou organizaci ČSOP, to vše stále při plném nasazení vědeckém a pedagogickém. Pokud víme, nikdy si nestěžoval na to, že ho tyto místní aktivity odvádějí od vědecké či pedagogické práce. Z pozdější činnosti stojí za zmínku jeho občanské aktivity na zachování klíčových prvků v Zákoně o ochraně přírody a krajiny při tvorbě jeho novely v r. 2017, ale i na podporu demokratických kandidátů na post prezidenta ČR. Ve všech svých aktivitách hájil demokratické principy a právo občanů rozhodovat o svém životním prostředí, a to i v době totality při nelehkých jednáních se socialistickými úředníky a funkcionáři.

Po přehledu Honyho působení ve vědě i veřejném životě pojďme ještě zmínit jednu méně nápadnou linku jeho života. Jak již bylo uvedeno výše, Hony za minulého režimu kvůli svému náboženskému přesvědčení oficiálně nikde neučil. Méně známé je, že byl v hledáčku Státní bezpečnosti a od svého návratu ze studijního pobytu v Oxfordu (1962-1963) byl opakovaně vyslýchán v Bartolomějské ulici i jinde. Odolal nátlaku StB, která se snažila přimět ho ke spolupráci, nicméně od r. 1966 až do sametové revoluce byl dále veden v jejích spisech jako „prověřovaná osoba“ pod krycím jménem „Kytička“. Jeho aktivity, zejména zahraniční cesty, byly pečlivě sledovány. Na třeboňském pracovišti Botanického ústavu ČSAV se v 70. letech sešel s dalšími “nepřáteli socialistického zřízení“: Dr. D. Dykyjovou, která nemohla učit, protože byla dcerou „kulaka“, a politickým vězněm a tajně vysvěceným knězem Dr. J. P. Ondokem. Tito tři lidé na pracovišti určovali kvalitu mezilidských vztahů. Vytvořil se tím ostrůvek pozitivní deviace, kde vládla důvěra, nadhled i laskavý humor. Jak moc to bylo nesamozřejmé, jsme si plně uvědomili až poté, když se členové pracovní skupiny po sametové revoluci vydali různými cestami. Podobný ostrůvek kolem sebe Hony vytváří, kdekoli po určitou dobu působí. Všichni, kteří jej známe, s vděčností vnímáme jeho laskavost, trpělivost a nezištné přátelství a pomoc, které podstatně přesahuje hranice kolegiálních vztahů a činí z nás členy jeho širší rodiny. Jeho dobrota, schopnost být tu pro druhé a hledat konstruktivní cesty v sebesložitějších situacích vycházejí z jeho hluboce prožívané křesťanské víry, o niž se opíral v dobách minulých, byť za cenu osobních obětí, i v dnešní době „postfaktické“. V diskusích se svými mladšími kolegy a přáteli osvědčuje neobyčejné znalosti nejrůznějších historických událostí a často dává k dobru působivé osobní vzpomínky na ně. Jeho vyprávění o těžkých i úsměvných okamžicích jeho života si můžeme poslechnout v sérii rozhovorů pro Paměť národa.

Devadesátiletý Jan Květ – Hony je stále v kontaktu s přáteli a kolegy doma i ve světě, ale hlavní pozornost věnuje své rodině: své ženě Radce, dvěma dcerám, šesti vnoučatům a čtyřem pravnoučatům. Milý Hony, do dalšího desetiletí Ti přejeme pevné zdraví a radost v kruhu svých blízkých, přátel i žáků!

Hana Čížková, Hana Šantrůčková, Andrea Kučerová, Lubomír Adamec, redakce Živy

Seminář k příležitosti životního jubilea „Honyho“ Květa

Na Přírodovědecké fakultě Jihočeské univerzity v Českých Budějovicích se **19. října 2023** uskuteční seminář (i se zahraničními řečníky) s názvem “Úloha mokřadů v krajině”, který je pořádán ku příležitosti 90. narozenin “Honyho” Květa, který se významnou měrou zasloužil za zachování a ochranu mokřadních ekosystémů nejen u nás, ale i ve světě. Hrál významnou úlohu při zakládání JU i jejím rozvoji.

Více informací bude ohlášeno počátkem září a my o tom budeme informovat na webových stránkách ČSE-BR a také v průběhu konference “Methods in Plant Sciences 2023”.

Martin Janda

Univerzitní Olomouc – pouto, které trvá už 450 let

Glosa rektora Univerzity Palackého v Olomouci prof. MUDr. Martina Procházky, Ph.D.

Olomoucká univerzita a univerzitní Olomouc. Pouto, které trvá už čtyři sta padesát let. Univerzitu nelze ve městě přehlédnout – a nejde zdaleka jen o budovy, které vzdělávací instituce využívá. Je jich bezmála sedm desítek po celém městě, od krásných barokních staveb zdobících historické centrum až po nové moderní objekty. Ovšem to skutečné univerzitní město tvoří hlavně lidé – studenti, pedagogové, vědci a další zaměstnanci, tedy komunita čítající téměř třicet tisíc osob. Nejvíce patrné je to právě v tuto roční dobu, kdy se zejména studenti po skončení akademického roku rozletí do světa a počet obyvatel Olomouce poklesne téměř o pětinu.

Univerzita je ale ve městě přítomná po celý rok a zvláště v tom letošním, kdy si celou řadou aktivit připomíná své velké výročí. Oslavy jsou v plném proudu, máme už úspěšně za sebou řadu akcí, kterými jsme si 450 let olomouckého vysokého učení připomněli, a další nás čekají. Dvěma hlavními liniemi, na které se při oslavách odkazujeme, jsou jezuité coby zakladatelé univerzity a osobnost Františka Palackého, jehož jméno získala škola ve své novodobé historii. Využili jsme tuto historickou příležitost, abychom „Otce národa“ představili spíše jako pracovitého, talentovaného a po vzdělání toužícího mladíka, který může být dobrou inspirací pro současnou generaci studentů.

Píle a touha po vzdělání stojí i na počátku olomouckého vysokého učení, kdy jezuitské snahy o šíření vzdělanosti ve světě vedly k založení druhé nejstarší univerzity v českých zemích. Se stopami jezuitských misionářů, kteří studovali v Olomouci, jsem se nedávno setkal na druhé straně naší zeměkoule. Uvědomil jsem si, že každý rok do světa vysíláme tisíce našich absolventů, kteří pak na mnoha místech a v nejrůznějších oborech dál úspěšně rozvíjejí své znalosti a dovednosti. Pomáhají šířit dobré jméno naší alma mater, ze které vzešli a která se tak navždy stala součástí jejich životů a příběhů.

Univerzita tu byla dříve než my, je tady s námi nyní a bude i po nás. Představuje pevný bod, kterého se můžeme přidržet, je jistotou a kotvou, jakých v životě nemáme mnoho. Věřím, že to tak bude minimálně dalších čtyři sta padesát let. Věřím také, že Olomouc už bude navždy univerzitní. Máme štěstí, že jsme její součástí a že se tak setkáváme v tomto inspirativním čase a prostoru.

Aleš Pěnčík, Jana Balarynová

Jana Albrechtová, Aleš Soukup

Medailon Olgy Votrubové u příležitosti jejích nedávných 80. narozenin

1 Autorský kolektiv knihy Obrazový průvodce anatomií rostlin (Academia 2017). Olga Votrubová uprostřed, zleva Aleš Soukup (oba z Přírodovědecké fakulty Univerzity Karlovy) a Milan Baláž (PřF Masarykovy univerzity), vpravo Alexander Lux (PřF Univerzity Komenského v Bratislavě) a Marie Kummerová (PřF MU). Brno 2017. Foto z archivu A. Luxe

2 Olga Votrubová při odběru vzorků na rybníku Rožmberk v r. 1997. Foto A. Soukup

RNDr. Olga Votrubová, CSc., se narodila 2. června 1942 v Táboře. Na Přírodovědeckou fakultu Univerzity Karlovy v Praze nastoupila jako studentka již v r. 1959 a své diplomové téma vypracovala na katedře fyziologie rostlin. Od té doby na této katedře trvale působila a postupně převzala praktická cvičení a posléze i přednášky a stala se hlavní osobností zajišťující kontinuitu oboru anatomie a cytologie rostlin na PřF UK na několik posledních dekád. Od 80. let 20. století také zaváděla nové kurzy anatomie a morfologie rostlin. Jelikož v té době neexistovaly internetové zdroje a nebyly dostupné ani anglické učebnice, rozhodla se vytvářet vlastní učební texty anatomie rostlin. Napsala řadu vynikajících skript a učebnic, nebo se na nich významně spoluautorsky podílela. Tím zcela zásadně přispěla k rozvoji nejstarší školy anatomie rostlin na území tehdejšího Československa, založené prof. Bohumilem Němcem již koncem 19. století. Kvalitu těchto textů dokumentuje jejich trvalá obliba mezi studenty na různých českých a slovenských univerzitách, ale i to, že jsou používány jako oborový standard.

Je třeba připomenout, že Olga Votrubová velkou část svého produktivního věku strávila na PřF UK v nelehké době socialismu, který tlumil vědeckou spolupráci se zeměmi za železnou oponou, a nedopřál rozvoje na fakultě těm, kteří nebyli politicky dostatečně přijatelní. Proto získala titul CSc. teprve v r. 1988, během určitého předrevolučního uvolnění poměrů po mnohaletém čekání od odevzdání práce. Dále se věnovala výzkumu v oboru anatomie rostlin – zejména strukturním adaptacím na faktory prostředí u kulturních rostlin a později především u mokřadních rostlin. Začala také intenzivně publikovat se svými žáky v pozici seniorní autorky v prestižních časopisech oboru, jako je New Phytologist (např. Soukup a kol. 2007) nebo Annals of Botany (Seago a kol. 2005). O získání vědecko-pedagogických titulů po revoluci již neusilovala – přesto se jí podařilo prokázat excelenci jak ve výuce, tak v anatomickém výzkumu. Více se o jejím vědeckém a pedagogickém působení především v nelehkých dobách za komunistického režimu dočtete v článku o historii školy anatomie rostlin na následujících stránkách. Do Živy přispěla několika články i v minulosti, poprvé v r. 1999, společně s druhým autorem tohoto medailonu, na téma mokřadních rostlin, je rovněž pravidelně recenzentkou článků z oboru rostlinné fyziologie.

Olga Votrubová je navíc nesmírně laskavý člověk, s opravdovým zájmem o obor. Navázala na nejlepší z tradice prof. Němce, jedním z jejích učitelů byl i prof. Jaroslav Pazourek. Rozvíjet obor a jeho výuku během let po válce nebylo jednoduché. Po revoluci v r. 1989 se situace změnila – Olga Votrubová se stala tajemnicí katedry, později zástupkyní vedoucího katedry, členkou oborové rady tehdejšího vzdělávacího programu anatomie a fyziologie rostlin na PřF UK. A dále usilovně pracovala na rozvoji anatomické školy na tomto pracovišti.

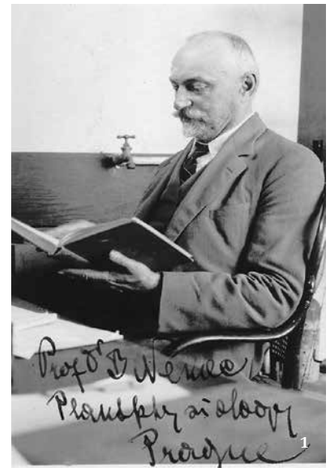
Dne 18. října 2022 se na katedře experimentální biologie rostlin konal seminář k příležitosti jejího životního jubilea, kde vystoupila s přednáškou Historie anatomie rostlin na katedře experimentální biologie rostlin PřF UK. Seminář byl hojně navštívený (okolo 70 osob) a závěrem se ukázalo, že přišlo také mnoho jejích bývalých žáků. Když bylo publikum dotázáno, kdo v místnosti někdy paní doktorku požádal o konzultaci nebo s ní vědecky spolupracoval, objevil se les rukou – naprostá většina přítomných. Přijeli kolegové z Bratislavy, Brna, Českých Budějovic, Třeboně.

Olga Votrubová byla v prosinci 2022 oceněna stříbrnou pamětní medailí Přírodovědecké fakulty Univerzity Karlovy, kterou jí udělil děkan fakulty prof. Jiří Zima za celoživotní působení na původně katedře fyziologie rostlin, nyní katedře experimentální biologie rostlin, a za rozvoj oboru anatomie rostlin. Jménem jejích žáků i dalších kolegů v Čechách i na Slovensku jí děkujeme za to, že nás zasvětila do tajů tohoto nádherného oboru a také za její neúnavnou podporu a šíření optimismu.

Za milou a vždy velmi vstřícnou spolupráci děkuje i celá redakce Živy. Blahopřejeme paní doktorce k jubileu a těšíme se na její další zapojení v pokračování odkazu významných osobností rostlinné anatomie a fyziologie také v Živě.

Olga Votrubová, Jana Albrechtová, Aleš Soukup

Nejstarší škola anatomie rostlin v českých zemích



1 Zakladatel nejstarší souvislé školy anatomie rostlin u nás prof. Bohumil Němec v r. 1912. Marine Biology Laboratory archive, University of Chicago, USA

Anatomie rostlin je základním přístupem ke studiu vnitřního uspořádání orgánů a pletiv, ať už ve vztahu k evoluci, ontogenezi, interakci s dalšími organismy nebo s okolním prostředím rostliny. Jde o tradiční disciplínu, která má na katedře experimentální biologie rostlin Přírodovědecké fakulty Univerzity Karlovy dlouholetou tradici – představuje nejstarší souvislé pokračování nejen v Čechách, ale i na Slovensku. Rozvoj oboru je spojen s řadou osobností, které jej formovaly a ovlivnily vývoj této vědní disciplíny i na řadě jiných pracovišť. Historii rostlinné anatomie nelze nepropojit s rozvojem ostatních oblastí experimentální biologie rostlin – s cytologií, fyziologií, vývojovou biologií a ekologií nebo s aplikovaným výzkumem. Tato historie, stejně jako historie celé katedry, měla lepší a horší období, z nichž mnohá souvisela s politickými poměry v naší zemi.

Počátky můžeme sledovat na konec 19. století a jsou spojeny s osobností prof. Bohumila Němce (1873–1966). Již před ním však lze najít osobnosti, které výrazně ovlivnily rozvoj této vědní disciplíny. Tou nejvýznamnější byl nepochybně Julius Sachs (1832–1897; viz také Živa 2014, 4: LXXII–LXXIII). Do Prahy přišel v 16 letech jako sirotek z tehdy pruské Vratislavi na pozvání Jana Evangelisty Purkyně, který si ho povšiml jako mimořádně nadaného vědeckého ilustrátora. S Purkyněm spolupracoval a během studií také žil v jeho rodině. Vystudoval v Praze na Filozofické fakultě Karlo-Ferdinandovy univerzity. Studia ukončil v r. 1857, několik let v Praze pracoval a krátce na fakultě i přednášel. Julius Sachs je znám především jako průkopník rostlinné fyziologie, v r. 1857 se v Praze jako vůbec první na světě habilitoval jako docent rostlinné fyziologie, zabýval se však i cytologií a anatomií. V r. 2022 uplynulo 165 let od této události, kterou bychom mohli považovat za počáteční impulz vývoje nejstarší školy rostlinné anatomie v Čechách. Studoval tvorbu letokruhů, vliv světla a gravitace na jejich charakter, věnoval se i větvení kořenů a stavbě apikálních meristémů. Popsal vztah mezi osvětlením a vznikem škrobových zrn v chloroplastech. Zavedl dnes běžně užívanou klasifikaci trvalých rostlinných pletiv.

Na počátku byl Bohumil Němec

Bohumil Němec, který je jednoznačně považován za zakladatele nejstarší souvislé školy anatomie rostlin v českých zemích, ale i na Slovensku, vystudoval přírodní vědy na Filozofické fakultě Karlo-Ferdinandovy univerzity v Praze. Zpočátku se zabýval zoologií na pracovišti prof. Františka Vejdovského, ale později se jeho zájem soustředil na botaniku. Zkušenosti získané při studiu zoologie pak uplatnil v experimentálních metodách, které vnesl do botaniky. Roku 1895, před ukončením studií, získal místo asistenta v Botanickém ústavu na české části tehdy rozdělené pražské univerzity u prof. Ladislava Čelakovského. Brzy poté, co byla dostavěna nová budova v Benátské 2 pro Botanický ústav české části univerzity (současně se stejnou budovou ve Viničné 5 pro německou část), byl B. Němec pověřen vybudováním Ústavu pro anatomii a fyziologii rostlin, předchůdce dnešní katedry experimentální biologie rostlin PřF UK. Roku 1899 se pro tyto obory habilitoval a byl jmenován přednostou nového ústavu. Roku 1907 byl jmenován profesorem a přednášel zde anatomii a fyziologii rostlin. Počátky zřejmě nebyly jednoduché, jak vyplývá ze vzpomínek prof. Silvestra Práta (Mladá farmacie 1939, 7: 79–82): „Bez vzoru a z prostředků velmi skrovných dovedl Němec vybudovat Ústav, jenž velmi rychle se stal známým v cizině a už před první světovou válkou byl pokládán za vzorné centrum vědecké práce.“

K tomu nepochybně přispělo, že B. Němec hodně cestoval. V r. 1898 pobýval na univerzitě v Jeně a navštívil i botanické ústavy v řadě dalších zemí Evropy. Hovořil 6 jazyky a setkával se s významnými osobnostmi, např. Albertem Einsteinem nebo Ivanem Petrovičem Pavlovem. Byl pokračovatelem vědeckého odkazu J. Sachse. Uznával jeho práci, propagoval ji a stýkal se s jeho žáky v zahraničí. O blízkém vztahu k Sachsovi svědčí i článek nazvaný Julius Sachs v Praze, který vyšel v Živě r. 1953 (6: 206–207).

Po vzniku samostatného československého státu se B. Němec stal v r. 1919 děkanem Filozofické fakulty UK a významně se v pozici rektora české Univerzity Karlovy, kterým byl v letech 1921–22, podílel na jejím rozdělení na Filozofickou a Přírodovědeckou fakultu. Ve 30. letech 20. století měl Ústav pro anatomii a fyziologii rostlin pod jeho vedením velmi dobrou pověst i v cizině, stal se členem řady zahraničních učených společností. Ústav vedl až do r. 1939. V tomto roce, kdy byly uzavřeny české vysoké školy, odešel do důchodu. Bylo mu 66 let, ale ještě téměř 30 dalších let zůstal vědecky aktivní. Po skončení války sehrál významnou úlohu při vzniku Ústavu pro fyziologii rostlin Univerzity Komenského v Bratislavě, který krátce vedl a kde také přednášel rostlinnou fyziologii a anatomii. I na Slovensku je tedy možné přičítat založení dodnes trvající školy anatomie rostlin Bohumilu Němcovi. Po válce, zejména po r. 1948, žil po léta v nuceném ústraní vyvolaném tehdejším politickým režimem, a to vzhledem ke svým názorům i politickým aktivitám v dobách první republiky. Byl vytlačen ze všech funkcí ve vědecké komunitě. Ke změně došlo až za komunistického prezidenta Antonína Zápotockého. Tuto změnu popsal prof. Josef Koutecký (Vesmír 1997, 4: 212–214): „V 50. letech, kdy jsme se dusili pod politickým tlakem, navštívila Československou akademii věd sovětská vědecká delegace vedená akademikem Borisem Denisovičem Sočavou (ruským geobotanikem, zakladatelem moderní vědy o krajině). Prvním, po kom se sháněli, byl Němec. Marně je přesvědčovali, že je nežádoucí. Rychle pro něho tedy poslali auto. Prezident Zápotocký pak podepsal jmenování Bohumila Němce akademikem. Od té doby byl trpně ‚hájen‘ a dokonce mu zvýšili penzi.“

Pro B. Němce byla věda povoláním i posláním. Považoval ji za hnací sílu lidského pokroku. Rozsah jeho aktivit byl neuvěřitelný. Vedle vědeckého výzkumu se věnoval i pedagogické práci, byl přesvědčeným popularizátorem vědy a před druhou světovou válkou byl velmi činný také v politice. Jeho práce se týkají nejen všech oblastí rostlinné anatomie, ale i nauky o buňce a fyziologie.

K jeho nejznámějším objevům patří statolitová teorie formulovaná ve dvou publikacích z r. 1900, která položila základy

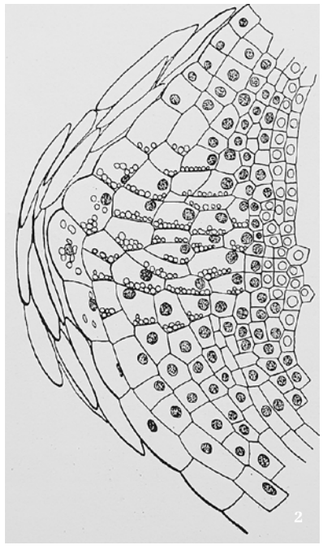
pro vysvětlení reakce rostlin na působení gravitace. Tento objev byl nepochybně ovlivněn jeho předchozími studiemi korýšů u prof. F. Vejdovského. Jak sám uvádí v článku Ueber die Art der Wahrnehmung des Schwerkraftreizes bei den Pflanzen (O vnímání gravitace u rostlin): „Vlastní pozorování mě přivedla k poznání, že buňky s tělísky, která se chovají jako specificky těžší tělesa v kapalině, jsou v rostlinné říši velmi rozšířeny. Starší studie sluchových orgánů nižších živočichů, zejména korýšů, ve mně vzbudily myšlenku, že toto by mohla být zařízení umožňující rostlinám percepci směru působení zemské tíže. Posléze byla správnost této myšlenky potvrzena experimentálně, což jsem předběžně uvedl ve vztahu ke kořenům.“

Statolitová teorie byla nejprve publikována r. 1900 v časopise Biologisches Zentralblatt a krátce nato ve výše uvedené publikaci v časopise Berichte der deutschen botanischen Gesellschaft. Ve stejném čísle byla zveřejněna podobná práce významného rakouského anatoma Gottlieba Haberlandta, která byla dodána do redakce o pouhých 10 dnů později. Haberlandt zde už cituje dřívější Němcovu práci: „Němcova zpráva mě přiměla k tomu, abych svá, dosud kusá pozorování tohoto jevu už nyní v krátkosti publikoval.“ Němec i Haberlandt došli ke shodnému závěru, že jako statolity fungují amyloplasty, které jsou v určitých typech buněk schopné měnit polohu v souladu se změnou orientace orgánu díky působení gravitace.

Vědecká činnost B. Němce zasahovala do mnoha dalších oblastí, takže je oprávněně uznáván jeho přínos nejen v rostlinné anatomii, ale také v cytologii, fyziologii a obecné botanice. Dokládá to několik set jeho původních prací i obsáhlé učebnice a monografie. Dále jsou význačné studie buněčného jádra – věnoval se např. polyploidii a mixoploidii, bývá mu připisováno rozdělení organismů na Eukaryota a Akaryota (dnes Prokaryota). Významné jsou i jeho výzkumy zabývající se oplodněním a partenogenezí. Popsal velmi zajímavý jev, zvaný též Němcův fenomén, kdy v petaloidních tyčinkách hyacintu východního (*Hyacinthus orientalis*) vznikají velká pylová zrna, která nevytvářejí pylovou láčku, ale útvar s 8 jádry, obdobný zárodečnému vaku. Toto pozorování možné změny samčího na samičí gametofyt vzbudilo velký zájem (viz Živa 2007, 3: 101–103). Na pracích prof. Němce bylo důležité, že se vždy snažil propojovat strukturu a funkci na úrovni buněk, pletiv, orgánů i celých organismů. Tento přístup a jeho výsledky jsou dodnes zdrojem inspirace.

V r. 1959 stál v čele rostlinných biologů, kteří iniciovali vznik odborného časopisu Biologia plantarum, jenž je dodnes důležitým vědeckým impaktovaným časopisem. V době tzv. železné opony to mělo velký význam, protože naši vědci měli značně omezené možnosti komunikace s kolegy mimo východní blok. Časopis vydávaný v Československu, ale distribuovaný mezinárodně byl proto zásadní.

Prof. Němec vychoval několik generací botaniků. Jeho heslem, kterého bychom se měli i my držet, je „Věřím, že lze i nejsložitější věci podat způsobem jednoduchým.“ Byl autorem několika učebnic, které mají



2 Ilustrace z článku B. Němce Ueber die Art der Wahrnehmung des Schwerkraftreizes bei den Pflanzen (O vnímání gravitace u rostlin). Kresba zobrazuje sedimentaci škrobu kořenové čepičky ve směru gravitace. Berichte der deutschen botanischen Gesellschaft (1900)

i podle dnešních parametrů výbornou úroveň jazykovou a dokumentační. I dnes v nich lze nacházet cenné informace. Tou nejvýznamnější je asi Nauka o buňce, Anatomie rostlin, součást tzv. Aventinského rostlinopisu z r. 1930. Další důležitá je Učebnice anatomie a fyziologie rostlin pro farmaceuty a přírodovědce, kterou napsal spolu se svými žáky prof. S. Prátem a prof. Janem Kořínkem a která vyšla r. 1945 jako vůbec první poválečná vysokoškolská učebnice. Na těchto učebnicích je fascinující preciznost, s jakou byly sepsány, a dokonalá obrazová dokumentace, často s originálními kresbami od prof. Němce, která v té době musela být časově nesmírně náročná.

Působení B. Němce na Slovensku vyústilo ve vydání dvoudílné učebnice Všeobecná botanika (1948 a 1949), kterou připravil spolu s bratislavským kolegou prof. Ľudovítem Pastýrikem. Spolu s ním a Máriou Luxovou vydali v r. 1958 knihu Jak žije ovocný strom. Významná ve své době a stále ještě v mnohém využitelná je Botanická mikrotechnika (1962), na které se s ním podílelo 11 spoluautorů včetně prof. Jaroslava Pazourka, dalšího význačného představitele rostlinné anatomie.

Prof. Němec se v řadě monografií snažil přiblížit svět rostlin nejen rostlinným biologům, ale i širší vědecké komunitě a v mnohých z nich i širší veřejnosti. K těm nejznámějším patří Dějiny rostlinstva na zeměkouli (1916), O původu a vývoji života (1916), Ze života rostlin (1924), Zelené království (1939), Život rostlin I a II (1941), Jak rostou rostliny (1943), Dějiny ovocnictví (1958). Ke knize Život rostlin napsal zajímavý doslov: „Jsme právem pyšni na to, čeho se věda o životě dopracovala od dob Linnéových. Čeho se dopracuje za dalších 200 let? Chtěl bych číst život rostlin, který bude napsán za 100 nebo 200 let. Chtěl bych se dovědět, co zbylo z toho, co jsem napsal v této knize, do které jsem vybral podle své volby tak asi to nejdůležitější, co vím o životě rostlin.“

Zajímavá a ve své době oceňovaná je Duše rostlin (1937, 1938, 1942), kde si všímá u rostlin jejich odolnosti, přizpůsobivosti, vůle žít, množení a zachování rodu. A že to byla tehdy opravdu populární kniha, o tom svědčí i fakt, že se o ní objevil r. 1937 článek v časopise Nature. Prof. Němec by si dnes určitě s velkým zájmem přečetl knihu Daniela Chamowitze Co rostlina ví (Academia 2020) o senzorických systémech rostliny.

Za pozornost také stojí jeho zápisky z 50. let, vydané v knize Vzpomínky (2002 a 2021). Její editoři k ní poznamenávají: „Své zápisky Němec koncipoval v době, která precizním pamětem nepřála. Komunistický tlak, v 50. letech nejdůraznější, způsobil, že ožehavá politická místa posouval do pozadí a věnoval se spíše svým cestám, setkáním se zahraničními kolegy a komentářům o vývoji věd. Přesto jeho vzpomínání je barvitým záznamem dlouhého a přes všechny peripetie úspěšného života vědce.“

Bohumil Němec stál rovněž u budování prestiže a tradice dvou nejvýznamnějších českých vědecky popularizačních časopisů. V r. 1923 obnovil na popud Aloise Rašína časopis Vesmír a spolupracoval se Živou, kde publikoval množství příspěvků s nejrůznější tematikou – první ještě o korýších. V letech 1910–15 působil dokonce jako redaktor Živy. Více se o něm dočtete např. v Živě 2006, 6: LXXXI; 2007, 1–5; nebo 2014, 4: 148–150; 2015, 4: 150–152.

Ovlivnil vývoj biologie rostlin i v dalším centru oboru, a to v Brně. Na Masarykově univerzitě získal r. 1938 čestný doktorát.

Významným Němcovým žákem byl prof. Rudolf Dostál (1885–1973). Vystudoval Filozofickou fakultu UK a později přešel do Ústavu pro anatomii a fyziologii rostlin. Pod Němcovým vedením se věnoval experimentální morfologii rostlin a publikoval první studie o růstových korelacích. V r. 1919 se u něj habilitoval a jako docent odešel do Brna, kde pracoval na Vysoké škole zemědělské a lesnické a na Vysoké škole zvěrolékařské. Dostálovy práce v oblasti rostlinné morfologie, výzkumu stimulátorů růstu, fotoperiodismu rostlin a rostlinných hormonů byly kladně přijaty ve vědeckém světě té doby. Na brněnských vysokých školách měla jeho práce mnoho pokračovatelů. O kontinuitu se zasloužil např. profesor Mendelovy zemědělské a lesnické univerzity v Brně Jiří Šebánek. Na Přírodovědecké fakultě Masarykovy univerzity rozvíjel obor rostlinné morfologie a anatomie prof. Zdeněk Sladký, rovněž motivovaný R. Dostálem. Důkazem jejich spolupráce jsou monografie, např. Experimentální morfologie rostlin z r. 1983, jejímiž autory jsou J. Šebánek, Z. Sladký a Stanislav Procházka, který je na Mendelově univerzitě dalším pokračovatelem tohoto směru. Stopu působení prof. Bohumila Němce tak najdeme napříč celým Československem.

Poválečný vývoj

Nástupcem B. Němce ve vedení katedry, tehdejšího Ústavu pro anatomii a fyziologii rostlin, se stal v r. 1939 prof. Silvestr Prát (1895–1990). Nová etapa dějin se ale začala psát hlavně po válce, po znovuotevření vysokých škol, a to již na novém místě, protože pracovišti byla přidělena celá budova německých botanických ústavů ve Viničné 5. Prof. Prát je znám především jako fyziolog, zapsal se ale krátce i do historie anatomie, a sice publikací Rostlina pod drobnohledem (1945, 1952), která je nejen příručkou praktické mikroskopie a mikroskopické techniky, ale také návodem k amatérskému pozorování stavby těla rostlin.

Po válce nastoupil na katedru Stanislav Lhotský (narodil se v r. 1911, rok úmrtí není znám). Jeho jméno není často zmiňováno, bádání se příliš nevěnoval, ale přispěl k rozvoji výuky anatomie. Ujal se nejprve cvičení a posléze přednášek; později sepsal pojednání o vývoji katedry. Období po druhé světové válce vůbec nebylo snadné, jak uvádí. Popisuje těžké počátky v budově s rozbitou střechou, poškozenými aparaturami, v obrovském nepořádku a s chybějícími základními pracovními prostředky. Vědecko-výzkumná práce byla bezprostředně po válce vlivem návalu prací reorganizačních a přemírou pedagogických úvazků minimální. Nejnaléhavějšími úkoly bylo rychle vyškolit velký příliv studentů, který nastal po znovuotevření vysokých škol. Přednášky a cvičení se r. 1945 konaly i během hlavních prázdnin. V r. 1946 byl počet posluchačů šestinásobný oproti předválečnému. Výuce se v té době věnoval hlavně S. Lhotský a vztah k ní už mu zůstal – napsal skripta Cytologie a anatomie rostlin (první vydání 1953, druhé, opravené 1962, dotisk 1963) a rovněž za jeho redakce vyšel v r. 1954 překlad vysokoškolské učebnice Anatomie rostlin Vladimira F. Razdorského.

Období profesora Pazourka

Roku 1949 přichází na katedru Jaroslav Pazourek (1923–1999), který představuje v novější době nejvýznamnější osobnost oboru anatomie rostlin, ale i osobu ve vývoji celé katedry. Od jeho nástupu výuku anatomie zajišťovali dva pedagogové a navíc se prof. Pazourek od začátku intenzivně zabýval i výzkumem. O tehdejším rozvoji anatomie svědčí, že v září 1953 bylo interně zřízeno oddělení rostlinné cytologie a anatomie.

Možnost profesionálního rozvoje J. Pazourka byla bohužel negativně ovlivněna režimem, se kterým v mnohém nesouzněl. Hlavní problém, kromě jeho názorů, byly styky s B. Němcem v době, kdy „nebyl žádoucí“. Považoval ho nejen za svého učitele, ale vázaly je i silné přátelské vztahy, Němec Pazourka na katedře často navštěvoval. Po úspěšném rozvoji Pazourkovy práce v 60. letech, kdy získal docenturu, nastaly další problémy po okupaci země vojsky Varšavské smlouvy v r. 1968. Během Pražského jara byl aktivním předsedou organizace ROH (Revolučního odborového hnutí – tehdy jediné odborové organizace) na fakultě a výrazně se angažoval v politickém dění. Vzhledem k tomu mu na počátku 70. let, v období normalizace, byla

3 Prof. Jaroslav Pazourek při přednášce na konferenci ke 100. výročí narození prof. Silvestra Práta v r. 1995 na Přírodovědecké fakultě UK. Foto L. Daněk

zakázána veškerá pedagogická činnost. V r. 1971 naposledy přednášel, ale už nesměl zkoušet a v témže roce obhájil práci jeho na dlouhou dobu poslední diplomant. Přestože byl odstaven od výuky a kontaktu se studenty, nadále se zabýval výzkumnou činností, publikoval řadu odborných studií a popularizoval vědu.

V 80. letech, po nástupu prof. Lubomíra Nátra do vedení katedry, začalo pomalu docházet ke změnám. Prof. Nátr postupně navázal s J. Pazourkem dobré vztahy a začali spolupracovat na odborných tématech. Nebylo však možné zcela prolomit zákaz jeho pedagogické činnosti, i když publikovat s L. Nátrem mu bylo umožněno. K přednášení se J. Pazourek vrátil až po r. 1989, kdy získal i profesuru, ale bohužel už si to takříkajíc příliš neužil, protože se stal vedoucím katedry, dobu provázela spousta změn, někdy i zmatků, a už tedy neměl dost času.

Největším přínosem vědecké práce Jaroslava Pazourka bylo zavádění metod kvantitativní anatomie – této oblasti se věnoval od počátku 60. let. Tehdy se stále více ukazovalo, že kvantitativní údaje jako počty struktur (např. průduchů) a jejich rozložení a velikost nebo poměry jednotlivých pletiv uvnitř orgánů umožňují lépe propojovat strukturní a funkční parametry rostlinného těla a ukázat, jak se mění např. působením vnějších faktorů při diferenciaci pletiv a orgánů v průběhu ontogeneze rostliny. Ve spolupráci s třeboňskou částí Botanického ústavu ČSAV studoval mokřadní rostliny, a to v rámci Mezinárodního biologického programu (IBP), který byl oficiálně zahájen v r. 1964 a probíhal do r. 1971. V té době to byla jedna z mála možností pro naše vědecké instituce ke spolupráci v celosvětovém měřítku.

Zajímala ho především stavba listů různých mokřadních rostlin. Nejvíce se věnoval studiu hustoty a rozmístění průduchů, ale i poměru jednotlivých pletiv v listu. Tyto práce byly často propojovány s faktory prostředí, např. se světelnou intenzitou nebo dalšími podmínkami stanoviště, ale sledoval i vztah hustoty, velikosti a rozmístění průduchů k pozici listů na stonku. K mokřadním rostlinám se pak vrátil koncem 80. let jako vedoucí diplomové práce Hany Čížkové, dnes profesorky na Jihočeské univerzitě v Českých Budějovicích, s tématem zaměřeným na kořeny ostřice (*Carex* sp.). Další důležitou etapou byly práce na obilovinách, nejprve na pšenici ve vztahu k deficienci některých makrobiogenních prvků a pak spolu s prof. Nátrem na různých genotypech ječmene. Tyto výzkumy byly později využívány při studiích fotosyntézy a vodního hospodářství, např. v laboratoři Bohdana Slavíka v Ústavu experimentální botaniky AV ČR. Kvantitativně anatomické práce J. Pazourka získaly ocenění i v zahraničí. Začátkem 90. let navštívil pracoviště ve Viničné 5 významný izraelský anatom prof. Abraham Fahn, autor Plant Anatomy, jedné z nejlepších učebnic anatomie rostlin; jeho návštěvu inicioval především zájem o tyto metody.

Prof. Pazourek spolupracoval také hodně s aplikační sférou – využil např. mikroreliéfové metody při rozeznávání semen. Způsob byl patentován a zapsán do rejstříku v r. 1964 pod názvem Způsob rozlišování semen, např. krmné kapusty a řepky. Ve spolupráci se společností Oseva řešil význam anatomické stavby bramborových hlíz pro jejich poškozování nárazem. S Výzkumným ústavem potravinářského průmyslu a Výzkumným ústavem zemědělské techniky zase sledoval vliv různého způsobu sušení na změny vnitřní struktury mrkve a chmelových hlávek – snahou bylo najít optimální způsob sušení.

Jeho přednášky byly srozumitelné a v jeho přístupu k pedagogické činnosti se jasně odrážel osobní vztah k anatomii rostlin. Odráží se i v řadě skript a učebnic, z nichž mnohé napsal sám, na jiných se podílel. Nejúspěšnější byly Pracujeme s mikroskopem (1975) a Poznáváme vnitřní stavbu rostlin (1979), dále pak skripta Praktická cvičení z anatomie rostlin pro botaniky (1970). Byl přesvědčený, že pro kvalitu výuky na vysoké škole je důležitá příprava středoškolská. Jako spoluautor se podílel na učebnicích pro gymnázia, napsal řadu článků pro žáky i učitele středních škol, zejména pro časopis Přírodní vědy ve škole nebo ABC mladých techniků a přírodovědců.

Stejně jako prof. Němec se věnoval i popularizaci vědy. Kromě textů pro učitele a studenty středních škol publikoval články ve Vesmíru a v Živě. V 80. letech minulého století se podařilo v nakladatelství Artia vydat postupně v několika jazycích knihu The Secret Life of Plants, obsahující množství anatomických mikrofotografií. Zde využil svých vynikajících znalostí přípravy anatomických preparátů a jejich fotografování. Kromě anglické verze kniha vyšla i francouzsky, německy, dánsky a švédsky.

V r. 1997 se podařilo, spolu s první autorkou tohoto článku, vydat Atlas of Plant Anatomy, založený opět především na databázi jeho mikrofotografií. Tato publikace ale neměla štěstí. Snaha o její vydání začala na počátku 90. let, kdy Nakladatelství Academia, kde měla vyjít, nejprve změnilo jazykové požadavky z české na anglickou verzi a poté knihu nemohlo z finančních důvodů vydat. Nakonec se to podařilo v nakladatelství Peres, které ale v r. 2008 skončilo v likvidaci a knize se

nedostalo patřičné propagace a distribuce. Zbylé výtisky byly předány katedře a tím zachráněny. Využívají se víceméně pouze interně pro výuku, zejména pro studenty programu Erasmus. Krásnou a poslední publikací od prof. Pazourka je Vyprávění o rostlinách, přibližující život rostlin širší veřejnosti. Vyšla bohužel až posmrtně (Academia 2001), zásluhou prof. Nátra, který si J. Pazourka velmi vážil.

Nedávná historie

Období normalizace v 70. letech 20. století bylo pro budoucnost rostlinné anatomie na katedře kritické. Výuku musel zpočátku zajišťovat Stanislav Lhotský, který se chystal do důchodu, a situace ho zmátala. Proto byla pověřena výukou anatomických cvičení Olga Votrubová (její medailon uvádíme na str. LVIII této Živy), vedle svých ostatních činností v laboratoři rostlinné fyziologie, a to příkazem, o čemž se v té době nedalo příliš diskutovat. Po úplném odchodu S. Lhotského do penze přednášky převzal tehdejší vedoucí katedry prof. Jaromír Seifert, který byl ale odborníkem na půdní biologii. Anatomie se v té době dostala do pozadí a na katedře o ni nebyl mnoho let z řad studentů zájem. Nakonec byla O. Votrubová pověřena i převzetím přednášek. Zpočátku se věnovala pouze výuce anatomie, což jí významně usnadnili jak S. Lhotský, tak J. Pazourek, který ani po všech událostech nezahořkl a vždy říkal, že mu záleží na tom, aby se udržela solidní úroveň výuky a „němcovská" tradice. Převzetí výuky O. Votrubovou mělo být dočasné, ale vzhledem k tomu, že se nenašel nikdo, kdo by se mohl této části výuky věnovat, stalo se trvalým.

Vyučovat jakýkoli obor a přitom opomíjet jeho výzkumná témata nelze, a tak se začal formovat směr anatomického výzkumu. Práce O. Votrubové navázala na její předchozí činnost v laboratoři Miroslava Dvořáka na PřF UK, která se zabývala minerální výživou a vlivem stresových faktorů na příjem a distribuci živin. Jedním ze stěžejních témat se stal nadbytek obsahu toxického hliníku v půdách následkem kyselých dešťů a vliv hypoxie vznikající utužením zemědělsky využívaných půd těžkou technikou. Následoval postupný přechod od fyziologických studií k anatomickým, zůstal ale zachován princip propojení mezi strukturou a funkcí. Postupně v tematice výzkumu převládla hlavně studia vlivu hypoxie, nejprve na kulturních rostlinách, především na kukuřici seté (*Zea mays*). Později byla navázána spolupráce s kolegy z Botanického ústavu ČSAV (AV ČR) v Třeboni, především výše zmíněnou Hanou Čížkovou, ale i s Janem Květem, Janem Pokorným a Lubomírem Adamcem. V 90. letech vznikly evropské projekty EUREED I a II, které se zabývaly hlavně stavem porostů rákosu obecného (*Phragmites australis*) v evropských mokřadech a jejich odumíráním. Účastnily se týmy z celé Evropy od Švédska po Španělsko a od Anglie po Rumunsko. Za Českou republiku spolupráci zaštiťoval Botanický ústav a vedoucí byla H. Čížková. Náplň těchto projektů asi nejlépe formuloval prof. Hans Brix z univerzity v dánském Aarhusu, vedoucí EUREED II (ve volném překladu): „Rákosy v Evropě odumírají velkou rychlostí na značných plochách, se závažnými dopady na důležité funkce mokřadů (biodiverzitu, stabilitu říčních a rybničních břehů, kvalitu vody i místní ekonomiku). EUREED je evropskou strategickou iniciativou základního výzkumu, která má za cíl analyzovat mechanismy kontrolující dynamiku růstu a stabilitu ekosystémů s dominantním rákosem."

Práce na mokřadních rostlinách, a to nejen na rákosu, později i na zblochanu, orobinci a puškvorci, pokračovaly i po ukončení projektu a umožnily další plodnou spolupráci s BÚ v Třeboni, ale i možnost navázat spolupráci s řadou nejen evropských institucí – např. s univerzitami v Berlíně, Bernu, Aarhusu, Hullu, State University New York nebo Oswego (také v americkém státě New York).

Začátkem 90. let se na katedře rozvíjel další anatomický směr poté, co inspiraci získala prof. Jana Albrechtová při studijním pobytu v Bangoru na Univerzitě ve Walesu. Věnovala se ekofyziologickým studiím dřevin a výzkumu funkčních strukturálních znaků listoví ve vztahu k jejich optickým vlastnostem, a metodám dálkového průzkumu.

Současnost

Na katedře teď pracují dva týmy žáků Olgy Votrubové – jeden pod vedením Aleše Soukupa, druhý pod vedením Jany Albrechtové. Oba týmy dále propojují anatomii s dalšími metodickými přístupy – vývojovou biologií, ekofyziologií, metodami dálkového průzkumu. Aktuální výzkumné zaměření skupiny A. Soukupa a Edity Tylové je spojeno s anatomickou strukturou, vývojem a funkcí kořenového systému, který se vytváří v interakci s heterogenními podmínkami půdního prostředí. Tým J. Albrechtové se zaměřuje na ekofyziologické studie, pokračuje v tradici využití kvantitativních metod pro popis struktur, kterou založil prof. J. Pazourek.

Studenti O. Votrubové se velmi dobře uplatnili v mnoha oborech. Mnozí se stali uznávanými učiteli biologie na středních školách, další pracují ve vědeckých institucích nebo v aplikovaném výzkumu. Tři z nich (J. Albrechtová, A. Soukup a E. Tylová) se stali členy katedry a dále rozvíjejí anatomii rostlin jak vědeckým výzkumem, tak výukou. Kromě původního předmětu Anatomie a morfologie rostlin přednášeného O. Votrubovou společně s kolegy z katedry botaniky byla zavedena rozšířená verze kurzu Anatomie rostlin. S rozvojem internacionalizace fakulty přibyla anglická verze Anatomie rostlin a Botanické mikrotechniky. Byla zavedena přednáška Fyziologická anatomie rostlin pro magisterské studium, propojující strukturu, vývoj a funkci rostlinného těla. J. Albrechtová převzala v polovině 90. let od J. Pazourka výuku předmětu Kvantitativní anatomie rostlin, který se transformoval v Metody analýzy obrazu a stereologie pro biology a je vyučován nadále spolu s kolegy z Oddělení biomatematiky Fyziologického ústavu AV ČR. Cytologie rostlin, přednášená O. Votrubovou se zapojením Jaromíra Kutíka, byla postupně předána Kateřině Schwarzerové. Všechny uvedené přednášky jsou studenty dobře hodnoceny a mají vysokou návštěvnost. Přednášky i cvičení stále navazují na tradici výuky rozvíjející se od dob B. Němce, jsou modernizovány a průběžně aktualizovány.

Úspěšnou aktivitou je i kurz Svět rostlin Univerzity třetího věku, založený Lubomírem Nátrem, který O. Votrubová převzala a na němž se podílí řada pracovníků katedry. V současnosti je garantem kurzu K. Schwarzerová.

V tomto období vznikla také řada výukových materiálů. V r. 2010 vyšla skripta O. Votrubové Anatomie rostlin, která se dočkala několika dotisků. Jsou využívána i na jiných univerzitách jako základní učební text. Nyní je připravováno nové, upravené vydání (ve spolupráci O. Votrubové, A. Soukupa a E. Tylové). Pokračuje snaha popularizovat vědu příspěvky do časopisů, jako jsou Živa nebo Biologie – Chemie – Zeměpis pro střední školy.

Díky kontaktům s pracovníky bratislavské, pražské a brněnské univerzity vznikla pod vedením prof. Alexandera Luxe z Univerzity Komenského v Bratislavě dvojjazyčná kniha Obrazový průvodce anatomií rostlin – Visual Guide to Plant Anatomy (Academia 2017). Podíleli se na ní současní představitelé univerzit, které prof. Němec ovlivnil při zakládání tradice oboru. Z brněnské Masarykovy univerzity to jsou Milan Baláž a Marie Kummerová, z Univerzity Karlovy O. Votrubová a A. Soukup. Ke spoluautorům této významné učebnice patří Jun Abe a Morita Shigenori z Japonska a Thomas Rost z USA. Kniha získala cenu poroty za přírodovědnou encyklopedii ve 25. ročníku soutěže Slovník roku 2018, pořádané Jednotou tlumočníků a překladatelů (JTP) na 24. mezinárodním knižním veletrhu Svět knihy Praha 2018. V r. 2017 byla nominována na cenu Nakladatelství Academia.

Ohlédneme-li se za vývojem anatomie rostlin na katedře experimentální biologie rostlin PřF UK, můžeme sledovat přímou nástupnickou jednotku bývalého Ústavu pro anatomii a fyziologii rostlin prof. Němce, jejíž dlouhodobý vývoj naštěstí nenarušila ani těžká období 20. století – první světová válka, souboj mezi českou a německou vědeckou komunitou, období uzavření českých vysokých škol během druhé světové války, nástup komunistické ideologie v 50. letech ani politická normalizace 70. let. Na tomto pracovišti se i v těžkých dobách našli lidé schopní podpořit ty, kteří se ocitli v nemilosti. Doufejme, že tato etika vědeckého světa bude nadále vítězit. Současní anatomové rostlin na PřF UK nezapomínají na počátky svého pracoviště, jeho tradici, ale zároveň obor rozvíjejí v kontextu nových přístupů a technologií.

Závěrem je třeba říci, že na rozvoji nejstarší školy anatomie rostlin na území bývalého Československa se podílela nejen celá řada pedagogů a vědeckých pracovníků, ale neocenitelnou roli hráli vždy studenti, četní techničtí pracovníci, laboranti, zahradníci a sekretářky, bez nichž by se tato vědní disciplína nemohla úspěšně rozvíjet. Doufáme, a vše tomu nasvědčuje, že tradice tohoto přímého následnictví bude dále pokračovat.

Použitou literaturu uvádíme na webové stránce Živy.

Přetištěno s laskavým svolením redakce Živy.

ABSTRACT BOOK

17TH STUDENT DAYS IN PLANT BIOLOGY CS 2023

METHODS IN PLANT SCIENCES 2023

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Simona Koutková
Jiří Šantrůček

Introduction word for 17th Student Days in Plant Biology CS 2023 and Methods in Plant Sciences 2023

Dear colleagues, participants of our conferences,
we have two conferences coming up, each with its own history. But for us, the organisers, it is essentially one continuous event. Firstly, the student conference "17th Student Days in Plant Biology CS 2023" will occur. As the name implies this conference will take place for the seventeenth time and is traditionally organized by the Czech Society of Experimental Plant Biology (CSEPB). I have a sentimental relationship with this conference. As a student, I have attended it four times, and I had my first opportunity to give an oral presentation of my research at a conference-type event. I believe the conference will provide many participants with such an opportunity and experience this year. This conference has always served to give students a chance to present their work to a broader and "non-home" audience. Coincidentally, I was also a co-organizer of the last edition of “Student Days”, which was held in 2021. That was not a good year for conferences. So, last year's edition was held online. But better than nothing. I am very happy that this year the conference is already running under a regular regime.

The main conference (in my view) "Methods in Plant Sciences 2023", will start immediately after the student conference. This conference follows the tradition of the so-called "Metodické dny". The first event was in 1997 and was attended by 60 participants. The last “Metodické dny” which was already nine years ago in Seč had 150 participants. This year, we have a similar attendance with 180 participants (including organisers and sponsors). The former main organizer was always Jan (“Honza”) Martinec, but after years of organizing, he decided to “pass the baton” to others. I was the one who took it. I hope that the whole organizing team and I will manage to build on the quality and atmosphere of the past "Metodické dny" and keep this event alive for decades to come. It is not only the new organizing team that is new. This year's conference is for the first time entirely in English. This came up naturally. We have more and more foreign colleagues working at Czech institutions, and at the same time, English allows us to invite excellent speakers from abroad and thus increase the already traditionally high quality of the contributions. Another organizational change is that I, from my position as the President of CSEPB, have connected the conference more with our society, hence the connection with the student days.

The conference consisted mostly of invited lectures, and it was not easy to decide from received excellent abstracts which lectures would supplement the invited ones. Microscopy techniques and cell biology are strong themes in the conference, but there will be no shortage of traditionally strong analytical topics or new developments in genomics and genetic engineering. Structural biologists have their own section as well. I believe the conference has great potential to enrich all of us and allow us to have an inspiring and fruitful discussion with colleagues using different methods in plant research. I am glad that a substantial number of our participants represent PhD students.

The number of participants I consider as a good achievement because this year is the first normal year for conferences after COVID. Thus, the “competition” with the other events was tough. And it is clear to me that the financial possibilities of the laboratories are not unlimited. It is a big commitment not to disappoint your interest in the conference and to maintain it in the future.

Finally, I would like to thank all sponsors, partners and colleagues who helped us in any way to organize this event.

In Srní 22. 9. 2023
Martin Janda

PROGRAMME 17TH STUDENT DAYS IN PLANT BIOLOGY CS 2023

colour coding

- Registration
- Start/start of conference
- meal
- coffee break
- Invited lecture I
- Session – regular lectures
- Poster session / taking together

** the organisers have the right to change the programme*

		Presenting author	Tittle	Institution
Friday 22. 9. 2023				
13:00	Start of the registration			
16:00–16:30	Opening of the conference			
16:30–17:15	IL1	Tomáš Figura	Enslaved and abused - ecophysiology of plants that feed on fungi	*Charles University*
Phytohormones I				
17:15–17:30	1	Pavel Jelínek	The role of elevated auxin perception in the proliferation of auxin-autonomous cell lines	*Charles University*
17:30–17:45	2	Kristýna Bieleszová	New auxin derivatives: elucidation of their biological activity and potential applications	*Palacký University*
17:45–18:00	3	Pavel Hladík	Metabolic profiles of phenylacetic acid conjugates differ in various plant species	*Institute of Experimental Botany AS CR*
18:00–18:15	4	Lenka Helusová	Localization and connection of IAA metabolic enzymes in tobacco cells at subcellular level.	*Institute of Experimental Botany AS CR*
18:15–19:30	Dinner			
19:30–	Taking together			
Saturday 23. 9. 2023				
Plant Cell I				
9:00–9:15	5	Barbora Jelínková	ARP2/3 and the secretory pathway	*Charles University*
9:15–9:30	6	Michaela Neubergerová	Protein-lipid interfaces in plant cell trafficking studied by molecular dynamics	*Institute of Experimental Botany AS CR*
9:30–9:45	7	Dzmitry Pruchkouski	Methods for analysis of RNA – protein interactions	*Masaryk University*
9:45–10:00	8			
10:00–10:15	coffee break			
10:15–11:00	IL2	Jana Koller	Phylogeny of jasmonate signaling in carnivorous plants	*University of Ostrava*
Phytohormones II				
11:00–11:15	9	Lucas Amokrane	At the heart of the balance between immunity and growth in plants: effect of salicylic acid on plant metabolism enzymes	*Université de Technologie de Compiègne*
11:15–11:30	10	Martin Hřivňanský	Touch (in)sensitivity of anaesthetized plant leaves and how to measure it	*Palacký University*
11:30–11:45	11	Vojtěch Schmidt	Revealing the origins of phytohormones: a profiling perspective	*Institute of Experimental Botany AS CR*
11:45–12:00	12	Jing Xu	Predictions of PIN-Likes Protein structures	*University of Freiburg*
12:00–13:30	lunch			
Biotic stress				
13:30–13:45	13	Matěj Drs	Chitosan stimulates root hair callose deposition and inhibits root hair growth	*Institute of Experimental Botany AS CR*
13:45–14:00	14	Lukáš Gímeš	The role of silicon in plant defence against aphids: a comparative study of barley and sorghum	*Comenius University in Bratislava*
14:00–14:15	15	Jhonny Hernandez	"Healthy poppy": Immune responses in *Papaver somniferum*	*University of South Bohemia in České Budějovice*
14:15–15:00	IL3	Hana Leontovyčová	Phytohormones as universal means of communicati-on in plant-microbe interactions	*Institute of Experimental Botany AS CR*
15:00–15:15	coffee break			
Abiotic stress				
15:15–15:30	16	Ondřej Gargoš	Regulatory mechanisms of exodermis differentiation under nutrient deficiency	*Charles University*
15:30–15:45	17	Natálie Závorková	How Phototropins influence drought stress responses in *Arabidopsis thaliana*?	*Palacký University Olomouc*
15:45–16:00	18	Ann Kokavcová	The effect of root cap removal on root lignification and physiological responses to abiotic stress in Pistia stratiotes	*Charles University*
16:00–16:15	19	Anna Kampová	Looking for the anther dehydration trigger	*Charles University*
Reproduction				
16:15–16:30	20	Dalibor Novokmet	The m^{6}A RNA methyltransferase subunits are required for male fertility in the moss *Physcomitrium patens*	*Comenius University in Bratislava*
16:30–16:45	21	Janto Pieters	Identification and characterization of GDPdl6: a novel gpi-anchored protein involved in pollen development and ovular attraction	*Institute of Experimental Botany AS CR*
16:45–17:00	22	Ömer Iltas	Comparative transcriptomics reveals differences in pollen-expressed genes between the selfer and outcrosser populations of *A.lyrata*	*Charles University*
17:00–17:15	23	Vinod Kumar	Structural insight into eiF3E function in the gametophyte	*Institute of Experimental Botany AS CR*
17:15–17:30	24			
17:30–17:45	25			
18:00–19:00	Dinner			
19:00–	Poster session			
Sunday 24. 9. 2023				
Plant Cell II				
9:00–9:15	26	Elnura Torutaeva	Characterization of a novel set of bzip transcription factors in Arabidopsis.	*Institute of Experimental Botany AS CR*
9:15–9:30	27	Anna Fleyberková	Characterization of ALBA family proteins in Arabidopsis thaliana	*Institute of Experimental Botany AS CR*
9:30–9:45	28	Lorena Huffer	Using fret-flim to visualize protein-protein interactions in planta	*Charles University*
9:45–10:00	29			
10:00–10:15	coffee break			
Others				
10:15–10:30	30	Karel Raabe	A Poltergeist in your genome – Words of caution regarding T-DNA-induced chromosome rearrangements	*Institute of Experimental Botany AS CR*
10:30–10:45	31	B. A. Morris	Uncovering crassulacean acid metabolism: the methods behind a complex trait, in a complex plant	*Newcastle University*
10:45–11:00	32	Tomáš Janicek	Characterization of Sex-linked Genes in *Silene latifolia*	*Institute of Biophysics AS CR*
11:00–11:15	33	Petr Hošek	Modelování a simulace fyziologických procesů - nový volitelný předmět KEBR PřF UK	*Institute of Experimental Botany AS CR*
11:15–12:00	IL4	Adéla Přibylová	An overlooked DNA repair mechanism in plants and animals? 5' nucleotide microhomology-mediated DNA repair after CRISPR/Cas9 cut	*Charles University*
12:00–12:30	Closing remarks			
12:30	Lunch			

ABSTRACT BOOK

PROGRAMME METHODS IN PLANT SCIENCES 2023

** the organisers have the right to change the programme*

colour coding

- Registration
- Start/start of conference
- meal
- coffee break
- CSEPB council
- Keynote lecture I
- Session - regular lectures
- From other worlds
- Poster session
- Sponsor presentation

Sunday 24. 9. 2022

	Presenting author	Tittle	Institution
14:00–22:00		Registration	
16:00–16:30		Start of conference	
16:30–17:15	Jenny Russinova	Small-molecule dissection of steroid signalling in plants	*VIB Gent*
17:15–18:00	Michal Cifra	Academic Tasks Made Efficient: Maximizing Productivity with AI and Large Language Models	*Institute of Photonics and Electronics, AS CR*
18:30–21:00		dinner + welcome party	

Monday 25. 9. 2022

7:30–12:00		Registration	
Microscopy, cell biology I			
8:30–8:55	Matyáš Fendrych	Live-cell imaging of root physiology	*Charles University*
8:55–9:20	Stefanie Müller-Schüssele	Visualizing subcellular redox dynamics using genetically encoded biosensors	*TU Kaiseslautern*
9:20–9:45	Marek Cebecauer	Cell surface nanotopography studied by super-resolution imaging	*J. Heyrovský Institute*
9:45–9:55	Eastport	Maxwell nucleic acid isolators and its use for detecting plant pathogens	
9:55–10:10		coffee break	
10:10–10:35	Christine Faulkner	Intercellular connectivity in complex tissues - assaying for plasmodesmal function	*JIC Norwich*
10:35–11:00	Marie Vancová	3D electron microscopy of the complex biological structures: current developments, available techniques, and future outlook	*PARU BC CAS*
11:00–11:15	Stanislav Vosolsobě	Live imaging of anther opening	*Charles University*
11:15–11:30	Martina Dvořáčková	Click-it detection: superresolution and clem approaches to visualise the nuclear components in *Arabidopsis thaliana*	*CEITEC Brno*
11:30–11:40	SVEN Biolabs	KILOBASER SYNTHESIZER: Your fastest way to DNA & RNA	
11:40–12:10	Tomáš Pluskal	Decoding the chemical language of plants	*IOCB AS CR*
12:10–13:30		lunch	
Genomics and gene editing I			
13:30–14:05	Hana Šimková	Hi-C techniques: from genome assemblies to transcription regulation	*IEB AS CR*
14:05–14:30	Martin Mascher	Sequencing ever more genomes: pangenomics in crop evolutionary studies	*IPK Gartesleben*
14:30–14:55	Dominik Grimm	Efficient Permutation-based Genome-wide Association Studies for Normal and Skewed Phenotypic Distributions	*TU Munich*
14:55–15:05	Bio-Rad	BIO-RAD - your partner in genomics experiments; application possibilities of ddPCR	
15:05–15:25		coffee break	
15:25–15:50	Jan Skalák	Prime editing in Arabidopsis: using natural genetic variability of cytokinin receptors as a tool for drought-resilient varieties introduction	*CEITEC Brno*
15:50–16:15	Helena Štorchová	The assembly of plant mitogenomes from Oxford Nanopore reads	*IEB AS CR*
16:15–16:40	Vojtěch Hudzieczek	Functional genetics in non-model plants	*IBP AS CR*
Structural biology			
16:40–16:50	MGP	M.G.P. always active	
16:50–17:15	Karel Berka	Alphafoldology: Machine Learning Revolution in Structural Biology and How to Use It.	*Palacky University*
17:15–17:40	Hector Martinez-Seara Monne	Unlocking Nature's Secrets: Decoding Complex Biological Systems through Molecular Dynamics Simulations	*IOCB AS CR*
17:40–18:05	Petra Junková	Mass spectrometry in structural proteomics	*IOCB AS CR*
18:05–18:30	Roman Pleskot	Integrative structural biology - solving molecular architectures of large biomolecular assemblies	*IEB AS CR*
18:30–19:45		dinner	
19:45–21:30		Poster session (together with refreshment)	

Tuesday 26. 9. 2022

Microscopy, cell biology II			
8:30–9:10	Yvon Jaillais	Anionic phospholipids across scales: from plasma membrane nanodomains to plant development	*ENS Lyon*
9:10–9:25	Martin Potocký	Surveying The Landscape of Lipid-Protein Interactions in Plant Cells	*IEB AS CR*
9:25–9:50	Matias Zurbriggen	Plant synthetic and reconstruction biology approaches for the study and control of cellular processes in plants – optogenetics as an enabling technology	*HHU Düsledorf*
9:50–10:00	Sipoch	Influence of pipette technical specification to your pipetting results	
10:00–10:15		coffee break	
10:15–10:40	Karel Říha	Light sheet microscopy in plant cell biology	*CEITEC Brno*
10:40–11:05	Jiří Friml	Cyclic nucleotides as second messengers in auxin signaling and beyond	*ISTA Klosterneuburg*
11:05–11:30	Daniel van Damme	Novel tools to visualize protein-protein interactions in planta	*VIB Gent*
11:30–11:40	Trigon plus	Company presentation	
11:40–12:10	Matouš Glanc	Uniting Czech Scientists with International Experience	*Czexpats in Science*
12:10–13:30		lunch	
13:30–18:00	excursions / workshop	Clément Lafon Placette – 14:00–17:00, Introduction to methods fostering more effective teaching (30 slots)	*Charles University*
18:00–19:30		dinner	
18:30–20:00	CSEPB	The plenary council of the CSEPB	
19:30–21:00		Poster session (together with refreshment)	

Wednesday 27. 9. 2022

-omics			
8:30–8:55	Pavel Solanský	Single-cell transriptomics in the plant research	*ZMBP Tuebingen*
8:55–9:20	Martin Černý	Shotgun lipidomics - versatile and rapid tool for supplementing plant omics analyses	*MendelU Brno*
9:20–9:45	Małgorzata Kwaśniak-Owczarek	Analysis of translation in plant mitochondria	*University Wroclaw*
9:45–10:00	Michal Karady	Quantification of methionine derived compounds and selected phytohormones by LC-MS/MS in plants	
10:00–10:15		coffee break	
10:15–10:40	Ondřej Novák	Recent advances in plant hormone profiling	*IEB AS CR*
10:40–11:00	Marieke Dubois	Single-cell transcriptomics and long-term cell tracking to study the role and regulation of a new pavement cell fate regulator in Arabidopsis leaves	*Ghent Univerrsity*
11:00–11:10	Thermo-Fisher Scientific	Agrigenomics portfolio overview & Absolute Q – new dimension in quantitative PCR	

Unclassified			
11:10–11:25	Tetiana Kalachova	Imaging-based screening to study mechanisms of specific and non-host resistance to filamentous pathogens	*IEB AS CR*
11:25–11:40	Tomáš Moravec	How do you program your plant cells?	*IEB AS CR*
11:40–12:10	Josef Patzák	Hop plant - from history through -omics to beer	*Hop Research Institute*
12:10–13:30		lunch	
Unclassified			
13:30–13:55	Jiří Kubásek	Stable Isotopes – powerful tool in plant science	*University of South Bohemia*
13:55–14:15	Vít Gloser	Gas exchange response curves: gaining more from classical tools	*MUNI Brno*
14:15–14:30	Lena Hunt	Whole leaf method for detecting accumulation of ROS and flavonoids in Arabidopsis	*Charles University*
14:30–14:45	Ján Šmeringai	Root and shoot phenotyping in soil	*CEITEC Brno*
14:45–14:55	PSI	Company presentation	
Czech Infrastructure			
14:55–15:15	Jan Petrášek	Advanced imaging techniques - Czech Bioimaging Infrastructure	*IEB AS CR*
15:15–15:35	Jiří Vondrášek	ELIXIR and „Plant Science community“ - a carriage to tackle biological complexity via data	*IOCB AS CR*
15:35–15:55	Lukáš Spíchal	High-throughput, high-precision and affordable plant phenotyping	*CATRIN Olomouc*
15:55–16:05	KRD	Company presentation	
16:05–16:20		coffee break	
PhDs			
16:20–16:35	Monika Kubalová	Exploring auxin´s influence on cell wall of Arabidopsis root via pectin lyases	*Charles University / University of Leeds*
16:35–16:50	Karel Raabe	Implementation of Ribo-BiFC method to plant systems using splitted mVenus approach	*IEB AS CR / Charles University*
16:50–17:05	Pavel Krupař	A novel genetically encoded cell wall pH sensor	*Charles University*
16:05–17:20	Labdeers	Seeding, the bottleneck of plant phenotyping	
Genomics and gene editing II			
17:20–17:40	Tomáš Vlčko	Gene editing in barley	*IEB AS CR*
17:40–17:55	Ksenia Timofeyenko	CATSNAP - A machine learning tool revealing the plasticity of alternative splicing in plants and animals	*IEB AS CR*
17:55–18:10	Petra Procházková Schrumpfová	Golem: a tool for determining the distribution of gene regulatory elements within plant promoters	*MUNI Brno*
18:10–18:25	Aleš Kovařík	Cooperation is always advantageous: analysis of ribosomal RNA loci by cytogenetic, classical genomic and chromosome-scale assembly approaches	*IBP AS CR*
18:25–19:10	Hirofumi Nakagami	Phosphoproteomics to dissect Plant signaling pathways	*MPIPZ Cologne*
19:10–23:59		End of the coference	
19:45–23:59		Farewell dinner with music	

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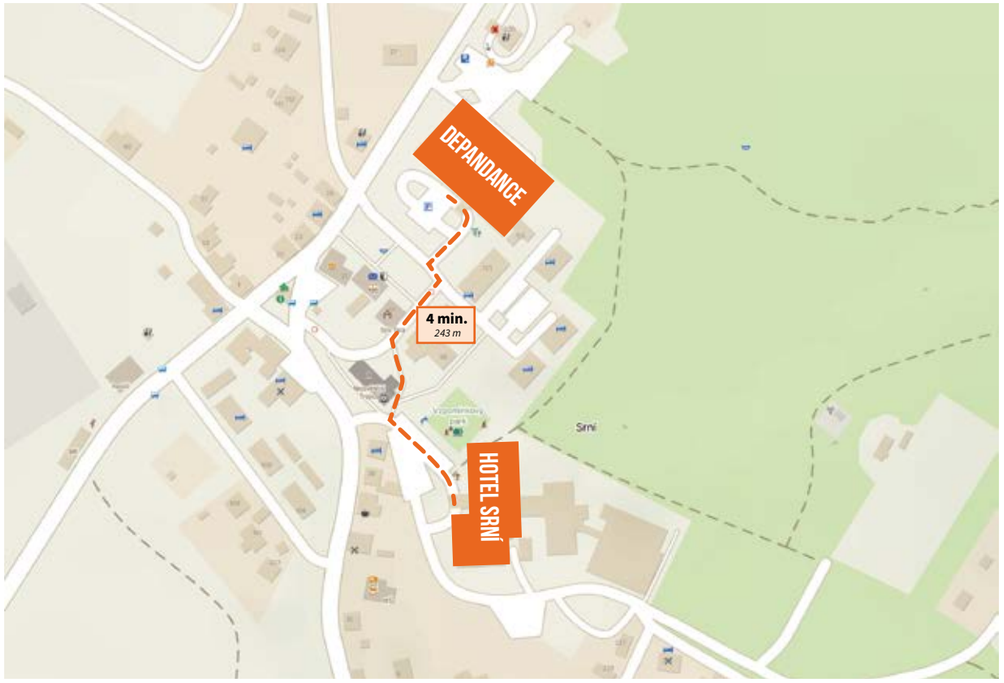

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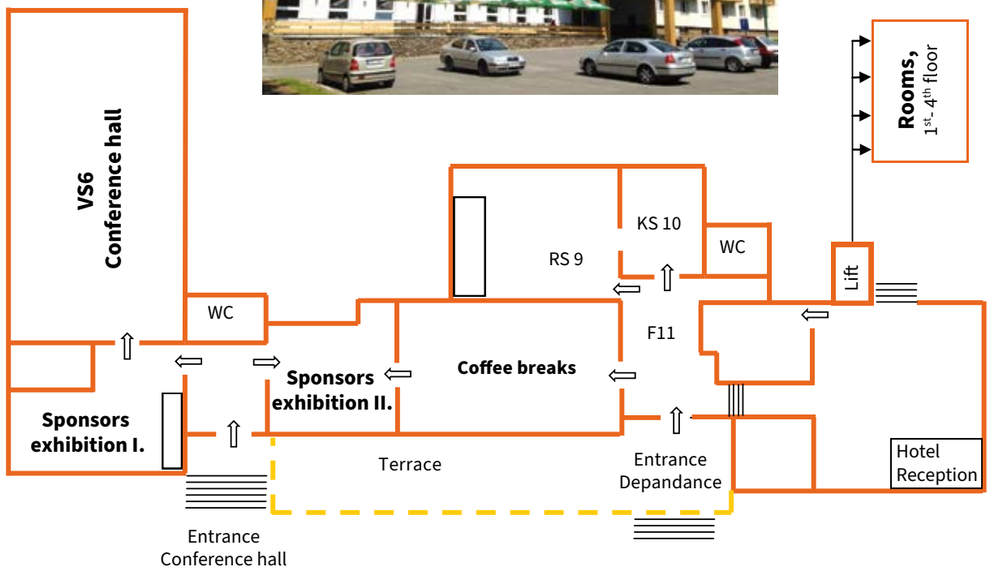

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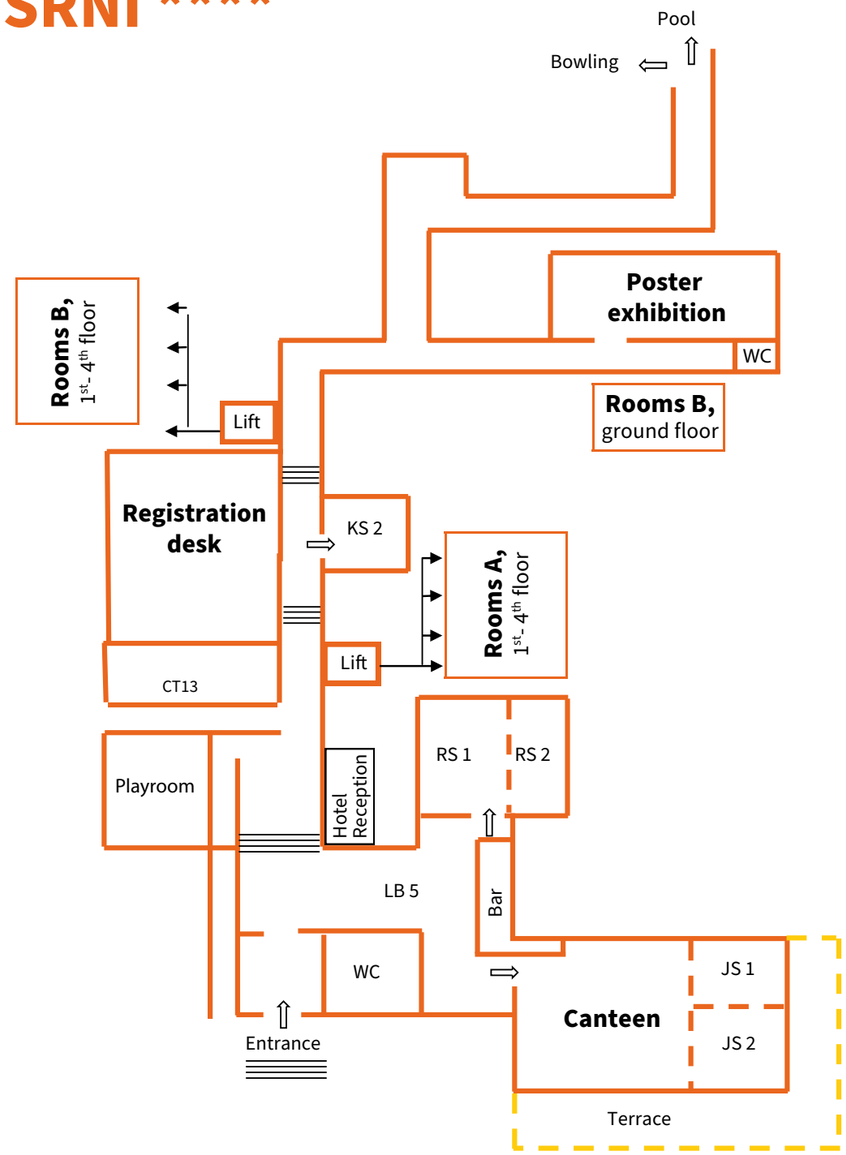

News, general informations and programme could be found on our webpage: https://csebr.cz/plantmethods2023/

ABSTRACT BOOK

22th–24th September 2023

17th Student Days in Plant Biology CS 2023

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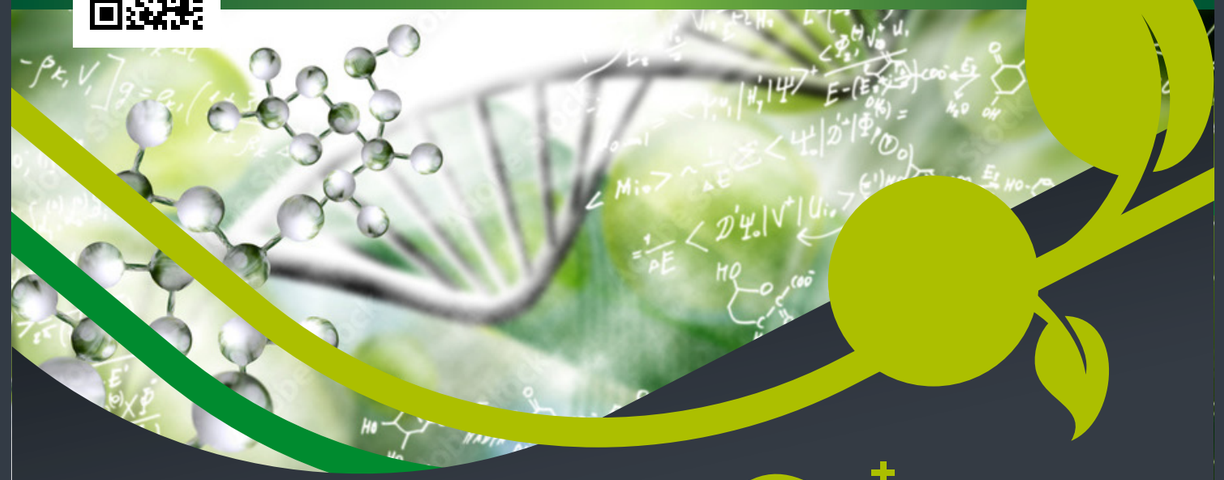

2023

Srní, Czech Republic

(in the heart of National Park Šumava)

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Přírodovědecká fakulta Faculty of Science

Jihočeská univerzita v Českých Budějovicích University of South Bohemia in České Budějovice

Česká společnost experimentální biologie rostlin

Institute of Experimental Botany of the AS CR, v. v. i.

Oral presentations

(alphabetical order)

Invited speakers

Tomáš Figura
Jana Koller
Hana Leontovyčová
Adéla Přibylová

Speakers

Lucas Amokrane
Kristýna Bieleszová
Matěj Drs
Anna Fleyberková
Ondřej Gargoš
Lukáš Gímeš
Lenka Helusová
Jhonny Hernandez
Pavel Hladík
Martin Hřivňacký
Lorena Huffer
Ömer Iltas
Tomáš Janíček
Pavel Jelínek
Barbora Jelínková
Anna Kampová
Anna Kokavcová
Vínod Kumar
Bethan Morris
Michaela Neubergerová
Dalibor Novokmeť
Janto Pieters
Dzmitry Pruchkouski
Karel Raabe
Vojtěch Schmidt
Elnura Torutaeva
David Ušák
Jing Xu
Natálie Závorková

ENSLAVED AND ABUSED - ECOPHYSIOLOGY OF PLANTS THAT FEED ON FUNGI

T. Figura [1,2,3,4], J. Ponert [4], E. Tylová [4], A. Gredová [1,4], M-A. Selosse [3]

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A typical plant is green, photosynthetic, mycorrhizal, and with ample seed reserves. However, there are also achlorophyllous plants with tiny seeds and virtually no seed reserves. Apart from typical parasites, that are directly connected to the phloem and xylem, there are also indirect parasites that feed on fungal carbon originally fixed by autotrophic plants. This phenomenon known as mycoheterotrophy has independently evolved several times in phylogenetically distant lineages. Despite considerable phylogenetic distance, these plants have developed similar traits convergently. In addition to distinctive morphological traits in embryogenesis, protocorm formation, having only one meristem, or leaf reduction, these plants exhibit various physiological features. These include carbon gain from fungi, phosphorous and nitrogen accumulation in their tissues or loss of photosynthetic pigments. Some specialize in parasitizing a single fungal strain, while others exploit multiple hosts. The transition from autotrophy to mycoheterotrophy is a gradual process involving multiple steps, one of which is mixotrophy. Začátek formuláře It's intriguing that evolution has independently reinvented similar concepts multiple times.

PHYLOGENY OF JASMONATE SIGNALING IN CARNIVOROUS PLANTS

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The carnivorous plants in the order Nepenthales co-opted jasmonate signaling from plant defence to botanical carnivory. After detection of prey, jasmonates induce expression and secretion of digestive enzymes. However, carnivorous plants have at least eleven independent origins and we asked whether jasmonate signaling has been co-opted repeatedly in different evolutionary lineages of carnivorous plants. We ascertained that although the carnivorous plants from different evolutionary lineages use the same digestive enzymes, the mechanism of their regulation differs. All investigated genera use jamonates for their ancient role - defense, but the co-option of jasmonate signalling for botanical carnivory probably occurred only once in the oldest lineage of carnivorous plant – Nepenthales, and has never been recruited again.

PHYTOHORMONES AS UNIVERSAL MEANS OF COMMUNICATION IN PLANT-MICROBE INTERACTIONS

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Phytohormones are small molecules essential for regulation of plant life processes including communication with other organisms in the environment. The surrounding organisms include pathogens, endophytes and symbionts and they trigger specific response of the plant phytohormone signalling which leads to activation of plant immunity. The plant immunity activation can be modulated by various resistance inducers, such as actin depolymerizing drugs, which often act via phytohormone pathway activation. Plant pathogens can also modulate phytohormone signalling to their advantage. Some pathogens can synthesize phytohormones themselves. Fungal pathogen *Leptosphaeria maculans* synthesizes a variety of growth-related phytohormones such as auxins and cytokinins as well as defence-related phytohormone salicylic acid (SA). The fungal-synthesized SA might then serve as means of communication between *L. maculans*, its host plant and other occuring microorganisms. The putative SA-biosynthetic pathway in *L. maculans* shares similarities with plant SA biosynthesis. While the synthesis of auxins and cytokinines has been described before this is the first evidence of salicylic acid being synthesized by a fungal pathogen.

AN OVERLOOKED DNA REPAIR MECHANISM IN PLANTS AND ANIMALS? 5' NUCLEOTIDE MICROHOMOLOGY-MEDIATED DNA REPAIR AFTER CRISPR/CAS9 CUT

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The CRISPR/Cas9 system is widely utilized across scientific disciplines, spanning from basic research, where it is used, for example, to generate knock-outs of specific genes to study their role, to applied research and plant breeding. This programmable target-specific nuclease induces double-strand DNA breaks, which are repaired by error-prone DNA-repair mechanisms that may result in deletions, insertions, or a combination of both. Those repair mechanisms are relatively conserved within plants and animals, but their activity differs within individual organisms, tissues, and the cell cycle stages.

In our recent study on *Nicotiana benthamiana* (Přibylová et al., 2022), we showed that a significant proportion of deletions might be directed by a 5' nucleotide at the PAM proximal end of the Cas9-induced double-strand break. To our knowledge, this type of DNA repair was not reported before. At the 17th Student Days in Plant Biology CS 2023 conference, I will discuss to what extent this type of repair is found in other plant species and how it is represented in the animal kingdom.

Understanding the repair processes at the tissue/cellular level is crucial for enhancing the accuracy and efficiency of CRISPR/Cas9 applications. By predicting mutagenesis outputs, we can minimize the number of required experiments, significantly reducing time and costs to achieve the desired results. Describing the overlooked 5' mediated DNA repair after Cas9 cleavage contributes to improving the prediction of CRISPR/Cas9 mutagenesis outcomes. … But. Wait. This is not a grant proposal, right? So, does it really contribute to improving the prediction? Why the 5' nucleotide-mediated DNA repair was not reported before? Are we sure we are seeing what we think we are seeing? I cordially invite you to my lecture, followed by a discussion in which we can critically evaluate the results of our work together.

AT THE HEART OF THE BALANCE BETWEEN IMMUNITY AND GROWTH IN PLANTS: EFFECT OF SALICYLIC ACID ON PLANT METABOLISM ENZYMES

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Salicylic acid (SA) is a crucial phytohormone in plant physiology. The accumulation of SA that occurs after a pathogen attack allows the induction of the main *PR* (Pathogenesis-related) genes that participate in the immunity response. This can be done through the activation of the so-called NPR1 (Non-expresser of Pathogenesis Related 1) pathway, which is a transcriptional regulator that requires a change in the intercellular redox balance to become activated.

In order to act on the cell machinery, SA must necessarily bind to one or more proteins, which will trigger a sequence of cellular events leading to the change in the cellular redox balance. The first identified Salicylic Acid Binding Proteins (SABPs) was catalase. Besides, a team recently identified different SABPs using a high throughput screen in *Arabidopsis thaliana*. What emerged from this study was the presence - among SABPs - of many proteins that are part of primary metabolism and certain enzymes involved in managing the redox balance.

My thesis work consists in deciphering the molecular determinants of the binding of SA to redox related enzymes. I have started this project with Arabidopsis catalase 2 (CAT2). CAT2 structure was modelized and simulated in presence of water using AMBER suite. Docking allowed to identify putative SA binding pockets. Of these pockets, one of them appeared consistent with SA inhibiting catalase activity. The role of this pocket in SA binding will then be experimentally proven by producing WT and mutated proteins in vitro and assessing SA binding and inhibiting effects on them.

NEW AUXIN DERIVATIVES: ELUCIDATION OF THEIR BIOLOGICAL ACTIVITY AND POTENTIAL APPLICATIONS

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Auxin is a major phytohormone that controls various aspects of plant growth and development. Many of these effects are orchestrated by asymmetric auxin distribution. Anti-auxins, on the other hand, have been developed to competitively inhibit auxin action. Synthetic auxins and anti-auxins have found a role as important auxiliaries in biological studies. Different approaches for visualization of their subcellular and tissue-specific localization are in process of development for decades. Indirect auxin detection using auxin-sensitive reporter lines such as DR5::GUS, DR5::GFP, etc., has been used to study auxin distribution patterns. As alternative, fluorescent labelling or click chemistry approach has been recognized as a possibility to visualize biomolecules *in vivo*.

Herein we report novel auxin derivatives and elucidation of their biological activity. The most promising compounds are further investigated for effect on auxin-mediated plant growth and development. Some of these compounds do not possess auxin activity but, on the contrary, they inhibit auxin-induced responses and had been demonstrated to be useful for manipulations of auxin-regulated processes, such as improving plant micropropagation. In addition, we focused our synthetic efforts on potentially suitable substrates for visualization of our derivatives *in planta*.

This work was supported by the Palacký University Young Researcher Grant Competition (JG_2020_002) and by the internal agency of Palacký University in Olomouc (IGA_2023_016, IGA_2023_031).

CHITOSAN STIMULATES ROOT HAIR CALLOSE DEPOSITION AND INHIBITS ROOT HAIR GROWTH

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Angiosperm plants have a general capacity to react after the treatment with immunity elicitors chitin or chitosan by the cell wall callose deposition. We are showing for the first time that the growing root hairs (RHs) of Arabidopsis can also respond to a mild (0.001%) chitosan treatment by the callose deposition, as well as by a deceleration of the RH growth. We demonstrate a key role for the glucan synthase-like 5 (GSL5)/PMR4 in this process. Upon the 10 x higher chitosan concentration (0.01%) treatments, the RH callose deposition response is abolished, while the RH growth inhibition is more prominent. Similar responses are also present in the functionally analogous, and evolutionarily only distantly related RH-like structures rhizophores (lycopod) and rhizoids (bryophytes). In order to understand the specificities of the low and high concentration chitosan treatments, we analysed the PTI signalling components, gene expression, and we also focused on the RH cellular endomembrane and cytoskeleton modifications in response to these treatments. Our results point to the RH callose deposition as a strategy of soil anchoring plant cells (rhizoids/rhizophores/RHs) to deal with the mild biotic stress, while the treatment with high chitosan concentration prominently disturbs overall cell machineries, dynamics of tip-localised endomembrane compartments and RH growth thus precluding callose deposition.

CHARACTERIZATION OF ALBA FAMILY PROTEINS IN ARABIDOPSIS THALIANA

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Acetylation-Lowers Binding Affinity (ALBA) proteins are highly conserved proteins with the structural capability to bind DNA/RNA. They are present in eukaryotes as well as archaea and serve various functions. In eukaryotes, ALBA proteins participate in RNA processing and storage while in parasitic protozoa are involved in stress responses and ontogenetic development. *Arabidopsis thaliana* possesses six *ALBA* genes distributed across two families: Rpp20-like and Rpp25-like. These genes are actively expressed in root meristematic zone, pollen developmental stages including mature pollen and pollen tubes. ALBA proteins are natively localised in cytoplasm without specific localisation (root) or in a reticular pattern (pollen). They play a role in plant thermotolerance and are associated with stress granules and P-bodies. Our *ALBA* genes study in Arabidopsis is based on transgenic plants with various genotypes, CRISPR-Cas9 derived mutants and *ALBA* expression controlled by various promoters.

We recorded no significant impact of the *alba456* triple mutant to plant development and morphology. However, we discovered that even single *ALBA* gene expression elevation in sporophyte significantly affects phenotypic manifestations in juvenile and reproductive ontogenetic stages. Consequently, our current objective is to investigate whether an excessive amount of ALBA proteins can impair the functioning of stress granules and P-bodies.

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REGULATORY MECHANISMS OF EXODERMIS DIFFERENTIATION UNDER NUTRIENT DEFICIENCY

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An effective root system is important for acquisition of soil resources and for stress tolerance. Apoplastic barriers (endodermis and exodermis) are essential functional parts of roots with a major influence on their performance. They represent an important regulatory mechanism for the selective uptake of water and nutrients from the environment, protect against unfavourable compounds and prevent leakage from the roots. Apoplastic barriers respond to environmental conditions by changing their rate of differentiation, which is an important mechanism for regulating root transport properties.

Our work investigates the effect of nutrient deficiency on exodermis differentiation in roots and analyses regulatory mechanisms, especially the role of phytohormones. We analyse the variability of responses in different plant species and the functional importance of localized and systemic responses in the regulation of root transport properties.

The results show that enhancement of exodermal differentiation in response to nitrogen and phosphorus deficiency is a common trend that helps to fine-tune the overall transport properties of the root system and thus increase the efficiency of nutrient capture from heterogeneous soil.

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THE ROLE OF SILICON IN PLANT DEFENCE AGAINST APHIDS: A COMPARATIVE STUDY OF BARLEY AND SORGHUM

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Aphids are one of the major global pests of agricultural and horticultural crops, and farmers rely mainly on insecticides to control their populations. Insecticides having negative impacts on the soil quality, groundwater, and other beneficial insects, could be potentially replaced by silicon, which enhances plant resistance to various abiotic and biotic stress factors. The aim of this study was to investigate whether silicon application to the substrate increased plant defence capability against an aphid infestation and reduced a damage caused by these hemipterans on the host plants. We also compared the results between two economically important cereal crops, *Hordeum vulgare* and *Sorghum bicolor.* We assessed the defence capability of the tested plants based on the number of aphids on the plants and the tolerance indices calculated from the morphological and production vondrduboisparameters, and concentrations of photosynthetic pigments. We also measured the activity of antioxidant enzymes, as reactive oxygen species production is a common response to different environmental stressors, including aphid infestation. Our comparative study of two Poaceae species revealed that the silicon-mediated resistance to aphid infestation varies. Despite their taxonomic similarity, these two species showed different levels of Si-induced enhancement of production and physiological parameters after aphid attack. However, in both cases, the younger plant organs compared to the older ones exhibited more pronounced benefits of silicon supplementation under this biotic stress. Moreover, we observed a reduction of aphid abundance in Si-treated plants in both species.

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Key words: aphids, defence capability, *Hordeum vulgare, Sorghum bicolor,* silicon

LOCALIZATION AND CONNECTION OF IAA METABOLIC ENZYMES IN TOBACCO CELLS AT SUBCELLULAR LEVEL.

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Besides transport, auxin biosynthesis and metabolism are the most important determinants of auxin concentration gradients. Although auxin has been studied for many years, the complexity of the metabolic pathways is still not understood in detail. In addition to the well-known enzymes GRETCHEN HAGEN 3 (GH3) and DIOXYGENASE FOR AUXIN OXIDATION 1 (DAO1), the IAA-LEUCINE RESISTANT 1-like (ILR1-like) gene family of enzymes that catalyze the hydrolysis of all amino acid conjugates of the major natural auxin, indole-3-acetic acid (IAA), has recently gained importance. We were the first to localize selected GH3 and ILR1-like enzymes in tobacco cells. In tobacco BY-2 cultures, GH3 enzymes were found in the cytoplasm and nucleus. ILR1-like enzymes, on the other hand, were localized in the endoplasmic reticulum. The separation of the conjugation and deconjugation reactions into different compartments indicates the specificity of these processes as well as the interdependence of the reversal processes, which are important for setting the required level of free IAA. These findings open new possibilities for the study of the mechanisms of interaction of the different elements of auxin metabolism.

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“HEALTHY POPPY”: IMMUNE RESPONSES IN PAPAVER SOMNIFERUM

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The Czech Republic is the world's biggest producer, consumer, and exporter of breadseed poppy (*Papaver somniferum subsp. Somniferum*). Recently, the Czech blue poppy got PGI (Protected Geographical Indication) mark from EU Commission. Despite of the fact that bread seed poppy has significant agricultural and economic importance in the Czech Republic (and partly in eighteen other countries) no research was focused on unraveling how poppy immunity works on a molecular level. However, such research has a high potential to gain knowledge to develop new strategies for improving poppy yield..

In our work, we established poppy cultivation in controlled conditions. We study typical immune responses of eight available poppy cultivars. In particular, it analyzed the burst of reactive oxygen species, seedlings and adult plant growth inhibition, callose deposition, gene expression, and salicylic acid concentration after treatment with typical microbe-associated molecular patterns (MAMPs)In parallel, three pathosystems were partially established: *P. somniferum x Xanthomonas* papavericola (bacterial pathogen), *P. somniferum x Botrytis cinerea* (fungal pathogen), and *P. somniferum x Sclerotinia sclerotiorum* (fungal pathogen) . Our results show a significant divergence between the poppy cultivars regarding how their immunity reacts under the same MAMP , providing a clear and engrossing insight into how plant immunity differs even among the same plant species.

Our long-term goal involves obtaining novel knowledge about the poppy immune responses, which we plan to use for gene editing to create novel poppy genotypes with higher resistance against common poppy pathogens

METABOLIC PROFILES OF PHENYLACETIC ACID CONJUGATES DIFFER IN VARIOUS PLANT SPECIES

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Auxins are group of plant hormones that are essential for the plant growth and development. Several endogenous compounds belong to this group, such as indol-3-acetic acid (IAA) and phenylacetic acid (PAA). Their proper function strictly depends on concentration gradients in plant organs and cells, which are maintained through processes, such as biosynthesis, transport, and conjugation. In most of plants species, PAA is more abundant than IAA, but the concentration needed for auxin-like response induction is also much higher. Thus far, PAA metabolism pathways seem similar to IAA as the conjugates are formed by the same enzyme. However, only three conjugates (PAA-aspartate, PAA-glutamate and PAA-tryptophan) formed by the group of Gretchen Hagen 3 enzymes have been identified in plants.

In our work, we developed a LC-MS/MS method for the quantification of the whole PAA metabolite profile. We identified and quantified three new amino acid metabolites: PAA-leucine, PAA-phenylalanine, and PAA-valine, as well as PAA-glucosyl ester in plants for the first time. Moreover, we quantified the PAA metabolite profile in various plant species. Our findings suggest that the dominant metabolic pathways differ among these species. We also performed PAA feeding experiments with *A. thaliana* GH3 knockout lines to observe how the metabolism would be altered. Additionally, we measured how PAA metabolism responds to drought and salinity stress. We hope that these results will contribute to a better understanding of PAA metabolism and degradation since these processes are not yet fully understood.

TOUCH (IN)SENSITIVITY OF ANAESTHETIZED PLANT LEAVES AND HOW TO MEASURE IT

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Nowadays, it is well-known that plants sense their environment through many different receptors and downstream signalling pathways. Much has been written to date about the perception of light, temperature, water, chemicals, or various biotic factors such as pathogens or wounding caused by herbivores. Recent research has shown that plants also sense touch, and interestingly plants can react to touch as to stress factor. Touch signalling in plants can work by at least two independent pathways: one dependent on jasmonates (JAs) and the JAs-independent pathway regulated by specific calmodulin binding transcription factors and therefore by Ca2+. Recent findings from our laboratory have shown that general volatile anaesthetic (GVA) diethyl ether can effectively inhibit JAs and Ca2+ systemic signalling in response to wounding in Arabidopsis thaliana. Here we investigated the effect of diethyl ether anaesthesia on cytosolic Ca2+ concentration, phytohormone levels and touch responsive genes expression in response to gentle brush touching on Arabidopsis thaliana leaves.

USING FRET-FLIM TO VISUALIZE PROTEIN-PROTEIN INTERACTIONS IN PLANTA

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Protein interactions are crucial for cellular processes, but visualizing them in live cells is challenging. Traditional methods lack visual output or are limited by the diffraction limit of light (~ 200 nm). Here we present a new approach: FRET-FLIM combined with a synthetic orthogonal auxin ligand-receptor pair.

FRET-FLIM utilizes Förster Resonance Energy Transfer and fluorescence lifetime changes to assess protein-protein interactions in vivo. Without interaction, the lifetime is similar to the donor fluorophore's lifetime (~2.5 ns in the case of GFP). Interaction leads to FRET and a substantial reduction in donor fluorophore lifetime. We have created a set of positive and negative controls for validation of the method: free GFP to measure the lifetime of GFP alone, free GFP co-expressed with mCherry to measure the lifetime change resulting from random FRET, and GFP coupled to mCherry via a short linker sequence to measure the shortened lifetime.

Auxin (IAA), a phytohormone with diverse regulatory roles in plants, impacts various cellular processes. It is perceived in the cell by a co-receptor comprised of one protein from the TIR1/AFB protein family and one protein from the Aux/IAA protein family. To investigate the interaction dynamics between auxin and its co-receptor in space and time, we use the synthetic orthogonal auxin-receptor pair comprised of concave TIR1 (ccvTIR1) and convex IAA (cvxIAA). We have also tested the method for putative interactors of IAA17.

In summary, we address the challenge of visualizing protein interactions in live cells by combining FRET-FLIM with a synthetic orthogonal auxin ligand-receptor pair. The method is validated through positive and negative controls. In the context of auxin signaling, the synthetic auxin-receptor pair enables the precise investigation of the dynamics between auxin and its co-receptor. This innovative approach not only enhances our understanding of auxin signaling but also holds broader applications in plant science research.

COMPARATIVE TRANSCRIPTOMICS REVEALS DIFFERENCES IN POLLEN-EXPRESSED GENES BETWEEN THE SELFER AND OUTCROSSER POPULATIONS OF A.LYRATA

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Pollen germination and pollen tube growth, known as pollen performance traits, are essential processes involved in double fertilization to facilitate the delivery of male gametophytes to the female gametophyte in flowering plants. These traits have not only evolutionary implications, as they are important for the male reproductive fitness, but they are also of great importance for plant breeding and the production of fruits. The development of pollen performance traits is underlain by appropriate specific gene expression, and knowledge of these genes is well documented through molecular studies, especially with the use of Arabidopsis mutants. Although such studies provide useful knowledge on the molecular basis of pollen germination and pollen tube growth, the genes discovered via knock-out mutant approaches may not be important for pollen trait variation observed in wild populations. Therefore, it is important to implement additional molecular studies in natural systems. In this study, we used different natural populations of *Arabidopsis lyrata* expected to vary for pollen performance traits, i.e. selfing and outcrossing populations. We first performed in vitro pollen germination assays to test whether pollen performance traits indeed show differences between the selfer and outcrosser populations. Further, we performed transcriptomics to elucidate the differences in gene expression between selfer and outcrosser *A. lyrata* pollen. Our in vitro results showed that the outcrosser population exhibits significantly higher pollen germination rate and longer pollen tubes than the selfer *A.lyrata*. Moreover, our transcriptomic results revealed that differentially expressed genes between outcrossers and selfers pollens are significantly enriched in the spliceosomal complex, a cellular component, known to play a role in pollen tube growth. Our result provides potential evidence for the molecular mechanisms underlying pollen tube growth in natural populations.

CHARACTERIZATION OF SEX-LINKED GENES IN SILENE LATIFOLIA

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The *HANABA TARANU (HAN)* is a transcription factor and a key regulator of the floral development of *Arabidopsis thaliana*. Although present in other species (eg. *Fragaria ananassa Duch*., *Zea mays*, and *Medicago truncatula*), its function appears to vary among each of them. Here, we investigate the sex-linked homolog of the *HANABA TARANU* gene in a dioecious plant *Silene latifolia* (*SlHAN*), and its role in male and female flower development. Significant differences were observed between the structure and expression of X and Y alleles. Notably, the Y-linked allele *of SlHAN* shows signs of genetic degeneration, suggesting epigenetic silencing of this allele. To further understand the *SlHAN* function, we explored the expression of the *SlHAN* in developing floral meristems using RNA *in situ* hybridization, revealing sex-specific expression preceding the formation of a distinct male or female meristem. Additionally, we are currently characterizing the *SlHAN* expression via RT-qPCR and evaluating its role using posttranscriptional silencing. Our findings shed light on the role of *SlHAN* in floral development and provide insights into its expression dynamics. This study contributes to a better understanding of the molecular mechanisms underlying floral development, particularly in the context of sex chromosomes in *Silene latifolia*.

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THE ROLE OF ELEVATED AUXIN PERCEPTION IN THE PROLIFERATION OF AUXIN-AUTONOMOUS CELL LINES

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Since the first derivation of exogenous auxin- and cytokinin-dependent cell lines in the 1960s, attempts have been made to grow the cells in media without these phytohormones. Cytokinin habituation was achieved first, followed by auxin. Despite more than fifty years of knowledge of the process, the underlying mechanisms of habituation are still not understood. Our work focuses on two tobacco cell lines that have achieved auxin autonomy and can proliferate in phytohormone-free media.

We performed RNA-sequencing analysis of four tobacco cell lines: BY-2, BY-2H, VBI-0 and VBI-2b. BY-2 and VBI-0 represent exogenous auxin-dependent lines, whereas BY-2H and VBI-2b are auxin-autonomous lines. Transcriptomic comparison of the auxin-dependent and auxin-autonomous BY-2 lines revealed the 236-fold up-regulation of the tobacco homolog of the auxin receptor TIR1 in the BY-2H culture where we also observed increased transcript levels of auxin biosynthetic genes such as TAA and YUCCA. No such changes were observed in the VBI-0xVBI-2b pair, suggesting various mechanisms of auxin autonomy in different lines. We hypothesised that the BY-2H culture synthesises more endogenous auxin and is more sensitive to it. Metabolomic analysis showed increased levels of IAA and its conjugates in the BY-2H culture compared to BY-2, supporting this hypothesis. Whole genome sequencing showed that the BY-2H culture has 200 copies of the TIR1 genomic region, which appears to be distributed throughout its genome. The potential genomic spread would point to specific activation of the TIR1 adjacent transposon. Using detailed phylogenetic analysis, we identified four tobacco homologs of the Arabidopsis F-box auxin receptor TIR1/AFB1 family. To demonstrate the role of TIR1/AFB1 genes in promoting auxin autonomy, we overexpressed tobacco TIR1 homologs in BY-2 cultures.

Our results suggest that elevated levels of endogenous auxin and increased perception of the hormone may be sufficient to ensure the proliferation of BY-2H cells.

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ARP2/3 AND THE SECRETORY PATHWAY

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The ARP2/3 protein complex, a known nucleator of fine actin meshwork, has been extensively studied in animals and yeast due to its major impact on membrane remodelling and endomembrane regulation. The phenotypes of plants that lack ARP2/3 subunits prompt us to look for similar activity in plants. The ARP2/3 mutants display cell-cell adhesion defects likely caused by defective cell wall synthesis/modification, which heavily relies on an intact endomembrane system. Using TIRF/VAE microscopy, we have found the ARP2/3 complex to localise near the plasmatic membrane very dynamically. The ARP2/3 also interacts with the exocyst complex and impacts its dynamics. Intriguingly, we have found disturbances in the retrograde pathway such as pharmacological inhibition by BFA and mutation in GNL1, a regulator of COPI vesicle formation[1] also to cause similar adhesion phenotype. These findings propose a versatile role of the ARP2/3 in the endomembrane regulation beyond the secretory pathway[2] . Notably, we have identified a comparable localisation pattern between the ARP2/3 activator, NAP1, and previously reported pectin localisation[3] in the cell wall, further emphasising the potential significance of ARP2/3 in plant cell wall synthesis and modification. These findings contribute to a better understanding of the diverse functions of ARP2/3 in plants and shed light on its involvement in various aspects of endomembrane dynamics and regulation.

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LOOKING FOR THE ANTHER DEHYDRATION TRIGGER

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Anther dehiscence is a developmental process that occurs at the end of the plant life cycle. It enables the dispersal of pollen grains, allowing them to participate in pollination and fertilization. Prior to anther opening, several essential events must take place, including the deposition of secondary thickenings in cells called endothecium, as well as programmed cell death of certain tissues. Subsequently, the anther walls dehydrate, leading to the outward bending of anther walls and thus the opening of the anther. The aim of this study is to uncover the mechanism of dehydration. To investigate the impact of external conditions on dehiscence, we examined the influence of high humidity, confirming its significant effect on dehydration. However, our analysis of *Arabidopsis thaliana* fluorescent marker lines for vacuole and plasma membrane integrity revealed that anther walls cells remain intact only minutes before sudden programmed cell death occurs. This suggests that the timing of dehydration may be internally regulated. Additionally, we conducted observations on stomata mutant lines. Mutant lines with an increased number of stomata exhibited faster anther opening compared to both the wild-type and a mutant line with a reduced stomata number, which showed delayed dehiscence. Our findings demonstrate that a combination of external and internal triggers is necessary for anther opening, with stomata potentially facilitating the crosstalk between these ambiguous signals.

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THE EFFECT OF ROOT CAP REMOVAL ON ROOT LIGNIFICATION AND PHYSIOLOGICAL RESPONSES TO ABIOTIC STRESS IN PISTIA STRATIOTES

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The specific role of the root caps of terrestrial plants is well known and described. However, some aquatic plants, including *Pistia stratiotes* (L.), have developed a particular type of the root cap which is slightly different in comparison with the caps of the terrestrial plants. The root cap of this floating aquatic macrophyte is very easy to remove, which makes it a suitable model for studying their role in the metal uptake. The purpose of this study was to determine the effect of root cap removal on physiological activity and lignification of root tissues in response to the various concentrations of antimony in the nutrient solution. Intact plants were cultivated in the Hoagland nutrient solution under control conditions and with additional supply of Sb in 10 mg L^{-1} and 20 mg L^{-1} concentrations. Parallel treatments were done with plants on which the root caps were removed before the start of the treatment. The amount of Sb in the plant biomass was analysed, however no significant differences among the treatments were detected. Furthermore, activity of antioxidant enzymes, specific proteins responsible for scavenging of reactive oxygen species, was determined in the plant roots and leaves after 3 and 7 days of treatment. The most interesting differences were observed in the G-POX activity. Histochemical analysis was performed to visualise lignin deposition in the root tips, since lignin is one of the phenolic biopolymers responsible for the formation of Casparian bands, the first phase of endodermal or exodermal cell wall thickening. Development of Casparian bands was detected closer to the root apex in the roots grown without the root cap which demonstrates their importance in the protection from excessive metal uptake.

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Key words: antimony, aquatic plants, Pistia stratiotes, root cap, stress physiology.

STRUCTURAL INSIGHT INTO EIF3E FUNCTION IN THE GAMETOPHYTE

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In flowering plants reproduction, a vegetative cell forms a polarized single cell extension, termed pollen tube (PT), responsible for dispensing two non-motile sperm cells into the female gametophyte. Previous reports indicate that eukaryotic translation initiation factor 3 subunits (eIF3), are predominantly present in mRNA storage compartment of mature pollen. Among all eIFs, eIF3 is contemplated to be the largest known complex elaborated in both sporophytic and gametophytic development. Here, we account that eIF3E subunit in *Arabidopsis thaliana* is vital for male and female gametogenesis. Our groundwork indicates that loss-of-function of *eif3e* not only affects pollen development at post pollen mitosis I (PMI), but also pollen germination, embryo-sac cell fate specification and defect in fertilization. eIF3E is ubiquitously expressed on the vegetative cell membrane and the cytoplasm. Concurrently, regulators of mRNA translation, PABP5 co-localize with eIF3E in pollen and PT, implying a possible association of eIF3E with RNA. Our findings disclose that all truncated eIF3E domains induce a dominant loss-of-function giving rise strong pollen phenotype and resulting in protein aggregates in *in vitro* grown PT. Additionally, structural studies in tobacco PT unveil the dynamic regulation of PT growth in an oscillating manner. Collectively, our outcomes exposed the mechanistic properties of eIF3E on the control of mRNA transability which impacts the vital role of eIF3E during pre and post fertilization events.

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UNCOVERING CRASSULACEAN ACID METABOLISM: THE METHODS BEHIND A COPLEX TRAIT, IN A COMPLEX PLANT

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Agricultural land is becoming increasingly more droughted, causing crop loss. Crassulacean acid metabolism (CAM) is a carbon capture mechanism that exists in ~7% of all plants. By temporally separating carbon fixation and photosynthesis, these plants are capable of increasing their water-use efficiency 10-fold. CAM can be constitutive, facultative or inducible.

Kalanchoe blossfeldiana is an inducible CAM plant. Leaves use C_3 photosynthesis early in their development and then transition to use CAM as they age, or due to an abiotic stress. To investigate this plant, we used an -omics approach alongside, conventional and modern gas exchange, titrations and plant physiology.

Our study has shown how metabolisms can mask each other and discovered more complexity behind CAM. To uncover plants with a CAM cycle we need to develop new methods, modernize old methods, and reapply knowledge.

PROTEIN-LIPID INTERFACES IN PLANT CELL TRAFFICKING STUDIED BY MOLECULAR DYNAMICS

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Anionic phospholipids (phosphatidic acid, phosphatidylserine, phosphatidylinositol, and its phosphorylated derivates phosphoinositides) are essential regulators of many cellular processes in plants, including signalling, cell trafficking, cell growth and division. They can modulate the physical properties of membranes, establish cell polarity, act as signalling molecules and finally also mediate interactions with peripheral membrane proteins (PMPs).

This project aims to identify PMPs of *Nicotiana tabacum* and unravel their specificity to anionic phospholipids using growing pollen tubes as a model system. To reach the goal, we first obtained a dataset of PMPs of *N. tabacum* that bind to anionic phospholipids of the plasma membrane. Next, we selected proteins of our interest (ones possibly involved in cell trafficking events emphasizing endo- and exocytosis) for subsequent experiments, including molecular dynamics (MD) simulations.

MD simulations can be seen as a computational microscope enabling the study of biological systems in unprecedented detail. Here, we used coarse-grained MD simulations to investigate the membrane binding of several *N. tabacum* endocytic adaptor proteins that possess an ANTH domain fold (identified in our dataset). MD simulations revealed differences in the ability of these proteins to bind anionic phospholipids, mostly in good agreement with experimental results. Moreover, this technique gave us insight into molecular details of the interactions enabling a more detailed understanding of the protein-membrane interfaces in general.

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THE M^{6}A RNA METHYLTRANSFERASE SUBUNITS ARE REQUIRED FOR MALE FERTILITY IN THE MOSS PHYSCOMITRIUM PATENS

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Keywords: epigenetics, male sterility; mRNA methylation; plant reproduction

Methylation of mRNA is an epigenetic mechanism of gene expression control conserved across all eukaryotes. The methylation of adenine nucleotide (m^{6}A) affects gene expression by modulating many aspects of mRNA metabolism such as maturation, export, and stability. *Arabidopsis thaliana* mutants lacking the core components of the m^{6}A methyltransferase complex, MTA and FIP37, are embryo-lethal and complement rescued plants with decreased m^{6}A methylation levels showing massive over-proliferation of the shoot apical meristem. Epimethylome analysis of these mutants showed that m^{6}A methylation affects the transcript stability of key meristem regulators *STM* and *WUS*. The core components of m^{6}A methyltransferase are present in the moss *Physcomitrium patens*, a representative of early land plants. To understand the function of m^{6}A RNA modification in *P. patens*, we generated knock-out mutants of the *PpMTA* and *PpFIP37* homologs, respectively. The deletion mutant plants formed fully developed leafy gametophores as well as both male and female reproductive organs. The *Dmta* and *Dfip37* mutants however were unable to produce sporophytes. To determine whether the lack of sporophytes is caused by male or female germ line sterility, we crossed the mutants with fertile fluorescent strain *Vx-dsRed* and male sterile strain *ccdc39*. Crossing *Dmta* and *Dfip37* mutants with a fertile *Vx-dsRed* strain yielded hybrid fluorescent sporophytes. We did not detect sporophytes after crossing with the male sterile strain. These results indicate that the deletion of *PpMTA* and *PpFIP37* cause male sterility in *P. patens.*

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IDENTIFICATION AND CHARACTERIZATION OF GDPDL6: A NOVEL GPI-ANCHORED PROTEIN INVOLVED IN POLLEN DEVELOPMENT AND OVULAR ATTRACTION

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Glycerophosphatidylinsitol (GPI)-anchored proteins (GAPs) are a ubiquitous feature of eukaryotic organisms. GAPs are targeted to the extracellular side of the plasma membrane via post-translational, C-terminal modifications known as GPI-anchoring. GPI-anchor synthesis mutants are embryo lethal, and have been shown to be critical for pollen tube (PT) targeting and attraction. As extracellular proteins, GAPs play a crucial role in environmental interactions and cellular perception. Consequently, pollen-expressed GAPs are prime targets for further investigation to elucidate their role in PT development, growth, and pollen-ovule intercommunication.

Here, we identified pollen-expressed GAPs and screened selected T-DNA mutants. Our results indicate that Glycerophosphodiester phosphodiesterase-like (GDPDL) genes could play an essential role in fertilization, as *gdpdl6* and *gdpdl7* showed reduced seed sets. GDPDLs have been implicated in cell-wall deposition and PT growth. Characterization of our novel *gdpdl6* T-DNA mutants revealed that the severe loss of seed set was due to pollen abortion and loss of PT attraction in mutant ovules. Reduced allele transmission was observed through the female gamete, confirming GDPDL6's vital function in female gametophyte. GDPDL6 localization confirmed the pollen plasma membrane targeting and additionally showed ovule filiform apparatus enrichment. Complementation rescued the phenotypes and recovered homozygous *gdpdl6* plants.GDPDL6 pollen and PT co-immunoprecipitation pulldown revealed interactions with pectin modifying enzymes elucidating to the potential function in pollen development. GDPDL6 enzyme characterization confirmed loss of canonical glycerophosphodiester activity.

Thus, GDPDL6 is a pollen-enhanced GAP but also expressed in ovule, and is required for pollen development and ovular attraction of the PT.

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METHODS FOR ANALYSIS OF RNA – PROTEIN INTERACTIONS

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Many RNAs and proteins inside a cell realise their functions by forming complexes with each other. High-throughput methods such as TRIP, iCLIP etc., provide a wealth of data, but are difficult to implement due to their high cost, equipment and data analysis infrastructure demands. Here, we explore possibility of studying RNA-protein interactions in a more approachable and less demanding way. As a rule of thumb, no single method provides all characteristics of a given interactions and each of the approaches has its own advantages and limitations. Traditionally, the method of first choice to study nucleic acid (RNA as well as DNA) – protein interactions is EMSA (Electrophoretic Mobility Shift Assay) which is the easiest to perform and provides the possibility to determine Kd of RNA-protein complex formation. On the other hand, as our research has discovered, it requires extensive optimization and often provides an unclear picture of binding.

As a result, we have employed a number of traditional, but less commonplace techniques, one of which is North-Western blotting. This determines whether a protein of interest (PoI) binds to radioactively labelled RNA. NW blots are time consuming compared to EMSAs, but the requirements for protein purity are much lower to a degree that even crude extracts are applicable.

Conformational changes of a forming RNA – protein complex have also been explored using Circular dichroism (CD) analysis, monitoring the change of chirality of RNA. Also to accrue data on bulk spatial organisation of the molecular ensemble in question and its tendency to aggregate, AFM (Atomic Force Microscopy) was tested.

A POLTERGEIST IN YOUR GENOME – WORDS OF CAUTION REGARDING T-DNA-INDUCED CHROMOSOME REARRANGEMENTS

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The use of T-DNA transformation is prevalent in *Arabidopsis thaliana* research and has expanded to a broad range of model crops and other species in recent years. While the introduction of T-DNA using the *Agrobacterium*-mediated transformation is straightforward, it is important to verify whether the desired genotype has been obtained. Here, we show the characterization of SALK T-DNA line with insertion in the gene encoding the subunit A of the eukaryotic translation initiation factor 3 (eIF3A), where heterozygous mutants showed higher frequency of aborted gametes. Interestingly, this phenotype was not observed in all heterozygous individuals. The investigation of such genotype-phenotype distortion pointed to a frequently occurring T-DNA-induced genomic rearrangements. Here we present the story of the eIF3A T-DNA line characterization and typical characteristics of T-DNA lines containing such rearrangements as a cautionary note to the plant research community, and to students especially, who use T-DNA transgenesis in their research.

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REVEALING THE ORIGINS OF PHYTOHORMONES: A PROFILING PERSPECTIVE

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The genomes of streptophyte green algae, closely related to land plants, typically do not show signs of developmental regulation by phytohormones. However, scattered reports of endogenous phytohormone production in these organisms exist. To bridge the gaps between available genomic and metabolomic evidence, we performed a comprehensive LC/MS-based analysis of multiple phytohormones systematically across streptophyte algae, including several land plants and chlorophyte algae for comparison. We show that the biosynthesis of compounds recognized as active phytohormones preceded the evolution of their receptors. Auxin, tRNA-derived cytokinins and salicylic acid were found ubiquitously, whereas abscisic acid and jasmonates only occasionally. Land plants were unique in the consistent detection of abscisic acid and signs of active auxin and cytokinin homeostasis, indicating these compounds only became phytohormones in the ancestral land plant. The culture media and control media were likewise analyzed to account for phytohormone excretion and environmental contamination, adding an unprecedented robustness to the analysis. Auxins and cytokinins were frequently excreted into the culture medium, while agar constituted a significant external source of salicylic acid.

CHARACTERIZATION OF A NOVEL SET OF BZIP TRANSCRIPTION FACTORS IN ARABIDOPSIS

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Transcriptional control is one of the most important means for regulating gene expression. One of the largest groups of transcription factors (TFs) in plants is the bZIP family TFs. In 2018, the bZIP family was updated to include four additional members, bZIP76-bZIP79, classified into Group E, alongside bZIP61. Microarray data showed the expression of bZIP61, bZIP77, and bZIP78 in pollen. Promoter-GUS fusions revealed that bZIP61 is expressed in both reproductive and sporophytic tissues, while bZIP77 is expressed exclusively in pollen. Transient localization experiments in tobacco leaves revealed that bZIP77 and bZIP78 localize to granules in the cytoplasm, while bZIP61 localizes exclusively in the nucleus; transient localization in tobacco pollen tubes revealed that bZIP77 and bZIP78 have uniform localization in the cytoplasm, with no granule localization. bZIP61 localized exclusively to the vegetative cell nucleus, but this time, in foci. Similar to the tobacco pollen tubes, in *Arabidopsis* pollen, bZIP77 and bZIP78 show uniform localization in the cytoplasm, while bZIP61 localized exclusively in the vegetative cell nucleus within foci. *bzip77/-* and *bzip61/-* single mutant lines showed phenotypic defects in pollen, while bzip*61/-* impacted ovule development. Protein-protein interaction assays showed that bZIP78 is unable to dimerize with other bZIP TFs, while bZIP77 and bZIP61 dimerize with members of Groups E and I. Our findings have started to unravel the function of these bZIP TFs in plant reproductive development.

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THEY SEE ME ROLLIN': CALLOSE SYNTHESIS BEHIND THE WHEEL OF PLANT CYTOKINESIS EVOLUTION

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In land plants, the separation of daughter nuclei during cytokinesis, the ultimate stage of cell division, is mediated by the inside-out formation of the membranous cell plate. The β-1,3-glucan polysaccharide callose is the first and the most abundant polysaccharide of the growing cell plate, with the unique properties of callose enabling the centrifugal expansion of the cell plate as well as the final attachment to the parental cell wall. Such centrifugal mechanism of cytokinesis is not conserved in all green plants, with several algal lineages exhibiting centripetal cytokinesis driven by actin-rich cleavage furrow, similar to animals and fungi. Nevertheless, even some furrowing plant species exhibit callose deposition into the centripetal cross wall, partially consistent with the highly conserved presence of callose synthesizing enzymes, callose synthases (CalSs), within the plant kingdom. What is the relationship between the evolution of CalS and plant cytokinesis? What facilitated the emergence of such an innovative centrifugal mechanism in land plants? Our robust unbiased evolutionary analysis of the CalS family provides novel insight into the possible processes underlying the plant cell division evolution.

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PREDICTIONS OF PIN-LIKES PROTEIN STRUCTURES

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Auxin is crucial for various aspects of plant development, including cellular differentiation and the formation of distinct plant organs. The tissue distribution of auxin depends on an interplay of inter- and intracellular transport processes. Here, we focus on the PIN-likes (PILS) protein family of putative auxin carriers at the endoplasmic reticulum. PILS proteins limit nuclear auxin signalling, presumably by limiting auxin diffusion into the nucleus (Barbez et al., 2012; Feraru et al., 2019). To predict structural features within PILS proteins, we previously compared AlphaFold-based structural predictions of PILS proteins with available structural information on PIN8 (Ung et al., 2023). PILS proteins exhibit highly conserved regions within their scaffold and transport domains. Notably, PILS1 displays an additional alpha-helix in its N-terminal region, which is unique among the PILS proteins in Arabidopsis. This N-terminal domain of PILS1 shows similarities to prolyl isomerases. We aim to functionally characterize PILS1 in Arabidopsis and assess if the prolyl isomerase provides a distinct function to PILS1.

HOW PHOTOTROPINS INFLUENCE DROUGHT STRESS RESPONSES IN ARABIDOPSIS THALIANA?

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Phytohormones are widely involved in plant stress responses. Abscisic acid (ABA) level increases under water stress and leads to stimulation of root growth and stomata closure through ion channels, resulting in reduced water loss. Conversely, stomatal movement is stimulated by blue light through a signaling pathway involving specific blue light receptors phototropins. This raises the question, how do ABA and PHOT signaling interact when a plant is subjected to drought stress?

To answer these questions, we chose to study *Arabidopsis* thaliana mutant lines *phot1*, *phot2* and *phot1/2* defective in the perception of blue light by the phototropins PHOT1 and PHOT2. We have selected three different experimental procedures. The first approach was an *in vitro* study of the phenotype of seedlings grown on solid medium under 7-day drought stress conditions mimicked by poly(ethylene-glycol) in different light conditions (dark, white, blue, and red light). We used the same approach for the phenotypic analysis and changes in hormone levels after exposure of seedlings to exogenously applied ABA. The second approach was to grow seedlings *in vitro* in liquid medium and expose them to short-term drought stress mimicked by either poly(ethylene-glycol) or sorbitol, followed by analysis of phytohormone abundance and gene expression for enzymes involved in ABA metabolism. The third approach is to monitor phenotypic changes in 6-week-old plants exposed to drought stress in conditions *in vivo*/in pots and under different light conditions.

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Poster presentations

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Jedrzej Dobrogojski
Dagmar Hromadová
Veronika Jirásková
Tereza Kalistová
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Milana Perković
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Martina Šuleková
Kateřina Vejvodová
Klára Veselá
Andrea Zounková

PLANT CELL WALL CHARGE AND ITS IMPLICATIONS FOR NUTRIENT ACQUISITION

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The plant cell wall is a multifaceted extracellular matrix that encloses each cell providing structural support and maintaining the integrity of plant cells (Shin et al. 2021). Up to 30% of the cell wall consists of pectin which is a mixture of acidic heteropolysaccharides that is post-synthetically modified by enzymes of the PECTIN METHYL ESTERASE (PME) family. PME's remove methyl groups from the pectin and leave behind negatively charged carboxyl-groups which provide a tremendous cation binding capacity of the cell walls (Haynes, 1980). It is text book knowledge that the majority of non-esterified pectin in the cell-wall is bound with divalent Ca^{2+} ions to form a so-called egg box structure important for cell strength and rigidity (Taiz and Zeiger, 2010). In addition, Ca^{2+} also serves as an important signaling molecule largely stored in the extracellular space or cell wall (Demidchik et al., 2018).

We hypothesize that the negatively charged plant cell wall could be beneficial for the acquisition of Ca^{2+} from the growth environment. In addition, we assess how cell wall charge impacts on Ca^{2+}-cell wall binding dynamics and what are its implications for downstream signalling processes in plants. In order to assess this biological question, we combine growth assays, *in-vitro* cell wall charge assessment as well as life-cell-vertical-confocal microscopy.

Shin, Y., Chane, A., Jung, M. and Lee, Y., 2021. Recent advances in understanding the roles of pectin as an active participant in plant signaling networks. *Plants*, *10*(8), p.1712.

Haynes, R.J., 1980. Ion exchange properties of roots and ionic interactions within the root apoplasm: their role in ion accumulation by plants. *The Botanical Review*, *46*, pp.75-99.

Taiz, L. and Zeiger, E., 2010. Plant Physiology, Ed Fifth.

Demidchik, V., Shabala, S., Isayenkov, S., Cuin, T.A. and Pottosin, I., 2018. Calcium transport across plant membranes: mechanisms and functions. *New Phytologist*, *220*(1), pp.49-69.

THE ROLE OF FASCICLIN-LIKE ARABINOGALACTAN PROTEIN 9 IN ROOT GROWTH OF ARABIDOPSIS THALIANA

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Arabinogalactan proteins (AGPs) are cell-wall localized glycoproteins, widely distributed in plant tissues. AGPs play an important role in the regulation of plant growth and development. Fasciclin-like arabinogalactan proteins (FLAs) with one or two fasciclin-like domains are likely involved in pectin cross-linking, cell wall signaling, and cell wall adhesion through the interactions of their FLA domains or carbohydrate moieties, but their roles are still yet to be fully characterised.

We focus on the FLA9 protein and its role in *Arabidopsis thaliana* root growth. *Fla9* knock-down mutant plants exhibit reduced root length, especially of lateral roots, and reduced branching. Within the root system, *FLA9* is expressed primarily in the root cortex, and the protein is distributed across the cortical cell walls with preferential localisation in the corners surrounding the cortical intercellular spaces. FLA9 appears to be involved in shaping the architecture of the root system by influencing the growth and emergence of lateral roots. The results also show that in addition to the root system, FLA9 affects seeds maturation and germination.

INSIGHT INTO THE ROLE OF M6A READERS IN MALE GEMETOPHYTE DEVELOPMENT

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The process of pollen development and maturation is highly organized and controlled by numerous post-transcriptional regulatory mechanisms. One such mechanism involves mRNA modifications that affect mRNA metabolic processes (stability, translatability, splicing, etc.), where the most prevalent mRNA modification is the N6-methyladenosine (m6A). YTH domain-containing proteins, known as m6A readers, recognize and binds to these m6A marks, which leads to specific regulation of the transcript. Some of these reader proteins, the EVOLUTIONARILY CONSERVED C-TERMINAL REGION (ECT) family members, have been shown to regulate transcripts fate during the sporophyte development. However, their involvement in male gametophyte remains unexplored. To address this, we investigate the role of reader proteins associated with m6A modification in the course of male gametophyte development. We present here functional characterization of three m6A reader genes that are highly expressed in *Arabidopsis thaliana* pollen, ECT5, ECT7, and ECT10.

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EFFECTS OF SALICYLIC ACID ON PLANT CUTICLE COMPOSITION AND RENEWAL DYNAMICS IN ARABIDOPSIS THALIANA

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Salicylic acid (SA) is a vital phytohormone whose role in plants is mainly connected with the involvement in plant immunity and defence against pathogens. However, it is well-known that a high concentration of SA inhibits plant growth. This phenomenon is best described in SA-overaccumulating mutants that exhibit dwarf phenotypes. The general explanation for growth inhibition is that induced immunity redirects plant nutrient flow from growth to defence. We hypothesise that such a modification in nutrient flow would also affect cuticle composition and renewal dynamics. The cuticle is the first physical barrier between the plant and the outer environment. Its composition and renewal dynamics are tightly connected and dynamically regulated because the biosynthesis of the cuticle is highly energy-consuming. In this work, we focus on the effect of SA on cuticle permeability, composition, and renewal dynamics. We monitored the properties of cuticle wax using GC-MS in mutants of *Arabidopsis thaliana* with modulated SA concentration. Within the analysis of cuticular wax dynamics, plants were labelled in an atmosphere enriched with $^{13}CO_2$. The data obtained contribute to a better understanding of how SA inhibits plant growth.

IS THE AGO-HOOK DOMAIN OF THE HISTONE CHAPERONE SPT6L INVOLVED IN PROCESSING OF RNA POLYMERASE II TRANSCRIPTS?

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Argonaute proteins (AGO) are executive components of regulatory pathways depending on sRNAs. AGOs can recognize target sequences complementary to associated sRNA and influence their expression either post-transcriptionally or transcriptionally. To guide the complex of AGO with sRNA, some proteins acquired a specialized AGO-hook domain. AGO-hook domain is an intrinsically disordered domain with conserved GW/WG motifs that binds AGO proteins. In plants some proteins that acquired an AGO-hook domain play a vital role in the RNA-directed DNA methylation (RdDM) pathway. These proteins include the largest subunit of Pol V and its transcriptional factor SPT5L, and also SPT6L which we proposed to partake in RdDM pathway. SPT6L, an orthologue of SPT6, is an essential transcriptional factor of Pol II conserved in all eukaryotes. In the Streptophyta clade, SPT6L has acquired the AGO-hook domain, which appears to be involved in processing of Pol II transcripts.

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USER-FRIENDLY TOOLKIT FOR CRISPR/CAS9 MUTAGENESIS IN TOBACCO BY-2 CELL LINE: INTRODUCING INDUCIBLE SELF-EXCISION SYSTEM

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Tobacco BY-2 cell line is the most commonly used cytological model in plant research. It is uniform, can be simply treated by chemicals, synchronised and easily transformed. One of its disadvantages - a lack of accessible mutant lines - was recently overcome by establishing CRISPR/Cas9 mutagenesis. However, the Cas9 cassette cannot be easily removed due to clonal reproduction, so the usage of mutated lines for further experiments is limited.

In this study, we established a CRE/Lox self-excision system that enables the removal of the Cas9 cassette after chemical induction. Our constructs are based on the GoldenBraid cloning system, which allows rapid cloning and is easily adjustable to the user's needs. Besides the basic parts, it includes a single or a multiplex system for gRNA expression, different selection markers and two optional inducible systems (XVE, VGE).

To assess the functionality of our system, we targeted the native gene family encoding RNA-dependent RNA polymerases 6 (RDR6). The mutated *rdr6* lines, exhibiting reduced post-transcriptional silencing, may find biotechnological application.

The other possible GoldenBraid components and useful tricks for cloning will be discussed.

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IN SILICO PREDICTION OF BEL-KNOX DIMERIZATION IN POTATO

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Potato (*Solanum tuberosum*) is a significant food crop that consistently ranks fourth in global production among major food crops. It encompasses numerous cultivars with variations in tuberization onset and yield, including the total mass and number of tubers. Extensive research has been conducted on the search for a phloem-mobile signal molecule controlling tuberization, leading to the identification of promising candidates: a) Flowering locus T-like protein SP6A, and b) BEL transcription factors (TFs), long-distance mobile transcripts.

BEL TFs belong to the TALE family of TFs, characterized by the presence of a three-amino acid loop extension (TALE) within their DNA-binding homeodomain (HD). In animals, TALE proteins have the ability to dimerize with other proteins, thereby influencing their activity and subcellular localization. In plants, the heterodimerization of TALE proteins belonging to the *BEL* and *KNOX* families has been extensively studied. However, detailed information about their subcellular localization and the presence of nuclear localization signals is currently lacking.

In potato tuber induction and development, three BELs are involved: BEL5 protein - the tuberization activator, and BEL11 and BEL29 proteins functioning as antagonists. The precise mechanism of their antagonistic action remains unclear. All three BELs have been found to form dimers with the KNOX protein POTH1 *in vitro*. However, only the BEL5-POTH1 complex has been tested for binding the promotors of target genes. We performed *in silico* predictions of potato BEL-KNOX dimer structures (BEL5, -11 and -29 with POTH1 and related POTH15) to aid in proposing the nature of their antagonistic functions.

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CROSSTALK ACROSS THE CUTICLE: MOLECULAR ASPECTS OF INTERACTION IN STOMATAL DEVELOPMENT AND WAX SYNTHESIS IN THE PLANT CUTICLE

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The plant leaf epidermis is covered with a hydrophobic layer, called a cuticle, consisting mainly of cutin and waxes, which protects plants e.g. against water loss. Pores in the epidermis, called stomata, regulate gas exchange and transpiration. Published information as well as our data suggest that stomatal and cuticular development could be coordinated at the molecular level through SHN transcription factors. Here, we focus on three members of the *SHN* (*SHINE*) family genes (*SHN1, SHN2 and SHN3*), which encode these transcription factors belonging to the AP2/EREBP (APETALA2/ ethylene-responsive element binding protein) family. Stomatal mutants with increased or decreased stomatal density (SD) increased or decreased simultaneously total epicuticular wax content. On the other hand, plants with increased expression of *SHN* family genes have more waxes and decreased SD. Therefore they have better tolerance to drought.

We identified five SALK homozygous mutant lines of Arabidopsis thaliana (ecotype Columbia) with T-DNA insertion in the SHN1, SHN2, or SHN3 gene, separately. Although we have identified homozygous lines slightly overexpressed or downregulated in SHN, we have found only moderated effects on SD and the total amount of chosen wax components, measured by GC-MS. The natural leaf surface structures were visualised using Cryo-electron microscopy.The expression of selected genes involved either in the cuticle or stomatal development was estimated by qRT-PCR.

CELL WALL CHARGE IN PLANT ROOTS AND ITS ROLE IN NUTRIENT ACQUISITION

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Plant cell walls are highly complex structures composed of diverse polysaccharides and structural proteins, which serve as a reservoir for iron, a mineral nutrient important for growth and development in plants. One of the major components of the cell wall is pectin, a structural acidic heteropolysaccharide which can be processed by PECTIN METHYL ESTERASES (PMEs).

PMEs are a group of enzymes that catalyze demethylesterification of pectin, and then generate free carboxyl groups which are negatively charged. More or less PME activity results in higher and lower cell wall charge respectively. These pectin modulations are associated with changes in iron immobilization capacity of the cell wall.

Our work assesses the molecular mechanisms by which plants adapt their cell wall charge to iron availability in order to optimize its acquisition from the environment.

DOES ARSENIC AND SILICON AFFECT IMPORTANT PROCESSES OF THE PHENYLPROPANOID PATHWAY IN MAIZE LEAVES?

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In recent decades, extensive investigations have been conducted on various biotic and abiotic stresses, revealing that the application of exogenous silicon (Si) can effectively alleviate the impact of these stresses on globally significant economic crops. To effectively apply silicon in practice and address environmental pollution caused by heavy metals or semi-metals, a comprehensive understanding of its metabolic effects is necessary. Both arsenic (As) and silicon (Si) are metalloids. While As is generally toxic, Si is considered beneficial for plants. One of the most important pathways that helps plants overcome various types of stress is the phenylpropanoid pathway. It provides the biosynthesis of a large number of very important substances such as lignin, suberin and many phenolic or flavonoid substances, including anthocyanins.

The main objective of our study was to compare two maize (*Zea mays* L.) hybrids, namely Tweetor (with higher drought tolerance) and Luciana (sensitive to drought), under exposure to toxic levels of As alone (75 µM or 150 µM) or in combination with silicon (Si). The plants were cultivated hydroponically for a duration of 10 days, with a particular focus on the shoots, specifically the first and second leaves, which were separated into blades and sheaths. We monitored the activity of key enzymes involved in lignin polymerization in cell walls, including polyphenol oxidase (PPO) and peroxidase (G-POX). Lignin is responsible for enhancing plant cell wall strength and resistance to various environmental conditions and stresses. Additionally, we also monitored specific metabolites such as anthocyanins and total phenolics, which also play very important role in plant defence mechanisms.

The results showed that in the first leaves, the activity of both PPO and G-POX enzymes was significantly higher in the Tweetor hybrid when exposed to As treatments, and the exogenous application of Si increased the activity of these enzymes. In the second leaves, the activity of enzymes was higher in the Luciana hybrid, and Si decreased their activity. Both anthocyanins and phenolics occurred to a greater extent, mainly in the Tweetor hybrid, but anthocyanins were most abundant in the leaf sheaths and phenolics in the blades. The concentration of anthocyanins and phenolics increased with increasing As, and Si reduced their accumulation. Based on our results we assume that the increasing of key enzymes activities and secondary metabolites in the Tweetor hybrid may serve as a defence mechanism against toxic As, which is likely associated with its higher tolerance to drought.

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THE USE OF HIGH-PERFORMANCE SEPARATION TECHNIQUES FOR MONITORING THE BIOACCUMULATION OF SELECTED MICROPOLLUTANTS IN PLANTS OF MODEL ROOT TREATMENT PLANTS

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A threat to the environment occurs when the concentration of non-steroidal anti-inflammatory drugs (NSAID) in the water is increased. They can cause unwanted cell growth in mammals but also weaken the functional organs of aquatic animals. For this reason, it is required to look for alternatives to remove NSAIDs from water in WWTPs. It is possible to remove ibuprofen (IBU) from wastewater by phytoremediation. This work deals with the determination of IBU, which is one of the most widely used NSAID, in model root filters in *Phalaris arundinacea*. The aim was to find out if the plant is able to accumulate IBU from the aqueous solution. HPLC with UV/VIS detection to determine the IBU content. A further aim was to validate and optimize the analysis of IBU. An XBridge C18 column (4.6 mm × 150 mm × 3.5 µm) was used for analyses with isocratic program and mobile phase of methanol and an aqueous acetic acid solution of pH 3.27. As part of the validation of the method, the repeatability, robustness, and yield of the method were verified. The yield of the method was 99.04 %. The values of relative standard deviations (RSD) when monitoring repeatability did not exceed 2 %, so the method is considered sufficiently accurate. The critical parameters of the used separation method are the column temperature and the ratio of the aqueous and organic phases in the mobile phase. A change in column temperature of +/– 5 caused retention time variability of around 10 % (RSD) and a change in mobile phase composition of +/– 2 % (*v*/*v*) caused a change in retention time of 16–20 % (RSD). Furthermore, the linearity of the method from 0.01 mg ml^{-1} to 2.0 mg ml^{-1}, the limit of detection (LOD = 0.049 mg ml^{-1}) and the limit of quantification (LOQ = 0.16 mg ml^{-1}) were determined.

It was proven that *Phalaris arundinacea* has the ability to accumulate IBU from an aqueous solution and efficiently transports it from the underground parts to the above-ground organs of the plant. Subsequently, it was found that the transported IBU is unevenly distributed in the leaves. Bioaccumulation was demonstrated and the highest concentration was detected by HPLC, which was 7.63 mg g^{-1}.

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TOOLS TO STUDY DOSAGE COMPENSATION AND PARENTAL IMPRINTING IN SILENE LATIFOLIA

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Dosage compensatory mechanism (DC) refers to the equalization of X-linked gene products between males and females. In old sex chromosomes systems, DC usually affects the entire X chromosomes with only few genes capable of inactivation escape. Nevertheless, in species with evolutionary young sex chromosomes, DC exhibits rather local or incomplete X chromosome inactivation, proceeding gene-by-gene or by whole locus deregulation. Despite the evidence given for the main animal species, the mechanisms through X chromosome inactivation evolves remain still enigmatic. To study early stages of DC and expression balance between sexes, we use combination of cytogenetic and NGS techniques in a species with young sex chromosome system, *Silene latifolia*. Using X chromosome specific marker and EdU that is incorporated into newly synthesized DNA strands, we distinguished paternal and maternal Xs. We show that parental X chromosome in females is late replicated, supported by previous evidence of transcriptome profiling and localization of active histone marks. Interestingly, only a chromosomal arm carrying PAR shows replication delay that suggests locus specific incomplete DC. Further, we ask weather polyploidy may disturb dosage compensation pattern in this model plant. To answer this question, we developed a set of tetraploid and triploid individuals with different sex chromosome constitution. This will help us to understand how and what (epi)genetic processes take a role in DC evolution, and help us to shed more light on the most fundamental question of sex chromosome biology.

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DECODING THE BIOSYNTHETIC PATHWAY OF THE ANTICANCER ALKALOID PIPERLONGUMINE IN PIPERACEAE PLANTS

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Piperaceae plants have been traditionally used to alleviate cancer-like symptoms.[1] Piperlongumine and its analogs are bioactive alkaloids produced by some plant species of the genus Piper. Piperlongumine selectively induces apoptotic and autophagic cell death pathways in primary myeloid leukemia cells[2] and glioblastoma multiforme cells[3] via inhibition of GSTP1 and subsequent accumulation of ROS.[4] Because of ROS induction, the compound also exhibited selective SARS-CoV-2 antiviral activity in a mouse model.[5] We used mass spectrometry analysis and molecular networking to analyze fifteen Piper plant species to obtain insights into the diversity of piperlongumine-related compounds. The analysis revealed the presence of a high amount of piperlongumine in *P. fimbriulatum* species. Using computational mass spectrometry tools MZmine, GNPS and SIRIUS, we obtained clusters of Piper "chemical space" and focused on the piperlongumine cluster. We combined fragmentation spectra comparison and SIRIUS prediction to annotate some of the piperlongumine analogues which could be potential candidates for drug development. Using metabolomics, we selected *P. fimbriulatum* because this Piper plant accumulates more piperlongumine than the fifteen other species we tested. Furthermore, in order to understand the biosynthetic pathway of piperlongumine we have generated hypotheses that amidation of piperlongumine might be catalyzed in a similar fashion to piperine through BAHD acyltransferase-mediated catalysis, considering that piperlongumine displays the same amide moiety as piperine, another piper alkaloid for which the last enzymatic step of biosynthesis is catalyzed by a BAHD enzyme.[6] Afterwards, we approached RNA isolation to obtain the transcriptomes of three Piper species including *P. fimbriulatum*. After mRNA sequencing data was obtained, we assembled their transcriptomes *de novo* using transXpress pipeline. Through a comparative analysis of transcriptomes of different species to identify homologous proteins of BAHD, we are conducting functional characterization of these proteins using agrobacterium-mediated heterologous expression in *N. benthamiana.* The products of the transient expression will be analyzed by high-resolution mass spectrometry to confirm the function of the enzymes. Elucidating the entire pathway holds promise for the production of piperlongumine and its derivatives via metabolic engineering. Compared to traditional technologies, bioengineering provides a more sustainable, less time-consuming process and ecologically favoured strategies to synthesize high-value chemicals.

References:
1. Wang, Y.-H. et al. Anticancer principles from medicinal piper (hú jiāo) plants. Afr. J. Tradit. Complement. Altern. Med. ***4****, 8–16 (2014).*
2. Xiong, X.-X.*et al.* Piperlongumine induces apoptotic and autophagic death of the primary myeloid leukemia cells from patients via activation of ROS-p38/JNK pathways. *Acta Pharmacol. Sin.* **36**, 362–374 (2015).
3. Liu, J. M.*et al.* Piperlongumine selectively kills glioblastoma multiforme cells via reactive oxygen species accumulation dependent JNK and p38 activation.*Biochem. Biophys. Res. Commun.***437**, 87–93 (2013).
4. Harshbarger, W.*et al.* Structural and Biochemical Analyses Reveal the Mechanism of Glutathione S-Transferase Pi 1 Inhibition by the Anti-cancer Compound Piperlongumine. *J. Biol. Chem.***292**, 112–120 (2017).
5. Tang, C. *et al.* A Selective SARS-CoV-2 Host-Directed Antiviral Targeting Stress Response to Reactive Oxygen Species. *ACS Cent Sci* **9**, 109–121 (2023).
6. Schnabel, A. *et al.* Identification and characterization of piperine synthase from black pepper, Piper nigrum L. *Commun Biol* **4**, 445 (2021).

STRESS DEFENCE EFFICIENCY VERSUS LIFE STRATEGY IN SORBITOL PRODUCING PLANTS

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Sorbitol is sugar alcohol which is, together with sucrose, the primary photosynthetic product in the *Plantaginaceae*. Both are used for long-distance transport of fixed carbon via phloem. In addition, many plant species accumulate sugar alcohols under drought or salinity. The aim of this project is to contribute to the understanding of the mechanisms of tradeoff between growth and stress protection in the genus Plantago, a model characterized by a broad carbohydrate spectrum. The goal is to describe selected metabolic and structure features with a focus on the sorbitol and sucrose balance in several Plantago species, which differ in life strategies (*Plantago lanceolata*, *P. maritima*, *P. eriopoda*, *P. subnuda*, *P. psyllium*, *P. major*, *P. coronopus*, *P. asiatica*). Our preliminary results comparing *P. lanceolata* and *P. maritima* show that sorbitol is accumulated in leaves up to tenfold concentrations compared to sucrose, which is further enhanced by salinity. However, the plant maintains a different ratio of sucrose to sorbitol in the

vascular tissue and a different ratio in the phloem. We hypothesize that the tolerance of halophytic *P. maritima* is mainly due to the different distribution of assimilates throughout the plant, but also to their different partitioning between the metabolic, storage and transport pools within the production leaf. Although slight differences were found in net photosynthetic rates, the two studied genotypes differed in growth rates – glycophytic species grew faster than the halophytic one. On the level of individual organs, the *P. maritima* leaves contained more soluble sugars (including sorbitol) than *P. lanceolata*. The genotypes varied in diurnal carbohydrate balance, when *P. maritima* plants preserved larger amount of assimilation starch compared to *P. lanceolata* at the end of the night. To extend our knowledge about their life strategies under non-stress conditions and under stress conditions (salinity) I repeated above mentioned analyses with additional species of Plantago genus.

ESSENTIAL OIL SPRAY HAS A REPELLENT EFFECT AGAINST FUNGAL PATHOGENS AND PLANT MITES ON CANNABIS

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Cannabis sativa is an economically and industrially important plant mainly due to its various cannabinoids, but frequent infestations by fungal pathogens and pests cause significant yield losses. These infestations need to be prevented by commercial chemical-based products. In our study, we tested the effects of essential oil solution against fungal pathogens naturally occurring in the greenhouse environment. The treatments were applied as sprays to mature *C. sativa* plants. The results showed that the spray containing, among others, the essential oil of some representatives of the *Apiaceae* family effectively reduced infestations of two highly prevalent fungal pathogens and pests of the *Eriophyidae* and *Tetranychidae* families.

OCCUPATION: THE PUPPET MASTER OF PHOSPHATIDIC ACID THE TARGETED MANIPULATION OF PHOSPHOLIPID LEVELS IN CELLULAR COMPARTMENTS

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Phosphatidic acid (PA), the simplest phospholipid, plays a crucial role in diverse cellular responses, including vesicle trafficking and cytoskeleton regulation. In plant cells, PA is compartmentalized into two distinct pools: signalling PA, predominantly localized on the plasma membrane, and metabolic PA, which resides in the endoplasmic reticulum and serves as a precursor for the synthesis of other phospholipids. The generation of PA occurs through two pathways: direct cleavage of structural phospholipids by phospholipase D (PLD), or a cascade of reactions involving phospholipase C and diacylglycerol kinase. Conversely, PA degradation is mediated by multiple lipid phosphatases, resulting in the production of diacylglycerol. To investigate the importance of various cellular PA pools, we established a method of its selective generation and depletion. To this end, we utilized truncated bacterial PLD and yeast cytosolic PA phosphatase Pah1 and fused them with compartment-specific targeting sequence. By introducing PA-modifying enzymes to the plant, we can manipulate the levels of PA in specific compartments and observe the resulting changes in cellular physiology *in vivo*.

AUXIN SENSING IN DEK1 MUTANT LINES IN PHYSCOMITRIUM PATENS

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The calpain protease DEK1 is a protein essential for proper asymmetric cell division and plays a key role in proper 3D growth architecture in plants. Auxin is a plant hormone that affects almost every aspect of plant growth and development. Previous work suggests that developmental processes regulated by DEK1 activity are largely controlled by phytohormones auxins. However, the specific interaction between DEK1 and auxins in the regulation of developmental processes is not yet known. The R2D2 auxin reporter system may be useful to reveal the relationship between DEK1 and auxins. This reporter is very sensitive and offers a precise view of auxin activity in cells.

In this work, we used auxin signalling reporter lines for targeted genetic modifications of *DEK1* in the model organism *Physcomitrium patens*. Using the moss-specific ratiometric reporter PpR2D2, we sensed auxin gradients in tissues of *PpDEK1* mutant lines. We noticed that *PpDEK1* deletion line (*Δdek1/R2D2*) shows reduced auxin sensing in buds. *Δdek1/*R2D2 is characterized by the arrest of bud development and the absence of gametophores. These results suggest that PpDEK1 is important for maintaining proper auxin sensing during the transition from the filamentous to the 3D growth phase of *P. patens*.

Acknowledgement: This work was supported by VEGA 1/0352/21 and APVV-21-0227 grants.

RESOLVING THE ISSUE OF SPORE VIABILITY IN LYCOPHYTES: BIOCHEMICAL APPROACH

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Spore viability is one of the most important indicators of the reproductive capacity of an individual. This indicator is used to understand population dynamics and speciation. The proportion of aborted spores (spore abortion index, SAI) is usually determined by morphology, germination test or differential staining of the spore content. However, these methods fail with lycophytes. Their spores have a thick sporopollenin wall that (i) prevents the penetration of dyes used to stain viable spores and (ii) makes the physical or chemical disruption necessary for germination difficult. In addition, lycophyte spores exhibit considerable morphological variability that is not taxon-dependent. Therefore, there is no consensus on the shape of abortive spores.

We selected a model group *Huperzia selago* agg. Where we expect a wide range of spore abortions. Our aim was to validate SAI (counting visually aborted, empty spores) against biochemical parameters representing spore viability (assessed by Fourier transform infrared spectroscopy, FTIR, ATR mode) in only 2–4 mg spore samples. We focused on the absorption bands of sporopollenin compared to proteins and oils, as aborted spores will be richer in sporopollenin and poorer in proteins and oils, in contrast to well-developed spores.

To overcome the thick sporopollenin cell wall, we had to grind the spores. However, the ATR-FTIR overestimated the oils due to their better contact with the ATR crystal. Therefore, we extracted the oils with hexane and quantified them gravimetrically. We found a close correlation between the oil concentration and the relative (FTIR-derived) protein content as well as a negative correlation between these parameters and the relative sporopollenin content. This confirmed the biochemical trade-off between developed and aborted spores. Most importantly, all parameters correlated well (positively or negatively) with the SAI, confirming the suitability of simple visual identification of aborted spores).

INTERACTION OF BIOCHAR AND PHOSPHORUS IN MODELS OF WASTEWATER TREATMENT PLANTS

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Phosphorus is a pollutant that must be removed from waste water. Moreover the concentration of P on the drain is regulated by government regulation. The aim of this work was to validate an easy and functional method for the determination of P in water matrix, utilizing a complex with malachite green. This method could be used for the determination of P in the samples from model constructed wetlands. Validation of the method included robustness in relation to the use of biochar (BC), which is used as a soil additive. The work also deals with the possible interaction of phosphate with BC.

For the determination UV-VIS spectrophotometry was used. The complex of phosphate ion (PO_4^{3-}) and malachite green has a maximum of absorption at 610 nm. The method is accurate and it has good repeatability. It was found that this method is several times more sensitive than the certified method. It's linear up to the concentration 0.228 mg(P) l^{-1}. The method is true, the deviation from true correct value was 5.34 %. The assessment of method robustness showed that the maximal permissible dilution of samples with BC was 1:250 (0.005 mg(BC) ml^{-1}). The limit of detection was $4.3 \cdot 10^{-3}$ mg(P) l^{-1} and the limit of quantification was $14.5 \cdot 10^{-3}$ mg(P) l^{-1}. During the evaluation of constructed wetlands models, it was found that four days after the addition of fertilizer the concentration of PO_4^{3-} dropped to its half.

During the observation of the effect of added BC to the concentration of PO_4^{3-} in the samples there were used two types of BC – water-washed (W-W) and air-activated (A-A). It was found that W-W BC in the sample causes 30.6 % increase of absorbance,

however A-A BC causes 4.3 % increase. A-A BC shows better interception of P. At the initial concentration of 0.1 mg(P) l^{-1} the interception of A-A BC was 31.1 % and 9.3 % of W-W BC.

We acknowledge the support of the Special joint project of the Institute of Experimental Botany, AS CR and the Region Pardubice of the Czech Republic (R200382101).

STUDY OF POTATO MUTANTS WITH AN AFFECTED TUBERIGENIC SIGNALING PATHWAY

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Potato tuberization is a complex biological process governed by a network of molecular pathways influenced by the internal plant status and environmental conditions. Tubers serve as essential energy stores and enable potato plants to withstand challenging environments. Moreover, potatoes are a significant crop worldwide. Therefore, understanding the molecular pathways regulating tuberization is crucial for crop improvement and maximizing yields. Recent studies have revealed key components of the tuberigenic signal, including SP6A, an FT homologue, and BEL1-like transcription factors (BEL5, BEL11, and BEL29). These factors, in the form of mobile proteins and mRNAs, respectively, are transported from leaves to stolons via the phloem. While BEL5 and SP6A support tuberization, BEL11 and BEL29 act as repressors, but the precise mechanism of their action remains unclear.

To further investigate the function of BEL11, we generated BEL11 RNAi lines and evaluated parameters associated with tuberization potential and other traits that impact overall tuber yields, such as photosynthesis, carbohydrate distribution, and biomass allocation. We observed significant changes compared to the wild type, including enhanced tuber yields in vitro, preferential biomass allocation to underground organs, and increased net photosynthesis rate.

Overall, the use of RNAi lines for studying mobile signals in potatoes has limitations, as the effectiveness of silencing depends on promoter activity, and variations may occur among organs and developmental stages. Therefore, we propose the utilization of the CRISPR-Cas9 method to achieve complete gene knockout.

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METHODS IN PLANT SCIENCES

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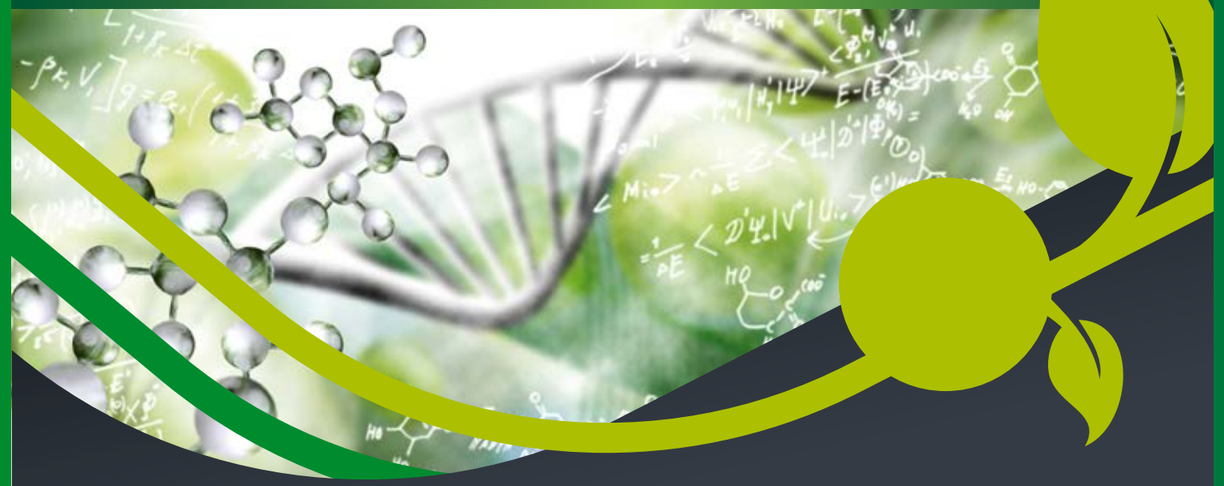

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ABSTRACT BOOK

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ČESKÁ SPOLEČNOST

Institute of Experimental

Methods in Plant Sciences 2023

Oral presentations

(alphabetical order)

List of speakers

Karel Berka
Marek Cebecauer
Michal Cifra
Martin Černý
Daniel van Damme
Tereza Dobisová (Labdeers)
Marieke Dubois
Martina Dvořáčková
Dominika Egermajerová (KRD)
Christine Faulkner
Matyáš Fendrych
Jiří Friml
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Vít Gloser
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Vojtěch Hudzieczek
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Katarzyna Kowalczyk (Bio-Rad)
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Marie Vancová
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Jiří Vondrášek
Stanislav Vosolsobě
Matias Zurbrigen

ALPHAFOLDOLOGY: MACHINE LEARNING REVOLUTION IN STRUCTURAL BIOLOGY AND HOW TO USE IT

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Machine learning is recently entering many scientific fields, but one of the most affected one is structural biology. Prediction of protein structure used to be an enigma, but the introduction of AlphaFold in 2020 has enabled unprecedented precision in protein structure prediction rivalling the experimental structures. As a result, many scientific tasks that requires protein structures are nowadays significantly easier or possible for the first time at all. But how to use it efficiently?

In this methodological lecture, we will show the basics of AlphaFold and algorithm as well as its possible uses and limitations.

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CELL SURFACE NANOTOPOGRAPHY STUDIED BY SUPER-RESOLUTION IMAGING

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T cells play a key role in the immune system of mammals. Their function is controlled by a panel of cell surface receptors. Non-random organisation of receptors enables that a limited number of signalling molecules controls diverse responses to ligands with similar physico-chemical properties. First, receptors were found in signalling microclusters, which, upon stimulation, accumulated in the central area of the contact between T cells and target cells, the immunological synapse. Improvements in biological imaging techniques enabled to uncover the origin of receptor microclusters. Nanoscopic T-cell surface morphologies, such as microvilli, drive segregation of receptors by spatio-temporal displacement. An example of CD4 and CD45 signalling molecules with opposite effect on T-cell activation will be used to demonstrate the importance of technological developments for a better understanding of immune response to pathogens under physiological and stress conditions.

Franke, C …. Cebecauer, M. Approach to map nanotopography of cell surface receptors. Comms Biol. 5: 218 (2022); DOI: 10.1038/s42003-022-03152-y

ACADEMIC TASKS MADE EFFICIENT: MAXIMIZING PRODUCTIVITY WITH AI AND LARGE LANGUAGE MODELS

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I will provide a range of practical instances that demonstrate how artificial intelligence tools can substantially enhance productivity within academic and scientific spheres. These examples span from specific technical undertakings to the composition of research papers, project proposals, and report writing.

The author generated the text in part with GPT-3.5, OpenAI's large-scale language-generation model. Upon generating draft language, the author reviewed, edited, and revised the language to their own liking and takes ultimate responsibility for the.

SHOTGUN LIPIDOMICS – VERSATILE AND RAPID TOOL FOR SUPPLEMENTING PLANT OMICS ANALYSES

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The lipidome represents one of the principal components of a cell. However, in contrast to other omics, its analysis is not as straightforward, and even the definition of lipids is not that simple. We have established a rapid screening protocol based on a chip nanoelectrospray source and a high-resolution mass spectrometer. The protocol is readily implemented in a routine proteome/metabolome workflow by aliquoting the nonpolar/semipolar fraction in the first step of the extraction protocol. The main advantage of the method is its low sample consumption and speed. The starting amount is as little as 10 mg fresh weight for Arabidopsis leaf tissue, and the direct infusion mass spectrometry analysis requires 2-5 min of measuring time per sample. In our experiments with contrasting plant tissues, including barley kernels, pea seeds, potato leaves, and poplar wood drillings, the technique confidently identified 200-300 most abundant lipid species. That number is far from the optimal lipidome coverage, but the list of identified compounds is larger than the corresponding set of confidently identified metabolites in an untargeted routine GC-MS metabolome profiling. The method's main limitations are in the quantitative analyses of isobaric molecules that can not be separated and are represented only by the highest-scoring compound in the corresponding MS2 spectra. In effect, their presence could interfere with the correct estimation of a lipid compound abundance. Further, for a reliable MS1 quantitation, the estimate of lipid content is needed, and reasonable relative comparisons are accessible only for samples that are not drastically different. This method is thus best applied in a combination with a TLC that provides rapid sample evaluation and semi-absolute quantitation.

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NOVEL TOOLS TO VISUALIZE PROTEIN-PROTEIN INTERACTIONS *IN PLANTA*

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Nearly all biological processes in every living organism depend on proteins and interactions occurring between proteins. Protein-protein interactions can range from short-lived, e.g. kinase-substrate interactions, to extremely long-lived, e.g. proteins assembling into protein complexes that remain stable over days. The investigation and identification of the protein interaction network surrounding a bait protein is one of the main approaches toward functional understanding of any specific protein of interest. Over the past years, the tools allowing the identification of protein interaction networks as well as the tools to independently confirm observed interactions have expanded greatly and several options have become available to visualize these interactions in planta. I will present some of the latest additions to the toolbox of protein-protein interactions for plant research and I will showcase the complementarity of the various tools we currently have at hand.

SEEDING, THE BOTTLENECK OF PLANT PHENOTYPING

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Background
Many of the Arabidopsis research programs are focused on the detailed characterization of early developmental growth, followed by costly and laborious omics data processing. To get maximum of these datasets it is crucial to reduce any source of variability. One of the most challenging sources of variability are the micro-sized seeds and their manual handling.

Technology
Here, we introduce Boxeed, the state-of-the-art technology intended for the non-invasive, dry-seeds phenotyping, sorting, counting and precise seeding to growth media. Boxeed is designed for 80µm–3mm seeds reaching the average working speed 600 seeds/hour. Phenotyping is based on 2D image analysis of individual seeds from multiple projections. The seed morphometric and fluorescence parameters are calculated in a real time, making it ideal for a seed selection and for single or multiple parameterized seed sorting directly from stocks.Results

In *Arabidopsis thaliana* Col-8 ecotype, we have identifiedthat just introducing proper seed-to-seed positioning and seed phenotyping is a key factor responsible for developmentally important impact during early postembryonic growth manifested as root growth variability. The described variability is quite considerable, and its understanding opens a bottleneck towards delicate characterization of the plant developmental processes.

SINGLE-CELL TRANSCRIPTOMICS AND LONG-TERM CELL TRACKING TO STUDY THE ROLE AND REGULATION OF A NEW PAVEMENT CELL FATE REGULATOR IN ARABIDOPSIS LEAVES

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The Arabidopsis leaf epidermis is a multifunctional tissue consisting of stomata and trichomes separated by puzzle-shaped pavement cells. Strikingly, whereas the ontogeny of the stomatal and trichome cells is well characterized, the **genetic pathways activating pavement cell fate are largely unknown**.

In our recent work (Dubois *et al*., 2023, *Nature Plants*), we reveal that the cell cycle inhibitor **SIAMESE-RELATED1 (SMR1)** is an important regulator of cell fate transition from stomatal lineage ground cells into pavement cells. Within the epidermis, *SMR1* is exclusively expressed in pavement cells and ectopic expression of *SMR1* in stomatal lineage cells forces them to acquire the pavement cell identity. By performing **cell tracking** we showed that the seemingly differentiated pavement cells of an *smr1* mutant lose pavement cell identity and divide to form stomata.

Currently, we are using **single-cell transcriptome analysis of *smr1* mutant leaves** to identify genes acting downstream of SMR1 in pavement cell fate acquisition. In addition, by studying the developmental trajectories of the epidermal cell populations in newly generated single-cell datasets of young, developing leaves (Tenorio Berrio *et al.*, 2022, *Plant Physiology*), we identified promising candidate transcription factors that could act **upstream of SMR1** in the process of pavement cell differentiation. We show that some of these transcription factors could indeed play a role in leaf differentiation, but additional molecular validation is still necessary. Finally, to screen for epidermal phenotypes, we developed a **semi-automated pipeline to track pavement cell behaviour** over a long period of leaf development. This non-invasive method based on leaf imprinting allows phenotyping of multiple lines in order to study these and other candidate regulators of pavement cell fate.

CLICK-IT DETECTION, SUPER-RESOLUTION AND CLEM APPROACHES TO VISUALISE THE NUCLEOLAR COMPONENTS IN ARABIDOPSIS THALIANA

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Nucleolus is a prominent membrane free nuclear domain formed by active transcription of 45S ribosomal RNA genes (rDNA). It is well structured into fibrillar center, granular component and dense fibrillar component, as revealed by electron microscopy. Nucleolar rDNA represents only about 10% of the total 570 copies found in the *A.thaliana* genome. We have previously isolated plant lines with reduced rDNA copy numbers and containing only the nucleolar rDNA fraction [1, 2], which facilitates investigation of rDNA organisation in the nucleolus. Deeper insight into the nucleolar space is possible due to the development of innovative super-resolution microscopy (SR) and we have previously implemented the Structured Illuminated microscopy and click iT chemistry to determine spatio-temporal distribution of rDNA during replication [3]. Click iT was also successfully used to label rRNA transcripts and visualise the nucleolus [4]. Recently, we focused on implementation of other SR approaches aiming to understand how active rDNA genes are organised in the nucleolus and how the epigenetic marks are distributed along rDNA fibers [5]. We found that further resolution can be achieved by the sample preparation, e.g. by extending DNA or chromatin into fibers or by isolation of naked nucleoli. Our latest research combines the correlative light and electron microscopy (CLEM) to monitor the distribution of replication foci in the nucleolus.

We will present the overview of abovementioned approaches and discuss their impact in the rDNA biology and investigation of the nucleolar processes.

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REFERENCES

Mozgova, I., P. Mokros, and J. Fajkus,. Plant Cell, 2010. **22**(8): p. 2768-80.; 2) Pavlistova, V., et al.,. Plant J, 2016. **88**(3): p. 411-424.; 3) Dvorackova, M., et al., J Cell Sci, 2018. **131**(2).; 4) Dvorackova, M. and J. Fajkus,. Front Plant Sci, 2018. **9**: p. 177.; 5) Kutashev, K.O., et al., Plant J, 2021. **105**(6): p. 1534-1548.

IBIDI: CELLS IN FOCUS

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INTERCELLULAR CONNECTIVITY IN COMPLEX TISSUES - ASSAYING FOR PLASMODESMAL FUNCTION

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Plant cells are connected by cytoplasmic bridges called plasmodesmata. Plasmodesmata are lined by the plasma membrane, essentially forming tunnels that directly connect the cytoplasm of adjacent cells through which soluble molecules can move from cell to cell. This cell-to-cell mobility is underpinned by cytoplasmic advection and diffusion in a manner dependent on molecular size, and whether plasmodesmata are open or closed defines the potential for molecular exchange between cells. GREEN FLUORESCENT PROTEIN (GFP) is a 27 kDa soluble protein that can move passively between cells via plasmodesmata. Thus, it serves as an ideal probe for assays to assess plasmodesmal aperture. GFP can be transgenically produced in single cells by microprojectile bombardment mediated transformation or by agroinfiltration, and its cell-to-cell mobility can be measured by live-cell imaging and counting the number of cells (or cell layers) to which it has moved. Thus, the number of cells in which GFP is visible serves as a measure of plasmodesmal aperture and functional cell to cell connectivity. In a complementary approach, live imaging can be used to quantify the callose at plasmodesmata, which is inversely correlated with functional aperture and GFP movement beween cells. All these experiments must be carefully designed and data arising from such assays is frequently heteroskedastic. Therefore, appropriate statistical analysis must be performed to extract meaningful and robust information from these experiments.

LIVE-CELL IMAGING OF ROOT PHYSIOLOGY

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Plants, often perceived as stoic organisms, live a very dynamic life constantly monitoring the internal and external environment and rapidly respond to a plethora of stimuli. Such signaling responses are manifested as cellular physiological outputs, making their analysis critical for unraveling the underlying molecular mechanisms of cellular behavior. In my contribution, I will present the hardware and tools that my team uses, encompassing live-cell imaging, microfluidics, fluorescence-based dyes, and genetic sensors. These techniques enable us to achieve cellular resolution in visualization of physiological processes in plant roots. I will focus on the root growth dynamics, kinetics of gravitropism, and visualization of apoplastic pH in roots to demonstrate the potential of these approaches. Furthermore, I will discuss the suitability of each technique for specific scenarios and demonstrate the remarkable potential of these approaches in unraveling the unforeseen aspects of plant hormonal signaling.

CYCLIC NUCLEOTIDES AS SECOND MESSENGERS IN AUXIN SIGNALING

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The phytohormone auxin acts in growth and development. Current paradigm says that the canonical TIR1/AFBs auxin receptors have E3 ubiquitin ligase activity and mediate transcriptional reprograming through ubiquitination and degradation of the Aux/IAAs transcriptional repressors. Accumulating evidence strongly suggests that TIR1/AFB receptors also mediate rapid auxin responses including cytosolic Ca^{2+} transients, plasma membrane depolarization and apoplast alkalinization. They all converge on root growth inhibition and are too fast to be explained by the transcriptional regulation. Recent discovery of an adenylate cyclase (AC) activity for TIR1/AFBs provided a plausible mechanism underlying these elusive rapid non-transcriptional responses. However, unexpectedly the TIR1 AC activity is not essential for the rapid but instead for the classical, transcriptional responses. Recently, we identified that TIR1/AFBs also have guanylate cyclase (GC) activity. The AC and GC activities are determined by adjacent but independent GC and AC motifs within the TIR1/AFB C-terminus. Our data suggest that in contrast to AC activity, which is crucial for transcriptional auxin responses, GC activity is specifically involved in rapid non-transcriptional auxin responses. Hence, TIR1/AFBs generate two major second messengers cAMP and cGMP in parallel, with each mediating a distinct set of transcriptional and non-transcriptional auxin responses. This unprecedented combination of AC and GC activities in a hormone receptor provides a new paradigm for how a single perception mechanism can mediate a multitude of diverse downstream responses.

UNITING CZECH SCIENTISTS WITH INTERNATIONAL EXPERIENCE

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Czexpats in Science is a community of more than a thousand Czech scientists who work abroad or have significant international experience. We started as a grassroots movement based on our own need to network with like-minded colleagues and stay connected with the Czech research environment during our own scientific stays abroad. Our mission is to connect Czech scientists with past, present and future international experience, and together inspire and positively influence the Czech scientific environment to make it as open and ambitious as possible.

Our main activities include networking events, such as the annual Christmas Conference, which brings together several hundred scientists each year who return to Czechia from abroad for the holidays. In cooperation with the embassies, we organise smaller meetings directly in the foreign scientific locations. We further build and develop the global community of Czech scientists online using the interactive *Map of Scientists* platform. We strive to inspire and influence Czech science both bottom-up by disseminating examples of the best practice from abroad, as well as top-down by advocating systemic changes in science policy at the institutional as well as governmental level.

GAS EXCHANGE RESPONSE CURVES: GAINING MORE FROM CLASSICAL TOOLS

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Methods based on the measurement of gas exchange (CO_2 and water vapour) between plants and the atmosphere have been well-established in many areas of plant science research. Simple monitoring of metabolic rates (photosynthesis and respiration) is a typical way of the use of this method on a routine basis. However, considerably more insight into plants' functional properties and acclimation ability provide response curves. These methods have been available for some time but undergo constant improvement. Namely, functional relationships between the rate of carbon assimilation and other parameters can be useful in many ways, but the outcomes are rarely fully appreciated. I would like to show recent trends in the use of three types of response curves and highlight some information that can also be gained.

New information can be obtained from the light curve – the relationship of the net carbon assimilation (P_n) and the irradiance provided in various parts of spectra using LED technology. We can see one of the practical outcomes of these measurements in the optimization of light supply in plant cultivations.

The A/Ci curve, relationship of P_n and concentration of CO_2 in the substomatal cavity provides substantial insight into processes limiting the carbon assimilation rate in the leaf. Modern instruments offer a new way of obtaining this relationship - "the CO_2 ramping" that shortens the measurement from 45 to 7 minutes. This new technique significantly increases measurements' productivity but should be used carefully. Combining the A/Ci curve data with simultaneous chlorophyll fluorescence measurements offers extra information about plant performance, but this option is not often used.

The relationship of stomatal response to changes of vapour-pressure deficit in the atmosphere is less widely employed. However, it provides data that can be used in selecting drought-tolerant genotypes or predicting plant response to climate change.

EFFICIENT PERMUTATION-BASED GENOME-WIDE ASSOCIATION STUDIES FOR NORMAL AND SKEWED PHENOTYPIC DISTRIBUTIONS

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Genome-wide association studies (GWAS) are a popular tool for analyzing the complex relationship between genotypes and phenotypes. Typically, linear mixed models (LMMs) are used to detect associations between genetic markers and complex phenotypic traits while correcting for population structure. Among other things, they assume normally distributed residuals and independent and identically distributed genetic markers. In real-world data, however, both assumptions are often violated. In a typical GWAS, up to millions of markers are analyzed simultaneously. Therefore, the critical step is to specify an appropriate threshold for distinguishing true from spurious associations while allowing for multiple hypothesis testing. However, the commonly used Bonferroni correction tends to be too conservative for the large number of tests, resulting in too many false negatives. On the other hand, for phenotypes that do not follow a Gaussian distribution, the Bonferroni correction may not be stringent enough, resulting in too many false positives. An alternative approach to overcome some of these limitations is to empirically estimate the family-wise error rate and to compute an adjusted significance threshold. This permutation-based threshold is able to account for multiple hypotheses, correlated markers, and skewed phenotype distributions. To efficiently compute these large numbers of permutations, we propose permGWAS, an efficient reformulation of LMMs using three- and four-dimensional tensors. We show that permGWAS outperforms current state-of-the-art LMMs in terms of runtime, and that the false discovery rate of permutation-based thresholds is lower compared to Bonferroni thresholds for skewed phenotype distributions. permGWAS is open source and publicly available on GitHub via: https://github.com/grimmlab/permGWAS.

FUNCTIONAL GENETICS IN NON-MODEL PLANTS

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Investigation of genome structure and function significantly improved our understanding of biological processes. Next-generation sequencing allowed large-scale genomic studies, leading to the identification of thousands of genes. Another methodical breakthrough, the introduction of genome editing tools, represents a powerful approach to functional genetics and genome engineering. However, the application of genome editing techniques is still limited due to the lack of effective delivery methods for non-model plant species. Major bottlenecks constraining the overall performance of genome editing in plants comprise the gene transfer to plant cells and subsequent recovery of modified plant individuals. We will show novel approaches to improve transformation efficiency and *in vitro* regeneration, such as the modulating plant susceptibility to *Agrobacterium*-mediated transformation and expression of developmental regulatory genes involved in callus initiation as well as shoot apical meristem maintenance. We will also discuss the associated technical issues, troubleshooting, and our experience with establishing the transgenic technology in order to apply the genome editing tools in non-model plant species.

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WHOLE-LEAF METHOD FOR DETECTING ACCUMULATION OF ROS AND FLAVONOIDS IN ARABIDOPSIS

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Flavonoids are an important group of plant secondary metabolites that have been around since plants first transitioned to life on land and were subjected to an abrupt increase in UV exposure. Flavonoids absorb UV radiation, and thus their accumulation in plant tissue serves as a natural UV shield, protecting cellular components from high-energy radiation. In addition, flavonoids have antioxidant properties, meaning they can scavenge reactive oxygen species (ROS) and reduce oxidative stress.

Flavonoid biosynthesis is known to be upregulated by exposure to high light conditions, although high energy short wavelengths (i.e., the blue and UV spectral regions) have been shown to be more influential and promote the biosynthesis of flavonoids with higher antioxidative capacity. In our current work, we aim to observe how exposure to light conditions with the same PAR intensity but different spectral compositions can influence stress tolerance in Arabidopsis exposed to UV-B radiation.

In this study, Arabidopsis plants were precultivated in 100 PAR of full-spectrum white light for 6 weeks, then 300 PAR composed of either red and green (RG), or red, green and blue (RGB) spectral components for two weeks, and finally exposed to 2 days of an additional 16 PPFD of UV-B radiation.

Previously, leaf-level accumulation of flavonoids was determined by treating cross-sections with a histochemical reagent to enhance natural flavonoid fluorescence (DBPA). However, the delicate nature of Arabidopsis leaves makes them an impractical substrate for this method.

Here, we present a new method of analysing whole Arabidopsis leaves (no cross-sectioning necessary) for both flavonoid accumulation as well as ROS accumulation. The results of these methods can then be analysed using image analysis, enabling visual and qualitative observation of oxidative stress and protective flavonoid accumulation in response to light conditions.

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ANIONIC PHOSPHOLIPIDS ACROSS SCALES: FROM PLASMA MEMBRANE NANODOMAINS TO PLANT DEVELOPMENT

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Membrane lipids are not only building blocks of membranes, they also act as privileged signaling relays within these biological interfaces. Membranes of eukaryotic cells are incredibly dynamic with constant molecule exchanges between compartments. In addition, lipids laterally diffuse in the plane of the bilayer. Local differences in lipid diffusion generate membrane domains that can be as small as few nanometers in size or up to an entire face of the cell. Lipid diffusion in membranes is complex as it depends on many factors, including for example, the type of lipid molecular species, the composition of the membrane, its shape and its phase behavior. It is also constrained by proteins and the extracellular matrix. The later emphasize the need to address lipid localization and dynamics in vivo. In this talk, I will describe the strategies that we are employing to study the localization of anionic phospholipids in plants, describe their dynamic behavior within membranes, and test their related function in development.

MASS SPECTROMETRY IN STRUCTURAL PROTEOMICS

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Advancements in the sensitivity and precision of mass spectrometry measurements have enabled the boom of methods using mass spectrometry (MS) detection. This applies also on methods of structural proteomics that can be used for studying protein structures and protein–protein interactions. These methods can complement the results of high-resolution techniques such as nuclear magnetic resonance and X-ray crystallography. Currently the most popular structural proteomic methods are protein cross-linking (XL-MS), protein covalent labelling, hydrogen/deuterium exchange (HDX), or native MS.

XL-MS relies on the use of bifunctional reagents known as cross-linkers. Cross-linkers connect two or more residues within proteins. The distance between the linked residues is defined by the length of the cross-linker. In this way, the cross-linker serves as a kind of molecular ruler that measures the maximal distance between protein residues or can be used to maintain the protein interactions. Covalent labelling is based on the site covalent modification of amino acid residues in proteins. The modification can be specific or non-specific, depending on the chemistry that is applied. Proteins studied by HDX are treated by deuterated water and the accessible parts of protein reversibly exchange their hydrogen to deuterium. In both cases, the accessibility of targeted residues reflects their position in protein structure or their involvement in the protein interactions. Unlike the aforementioned methods, native MS is based on the measurement of proteins and their complexes in native state that allows the topological and stoichiometry investigation of protein complexes with high sensitivity and a theoretically unrestricted mass range.

The different principles of the presented methods make them complementary and applicable to tackle various biological questions. The elucidation of protein structures or the structures of protein complexes helps to unravel the functions of proteins at different levels.

IMAGING-BASED SCREENING TO STUDY MECHANISMS OF SPECIFIC AND NON-HOST RESISTANCE TO FILAMENTOUS PATHOGENS

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Studying plant interactions between two or more organisms, researchers face twice as much of methodical challenges. Are the experimental conditions optimal for all players? Is it possible to evaluate contribution of each player reliably, and how to make output reproducible? Different pathosystems call for particular approaches, being limited by the model organism's features (i.e. size, optical properties, possibility for the modification). However, molecular biology and biochemistry often lack spatial resolution, which is particularly important for the multicellular pathogens like fungi.

We focus on interactions between oilseed rape *Brassica napus* and pathogenic fungus *Leptosphaeria maculans*, a major cause of a blackleg disease. We combined *in vivo* and *in situ* visualization of the dynamics of GFP-tagged mycelium growth in tissues and correlated it with activation of plant defense responses on transcriptional and biochemical level. We show the switch between biotrophic phase of asymptomatic growth of the fungus to toxin production, which is associated with necrotic lesions, and a novel function a fungal hexose transporter LmHxt1 in spore germination and fungal pathogenicity.

The developed methodology was then used study non-host resistance in *Arabidopsis thaliana* by parallel screening of the set of 186 *A.thaliana* ecotypes for support of *L.maculans* mycelium growth and symptoms development. We found several groups of ecotypes: hypersensitive, true susceptible, tolerant, true resistant, and identified genomic regions, polymorphism in which is likely associated with basal non-host resistance.

QUANTIFICATION OF METHIONINE DERIVED COMPOUNDS AND SELECTED PHYTOHORMONES BY LC-MS/MS IN PLANTS

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The amino acid methionine plays a pivotal role in plant metabolism. It exerts its influence on virtually all plant processes through a series of recycling reactions known as the "Yang cycle." The byproducts generated from these reactions have far-reaching effects on various aspects of plant ontogenesis. Quantifying Yang cycle metabolites, which are highly polar in nature, poses a challenge due to their limited retention on reversed-phase columns. Additionally, the chemical instability and low abundance of certain metabolites further compound the difficulty in their accurate quantification. Besides these metabolites, major phytohormone ethylene levels are determined by the production of 1-aminocyclopropane-1-carboxylic acid (ACC), arising from Yang cycle. New compelling evidence suggests, that ACC may act as a unique signaling molecule, pushing it into the direction of becoming a new plant hormone. ACC itself, its biosynthesis and the consequent metabolic steps have never been measured and quantified in a complete method from plants. Our results show, that this profiling can be accomplished, together with other phytohormones, by subsequent quantification from the same sample - thus placing ACC on phytohormonal map.

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COOPERATION IS ALWAYS ADVANTAGEOUS: ANALYSIS OF RIBOSOMAL RNA LOCI BY CYTOGENETIC, CLASSICAL GENOMIC AND CHROMOSOME-SCALE ASSEMBLY APPROACHES

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Ribosomal RNA loci (rDNA) are classical chromosome markers used for cytogenetic and phylogenetic studies. They encode four types of ribosomal RNA (5S, 5.8S, 18S and 26S) which are functioning in ribosomes. In chromosomes they are organized as tandem arrays comprising hundreds to thousands of highly similar units. These features complicate their detailed analysis which only until recently relied on classical cytogenetic and single clone sequencing. With the rise of long-read sequencers and long-range technologies, delivering high-quality plant genome assemblies is no longer reserved to large consortia. Not only sequencing techniques, but also computer algorithms have reached a point where the reconstruction of assemblies at the chromosome scale is now feasible at the laboratory scale. Here we present some examples of application of Illumina sequencing and Chromosome-Scale assembly in the studies of rDNA structure and evolution. The experiments performed in several plant systems revealed: (i) Illumina sequencing seems still to be a golden standard for determining variation at the single gene level and copy number estimation. (ii) Long-read assemblers allowing partial reconstruction of rDNA loci represent a significant advance in deciphering fine architecture of these complicated genomic regions. We infer that their reliable portrait can currently be achieved by a combination of methodical approaches.

BIO-RAD - YOUR PARTNER IN GENOMICS EXPERIMENTS; APPLICATION POSSIBILITIES OF DDPCR

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Droplet Digital PCR (ddPCR) provides ultrasensitive nucleic acid detection and direct absolute quantification without the need for standard curves. Not only does this simplify experiment set-up but it also increases reproducibility. In ddPCR, the PCR reaction is partitioned by droplet generator into 20,000 uniform sized droplets, which has been externally validated and shown to be highly reproducible.

In summary, this technology stands out by simplified quantification - no calibration standards or reference (ΔΔCq method) are required for absolute quantification. We could reduce consumable costs - reaction volumes range from pico to nanolitres, reducing reagent consumption and the amount of sample required for each data point. The emulsion-based reaction system means that PCR reactions can be performed in a standard thermocycler without complex chips or microfluidics. ddPCR technology allows 20,000 droplets per 20 µl sample, or almost two million partitioned PCR reactions in a 96-well plate which increases precision and sensitivity compared to qPCR or even dPCR.

This direct method of quantification can be applied to any application that uses primers or primers/ probes to detect nucleic acid sequences and we have tools to help you switch any qPCR assay over to ddPCR. Some but not all applications include environmental monitoring of pathogens, species determination, residual host cell contamination assessment, viral load analysis, mutation detection, copy number variation (CNV), minimal residual disease (MRD), microbial quantification, NGS library quantification, genome editing assessment (HDR and NHEJ), small-fold change gene expression analysis, miRNA quantification and methylation sensitive restriction enzyme (MSRE) ddPCR without the need for bisulfite conversion. Probe based analysis using a one-step kit for RT-ddPCR provides minimal hands-on time for reliable 4-plex (QX200) gene expression analysis.

A novel application of ddPCR is linkage assessment. Which can be used to identify intact proviral DNA, the integrity of viral vectors, inversion assessment, plasmid impurities, cis/trans mutations, and whole cell DNA analysis. Linkage works by using two or more assays (FAM and HEX) at either end of your target that are expected to be co-amplified (e.g. vectors, viral pathogen, suspected cis-mutations, whole cells isolated in droplets). The software can then determine the amount of linkage above that of random distribution to determine the concentration of linked loci and the %linkage, this technique is solely unique to digital PCR.

ddPCR provides reliable and accurate nucleic acid quantification and can be used for a wide variety of applications, some only application to ddPCR. Bio-Rad has a wide range of online tools, bulletins, kits, assays, online assay design tools, experienced specialist, specialized supermixes and optimized software (drop-off analysis function for mutation quantification and genome editing, 2D plate view for experimental optimization, amplitude multiplex, probe mix triplex, advanced classification mode, tilt correction, heat-map for thresholding, positive sample thresholding, regulatory editions and more). This enables Bio-Rad to provide the best possible support during experimental design, trouble shooting and data analysis that we need and want to use.

The Bio-Rad system is a well-established platform (>8300 publications across a wide range of applications), with easy-to-use software, high resolution data, experienced technical support, and continued product development to broaden the scope and applications of ddPCR. You can also search Bio - Rad publication list to look for applications of interest.

A NOVEL GENETICALLY ENCODED CELL WALL PH SENSOR

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Accurate determination of cell wall pH is of great importance for understanding plant tropisms and diverse developmental processes, among others root cell elongation, pavement cell lobing, and pollen tube growth. Although numerous dyes and sensors have been used to estimate cell wall pH, these methodologies suffer from significant limitations. Here, we introduce a novel assemblage of pH sensors comprising two fluorescent proteins and a cell wall binding module. Our approach capitalizes on the distinct sensitivity of diverse fluorophores to different pH. The cell wall binding modules originate from distinct cellulolytic bacteria and exhibit specific affinities for various cell wall components, such as cellulose or xylan. Collectively, the new cell wall-binding pH sensors enable ratiometric determination of cell wall relative pH *in vivo*, and when combined with live cell imaging, they furnish a helpful tool for investigating a wide array of plant developmental processes.

EXPLORING AUXIN'S INFLUENCE ON CELL WALL OF ARABIDOPSIS ROOT VIA PECTIN LYASES

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The phytohormone auxin plays a multifaceted role in the regulation of plant growth and development, with one of its essential functions being the regulation of cell wall properties. By directly influencing the expression of cell wall genes, auxin exerts control over the structural integrity, expansion, and overall growth of plant cells. Nevertheless, our understanding of the molecular mechanism through which auxin modulates cell wall composition and properties remains incomplete. To elucidate these phenomena, we employed a range of molecular techniques, including gene expression analysis, protein profiling, and comprehensive characterization of cell wall components. We characterized a set of auxin-regulated pectin lyases, proteins modifying the cell wall. These enzymes exhibited specific expression patterns in the root elongation zone and manipulating their expression levels resulted in significant alterations in root development. I will present an inducible overexpression system for cell wall-localized proteins based on the GoldenBraid cloning system. Additionally, I will outline a series of methods to characterize the cell wall modifications that the modified expression level of the pectin lyases triggered in the cell walls in Arabidopsis roots. Specifically, I will focus on immunoassay methods used to study how auxin affects the composition of pectin during root cell elongation.

STABLE ISOTOPES – POWERFUL TOOL IN PLANT SCIENCE

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Unequal natural abundance of stable isotopes (SI) of biogenic elements together with biological discrimination between lighter and heavier isotopologues provide powerful tool for plant scientists. Moreover, working with SI bring no health risk in comparison to radioactive isotopes and recent instrumentation deliver ultimate precision requiring typically only nanograms to milligrams of the sample. We can (1) measure biological discrimination since most processes prefer lighter isotopologues. This preference depends on environmental variables and plant species/type/conditions. Thus, we can, for instance, resolve C4 from C3 plant species just from 1 mg of dry plant fragment! Water use efficiency and paleoclimatic variables are commonly estimated from tree rings hundreds and even thousands years old. SI in water may help with calculation of hydraulic and evaporative resistances in water passing through the plant. We can also (2) label plants with minor isotopes (in CO_2, water etc.) and track their fate in different plant parts and/or metabolic compounds. By coupling high precision isotope ratio mass spectrometry (IRMS) with a separation techniques (GC, HPLC…) we obtain very sensitive metabolomics tool. In this presentation we would like to show you several applications we made in the past from methodical point of view. Moreover, technical aspects, advantages and limits will be discussed.

ANALYSIS OF TRANSLATION IN PLANT MITOCHONDRIA

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The plant mitochondrial proteome consists of at least 2000 different types of proteins. Only a handful of them are encoded in the mitochondrial genome and synthesized by mitochondrial ribosomes (mitoribosomes). Since plant mitochondrial translation products are mainly components of oxidative phosphorylation system, studying their production seems to be crucial for understanding plant growth and development. The talk aims at presenting the known assays to study translation in plant mitochondria, based on the model plant *Arabidopsis thaliana*.

The most straightforward approach to analysis of proteins synthesized in plant mitochondria is *in organello* assay. The principle of this method relies on analysis of the incorporation rate of radiolabelled amino acids into newly synthesized proteins directly in isolated, intact mitochondria. The other method, used to study the translational status of plant mitochondrial mRNA, is the profiling of polysomes, multiple ribosomes associated with an individual mRNA. In this approach two parameters related to translatability of mRNA can be assessed: ribosomal density (the number of ribosomes present on a transcript) and ribosomal loading (the proportion of ribosome-bound mRNA to total mRNA). Moreover, recent advances in NGS technology enable monitoring protein translation at nucleotide resolution *in vivo* by ribosome profiling (Ribo-seq), also in plant mitochondria. Ribo-seq relies on sequencing of ribosome protected mRNA fragments (so-called ribosomal footprints), allowing to map the exact positions of ribosomes on transcripts. The presentation will also discuss an exemplary results of various experiments that may be valuable for assessing the mechanistic aspects of translation in plant mitochondria.

Developing and implementing of the presented protocols was funded by the Ministry of Science and Higher Education, Poland (grant N N301 784940 to H.J.) and by the National Science Center, Poland (grant 2013/11/D/NZ1/00288 to M.K.-O. and grant 2014/15/B/NZ2/01065 to H.J.).

MAXWELL NUCLEIC ACID ISOLATORS AND THEIR USE FOR DETECTING PLANT PATHOGENS

Vojtěch Ledvina

Maxwell RSC is an automated benchtop platform for quick and reliable isolation of nucleic acids from various sample types. These include body fluids, eukaryotic cells, FFPE sections, swabs, and also plant tissues and food samples. This short talk will describe the basic features of the instrument and how it can be used for the detection of plant pathogens such as Xylella fastidiosa.

UNLOCKING NATURE'S SECRETS: DECODING COMPLEX BIOLOGICAL SYSTEMS THROUGH MOLECULAR DYNAMICS SIMULATIONS

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Advancements in computational hardware and novel scientific software methodologies have significantly expanded our capacity to simulate larger biomolecular structures over prolonged timescales with atomic precision. Here we underscore emerging opportunities in molecular dynamics, emphasizing our newly reached ability to study multi-million atom systems mimicking extensive biosystems, including cellular membranes containing biologically relevant mixtures by including lipids, proteins, saccharides, and ions simultaneously. We also explore the features of the extracellular environment rich in sugars. However, despite both being tiny on the cellular scale, these achieved massive scales introduced unique challenges, such as constructing complex biological systems, sampling them efficiently, analyzing them, and deriving meaningful insights. We complement these by showcasing recent breakthroughs in molecular simulations that have provided unprecedented insights into the organization of plasma membranes containing dozens of moieties and the glycocalyx.

SEQUENCING EVER MORE GENOMES: PANGENOMICS IN CROP EVOLUTIONARY STUDIES

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Pangenomes are collections of genome sequence assemblies that represent the entire diversity within a species and provide useful resources for evolutionary studies, functional genomics and breeding of cultivated plants. Cost reductions in high-throughput sequencing and advances in sequence assembly algorithms have made it possible to create multiple reference genome sequences along with a catalogue of all forms of genetic variations in plant species with large and complex or polyploid genomes. In this talk, we explain the design and implementation of a pangenome studies using the crop barley as an example. I will speak about the selection of representative core sets from germplasm collections, the assembly of chromosome-scale reference sequences, and the discovery of structural variants associated with agronomic traits. A remaining challenge is the development of interactive browser for easy access to pangenome resources.

HOW DO YOU PROGRAM YOUR PLANT CELLS?

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DNA serves as the fundamental programming language for all living cells. Its incredible capacity for encoding diverse functions poses a challenge when attempting to engineer complex DNA constructs in the laboratory. However, recent advancements in hierarchical and modular cloning techniques, such as MoClo (Modular Cloning) and GoldenBraid, have revolutionized the assembly of such complex DNA. Both MoClo and GoldenBraid employ the same standardized modular building blocks, allowing for the assembly of increasingly complex structures. Over the past several years, we have successfully usedthese techniques to generate a wide range of constructs, including protein overexpression in plants and E. coli, CRISPR/Cas9 constructs for gene editing, fluorescent tagging, synthetic metabolic pathways orinfectious clones of plant viruses. Here we share our extensive experience with these methods and present advanced strategies to enhance the efficiency of complex construct assembly. Additionally, we are pleased to offer the plant research community access to our extensive collection of useful parts and vectors, as well as our assistance in designing constructs for your specific experiments.

VISUALIZING SUBCELLULAR REDOX DYNAMICS USING GENETICALLY ENCODED BIOSENSORS

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In vivo, thiol redox state is the result of thiol reactivity as well as competing reduction and oxidation reactions. Reducing equivalents and photosynthetic light reactions channel electrons into reducing and ROS-scavenging systems that differ between subcellular compartments. Regulatory and catalytic cysteines in many proteins are coupled to several oxidising and reducing pathways and exhibit particularly dynamic redox kinetics. The impact of local redox dynamics on global metabolic and signalling responses is largely not understood in plants.

Dynamic *in vivo* imaging of organellar redox-states has become feasible with the development of various genetically-encoded redox sensors, such as redox-sensitive GFP2 (roGFP2), roGFP2-Orp1, HyPer or CROST. This talk will summarise the power and limitations of using *in vivo* biosensing using confocal laser scanning microscopy or plate-reader based read-out. Using examples of biological questions addressed by *in vivo* biosensing, I will highlight important considerations while analysing and interpreting biosensing data.

PHOSPHOPROTEOMICS TO DISSECT PLANT SIGNALING PATHWAYS

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Protein phosphorylation is a reversible post-translational modification that regulates activity, localization, stability, interaction, and liquid-liquid phase separation of proteins. Most plant genomes which have been sequenced thus far have been found to encode over a thousand protein kinases (PKs). PKs act as receptors of plant hormones and microbe-derived molecules, or as signal transducers of a wide range of physiological responses, suggesting that protein phosphorylation is an indispensable regulatory mechanism of the plant's ability to adapt to adverse environmental and biological conditions.

Phosphoproteomics, a proteome-wide phosphorylation status monitoring, is an attractive approach for identifying uncharacterized PKs and novel substrates in particular physiological processes. In this talk, I will introduce and discuss about the basics and potential pitfalls of recent liquid chromatography-mass spectrometry (LC-MS) based phosphoproteomics technologies, and its application to the plant research.

RECENT ADVANCES IN PLANT HORMONE PROFILING

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Phytohormones play crucial roles in the control of various physiological processes. Whilst metabolism provides the building blocks for plant growth and development, the phytohormonal groups are essential to control the rate of growth of individual plant parts and to integrate the activities of these parts. High-resolution measurements of phytohormones are therefore necessary for physiological studies of their mode of action.

We have developed several fast chromatographic separations and highly sensitive tandem mass spectrometry (LC-MS/MS) methods for simultaneous profiling of phytohormone metabolites. We also focused on efficient cell and organelle isolation, combining different approaches such as density gradient ultracentrifugation or fluorescence-assisted cell/organelle sorting (FACS/FAOS) with a simple one-step purification protocol based on in-tip micro-solid phase extraction and a class-specific miniaturized immunoaffinity chromatography method. New analytical tools provide a comprehensive insights into plant hormone regulatory networks, such as detailed distribution of plant hormones in specific tissues, cells and organelles. Our preliminary data point out to the fact that phytohormone profiles in the plant cell are quite complex and include not only expected active molecules but also other key representatives that covers phytohormone biosynthesis, conjugation and degradation. By employing these novel methods, we are able to gain a much better understanding of how genetic and experimental manipulations affect plant hormone levels, which will foster a more complete understanding of how these hormones act.

HOP PLANT – FROM HISTORY THROUGH OMICS TO BEER

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Hop (*Humulus lupulus* L.) is a diploid, dioecious, perennial climbing plant belonging to the Cannabaceae family. Female plants are cultivated for the commercial production of inflorescences (cones), which are mainly used in the brewing industry but also commonly used in the production of pharmaceuticals and cosmetics. In the early Middle Ages, wild and cultivated hops were found in many parts of central and northern Europe and they have been used for the preparation of beer since the ninth century. The names of traditional landrace cultivars refer to the regions and places of origin from this time. Saaz and Hersbruck landraces were historically the most important for genetic diversity of European wild hops. The active transfer of Saaz hop plants in Europe took place in the 14th century and subsequently also in the 19th century

A recent technical advance in next generation sequencers (NGS) opened a way to obtain huge amounts of transcriptome and whole genome sequence information, which together with proteome studies have provided a systematic understanding of secondary metabolite biosynthesis pathways, the role of structural and regulatory genes for their biosynthesis in lupulin glands (glandular trichomes). unique and exclusive organs, predominantly formed in hop cone inflorescence. From our results, we confirmed that the bitter acid content in lupulin glands is dependent on the last step of alpha bitter acid biosynthesis and lupulin gland density. We also studied gene expressions of transcription factors involved in regulatory network for secondary metabolite biosynthesis and lupulin glands development.

New Omics information can be useful for hop breeders because beer market, mainly craft breweries, is seeking for new hop cultivars with new flavours. Moreover, big brewery groups are afraid of climate changes to keep sustainable hop production and hop breeding is necessary to overcome these abiotic and biotic factors by new more tolerant cultivars.

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ADVANCED IMAGING TECHNIQUES OFFERED TO PLANT BIOLOGISTS BY THE CZECH BIO-IMAGING INFRASTRUCTURE

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Czech-BioImaging (CzBI) is a national biological and medical imaging infrastructure comprising imaging facilities of 10 institutions located in Prague, Brno, Olomouc and České Budějovice. The majority of them are also part of the Euro-Bioimaging (EuBI), a network of pan-European open-access services. CzBI provides open access to a spectrum of up-to-date imaging technologies and offers also expertise in image analysis.

Most of the technologies offered in this consortium are available for plant biologists. Imaging Facility of the Institute of Experimental Botany (IFIEB) of the Academy of Sciences of the Czech Republic (http://www.ueb.cas.cz/if) is directly focused on plants, however, other facilities also routinely meet plant biologists regularly. The advantage of having a network of facilities is that they are able to offer the user the most accessible and, at the same time, the most appropriate technology, thanks to their equipment and their expertise. The purpose of this talk is to provide an overview of methods that allow to study processes in plant cells in high spatial and time resolution. We present here a practical guide for plant biologists at all levels of microscopy expertise. We aim to help them in their cost-benefit considerations as well as in their understanding of the basic principles of what are often very advanced microscopy techniques.

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INTRODUCTION TO METHODS FOSTERING MORE EFFECTIVE TEACHING

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We may agree that the goal of teaching is that students learn. Does it seem like an obvious statement? Well, it does not seem to be obvious enough: decades of research suggest that what most of us experienced and are still experiencing, i.e. “frontal” teaching where students sit and listen and occasionally ask questions while the teacher speak, is actually not the most effective way for students to learn. In this workshop, we will practice different pedagogical methods that helps to develop a student-centered approach to teaching. That means focusing on what students do and building teaching strategies to train, monitor, and eventually assess students’ actions. For this purpose, in this workshop we will work with approaches such as learning outcome-based learning, Bloom’s taxonomy of learning, and the “growth mindset” from Carol Dweck’s research work.

INTEGRATIVE STRUCTURAL BIOLOGY - SOLVING MOLECULAR ARCHITECTURE OF LARGE BIOMOLECULAR ASSEMBLIES

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Delineation of the spatial and temporal arrangements of biological systems is essential in formulating hypotheses about their function and evolution. Classical methods such as X-ray crystallography and nuclear magnetic resonance spectroscopy are compelling in deciphering structural information of biological systems. Still, both are limited to relatively small proteins and a limited number of interacting components. Cryo-electron microscopy has revolutionized our view on structural biology and has been successfully used to depict structural aspects of many multimeric protein complexes, e.g. COPI vesicles or the clathrin cage. However, cryo-electron microscopy requires a sufficient amount of pure and homogeneous material, which is not always possible to obtain. In integrative approaches, diverse information at different levels of description is synthesized to yield a common view of a biological system. The integrative approach builds a system representation by simultaneously combining information from various sources, both experimental (such as chemical cross-linking with mass spectrometry, protein co-immunoprecipitation or yeast two-hybrid assays) and theoretical (physical theories, statistical analysis or evolutionary analysis). When all available information about the modelled system is used, the accuracy, precision, and completeness of the resulting model are maximized, thus significantly surpassing any of the single structural methods. Moreover, the integrative approaches provide unprecedented insight into the conformational dynamics of large biological assemblies.

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DECODING THE CHEMICAL LANGUAGE OF PLANTS

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Although plants are an incredibly rich source of pharmaceutically relevant specialized metabolites, biosynthetic pathway elucidation in plants has proven challenging. Unlike bacteria and many fungal species that contain biosynthetic operons, the genes of a given plant typically scatter randomly across the genome, making pathway discovery via genome mining nearly impossible. My lab is developing generalized workflows for connecting biosynthetic gene sequences (RNAseq data) to their downstream metabolites (LC-MS data). For this, I will demonstrate a top-down approach, which is based on correlating expression levels of enzymes with metabolite abundance across a plant family, and a bottom-up approach, which is based on predicting the substrate specificity and function of individual biosynthetic enzymes directly from their amino acid sequences using deep learning.

EXPLORING LIPID-PROTEIN INTERACTIONS IN PLANT CELLS

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The complex interplay between lipids and peripheral proteins at cellular membranes orchestrates critical processes in plants, including vesicle trafficking, cytoskeletal regulation, and stress responses. Anionic phospholipids define organelle membrane identities and recruit peripheral proteins in a spatiotemporal manner via lipid-binding domains. Despite the significant biological consequences, molecular details underlying the phospholipid-mediated protein recruitment to membranes remain unclear due to the limitations of structural techniques.

In my talk, I will present an overview of the cell biology and biochemistry approaches geared towards analyzing protein-lipid interactions that circumvent the need for specialized technologies. I will introduce protein–lipid overlay assays as an initial, straightforward approach for screening protein interactions with various lipids or lipid intermediates. Subsequently, specific interactions can be analyzed quantitatively using protein–liposome association assays. I will describe the utility of molecular dynamics simulations for identifying specific lipid-interacting amino acids. In parallel, I will demonstrate the application of live-cell imaging, which enables the analysis of fluorescently-tagged protein translocation to the target membrane upon cell stimulation, metabolic perturbations, or expression of lipid-modifying enzymes. Together, these multifaceted techniques can offer novel insights into the complex world of membrane-protein interactions.

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GOLEM: A TOOL FOR DETERMINING THE DISTRIBUTION OF GENE REGULATORY ELEMENTS WITHING PLANT PROMOTERS

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Regulation of gene expression is a highly complex process. Among various mechanisms involved in this regulation, the recognition and binding of transcription factors (TFs) to short cis-regulatory elements (CREs) within promoters regions play a crucial role. The accurate positioning of CREs near the transcription start site (TSS) is essential for controlling transcription probability, frequency, and rate. During tightly regulated processes like sexual reproduction, the transcription frequency of specific genes can vary significantly across different tissues and developmental stages. Existing transcriptomic databases focused on plant gametophyte development lack precise localization and visualization of CREs within gene promoters in relation to tissue-specific gene expression levels.

To overcome this limitation, we have developed GOLEM (Gene regulatOry eLEMents), an program that allows users to precisely determine the motif's position relative to the transcription or translation start sites. Our program offers a powerful tool for visualizing the precise localization and distribution of CREs in gene promoters, taking into account gene expression levels in specific tissues and across diverse plant species throughout the plant phylogenic tree.

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IMPLEMENTATION OF RIBO-BIFC METHOD TO PLANT SYSTEMS USING SPLITTED MVENUS APPROACH

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Translation is a fundamental process for every organism. In plants, the rate of translation is tightly modulated during development and stress responses. However, it is difficult to quantify the actual translation state of the tissues *in vivo*. Here, we report on the implementation of an *in vivo* translation marker based on ribosomal-derived Bimolecular Fluorescence Complementation, the Ribo-BiFC. We have combined this method originally described in the fruit-fly system with an improved low background splitted-mVenus BiFC method described in plants. We labelled small and large ribosomal protein with fragments of splitted mVenus fluorescent protein and showed these recombinant proteins integrate in 40S and 60S ribosomal subunits. Upon the assembly of the ribosome, complemented mVenus can be detected using fluorescent microscopy. This Ribo-BiFC method system could enable visualisation of translational rate in plant tissues and thus could be applied to study translation dynamics during plant development under abiotic stress or in mutant background.

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SMALL-MOLECULE DISSECTION OF STEROID SIGNALING IN PLANTS

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Chemistry and plants have always been tightly linked, as plants have been used as a source of diverse natural compounds and they have inspired synthetic chemists and drug developers. Furthermore, the discovery of phytohormones and their signalling pathways have stimulated the interest of many plant biologists to search the chemical space and identify functionally similar small bioactive molecules. In the last two decades, the focus has shifted toward more targeted approach wherein plant biologists have used small molecules to selectively modulate the function of a protein or of members of a protein family, phenotypically resembling genetic mutations of the protein-encoding gene. My talk will illustrate the use of chemical biology in our research to gain novel information about steroid signalling in plants.

LIGHT SHEET MICROSCOPY IN PLANT CELL BIOLOGY

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In higher plants, cellular processes crucial for plant reproduction, i.e. development of plant reproductive organs, germline differentiation, fertilization and embryo development occur in relatively short time window during development of flowers. Despite undisputable importance for agriculture and food production, our understanding of cellular mechanisms that govern plant reproduction still lags behind other plant developmental processes, such as root growth and formation. This is largely because these events take place in relatively few cells that are buried deep within floral tissues, which makes them notoriously difficult to study. To overcome this limitation, we have developed methodology for live imaging of cellar processes within developing floral organs by light sheet microscopy. We will demonstrate power of this approach to capture male and female meiosis, asymmetric pollen division, embryogenesis and quantitative imaging of plant floral meristem. Furthermore, we will show utility of this method for spatial and temporal protein localization, characterization of mutants, or application of drugs that specifically interfere with certain molecular processes. This demonstrates that light sheet fluorescent microscopy can be used as a versatile tool to study various aspects of plant sexual reproduction.

PRIME EDITING IN ARABIDOPSIS: USING NATURAL GENETIC VARIABILITY OF CYTOKININ RECEPTORS AS A TOOL FOR DROUGHT RESILIENT VARIETIES INTRODUCTION

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The manipulation of plant hormone signaling pathways enables the control over plant growth, development, and responses to biotic and abiotic stressors throughout their entire life cycle. Among the extensively researched plant hormones in this context, cytokinin stands out for its crucial role in key developmental processes and modulation of plant adaptation mechanisms under adverse environmental conditions. To modulate cytokinin signaling pathway, we employed an innovative genome editing technology called Prime Editing (PE) to create desired mutations at specific sites. We modified PE technology to introduce base-to-base conversions in the cytokinin receptor ARABIDOPSIS HISTIDINE KINASE 4 (AHK4), which naturally occur as missense mutations within the Arabidopsis accessions. Firstly, we studied the substitution of a single nucleotide at a specific position in the genome, known as single-nucleotide polymorphism (SNP), through an *in-vitro* AHK4 activity assay. Our findings revealed that the AHK4 variants displayed higher cytokinin responsiveness, suggesting that the identified SNPs enhance AHK4 function to transduce the signal downstream of the pathway. Thus, we employed the PE technology to exchange a single nucleotide within the AHK4 gene in planta resulting in the increased responsiveness of edited plants to cytokinins. Furthermore, our PE-edited plants exhibited increased sensitivity to drought conditions, as evidenced by earlier growth reduction reflecting phenotype of wild-type plants sprayed exogenously by cytokinins. Overall, our findings provide a novel genome editing strategy that enables precise modulation of signaling pathway dynamics by harnessing naturally occurring SNPs to further modify plant responses to the stress. These findings have significant implications for the field of synthetic biology, particularly in the development of methodologies aimed at enhancing plant resistance to abiotic stresses.

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SINGLE-CELL TRANSCRIPTOMICS IN PLANT RESEARCH

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Single-cell RNA sequencing (scRNA-seq) has become a powerful method for exploring the transcriptomic properties of various tissues and organs with unprecedented resolution and throughput. In contrast to the traditional bulk RNA-Seq, it allows us to estimate the transcriptomic profiles of specific cell types including rare cell types such as stem cells, and recover the highly dynamic processes within individual tissues without prior cell sorting or tedious manual dissection which may be not always possible. The increasing popularity of scRNA-seq led to the construction of extensive atlases containing a still increasing number of individual cells providing a vast and valuable source of information.

In this talk, I will introduce the main benefits and limitations of scRNA-seq, focusing on a droplet-based technology with illustrative examples from different fields of plant research such as development or plant-microbiome interactions. The talk will cover fundamental considerations in experimental design and describe the preparation of single-cell libraries. I will also discuss the analytical part of the experiments which is critical for proper data mining and interpretation. The current rapid development of novel data analysis tools, including machine and deep learning approaches, allows us to address a broad range of research questions using methods such as the construction of tissue-specific gene regulatory networks, the study of cis-regulatory elements, dynamics of mRNA splicing, or performing *in silico* gene perturbation.

In summary, this talk will provide an overview of the scRNA-seq, including data analysis, which may help researchers to adopt this novel technology.

HIGH-THROUGHPUT, HIGH-PRECISION, AND AFFORDABLE PHENOTYPING

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Several methods using semi-automated hardware and software approaches for phenotyping model plants and crops in multiple environmental conditions are available at CATRIN. Multi-Trait High-Throughput Screening, which can test higher tens of variants in one experimental run (in total >25,000 plants), uses simple RGB imaging of Arabidopsis in controlled conditions. The variants represent combinations of concentration ranges of tested chemicals/products, genotypes, individual abiotic stresses (water and nutrient limitation, salinity, heavy metals), individual biotic stresses (*Botrytis*, *Pseudomonas*), and their multiple combinations. The tested agents can be applied through seed/seedling priming or root absorption. Our software pipeline automatically processes the images and visualizes results with a neural network-based plant recognition algorithm, quantifying growth dynamics and morphological and stress response traits. Rapid assay of emergence dynamics, synchronicity, early development, and stress response in normal and abiotic stress conditions (water limitation and salinity) is further available for large-scale screening of various crops. High-precision phenotyping in controlled conditions using different non-invasive sensors (RGB, FluorCam, IR, VNIR, SWIR) for detailed inspection of numerous morphological and physiological traits in normal and stress situations can be performed with up to 640 plants in one run. To improve access to phenotyping technologies, we have developed several affordable devices, including “Vertical phenotyper (VP)” – a device for high-throughput automated screening and activity testing, easily fitting into laboratory space and designed to grow up to thousands of small plants in controlled conditions. RGB cameras enable top, bottom, and side views for shoot size, height, and root imaging. A significant advantage of the system is scalability by multiplying the VP units to study combinations of environmental conditions and various treatments. All datasets resulting from the experiments are managed through an automated data pipeline compatible with BrAPI following FAIR principles.

3C TECHNIQUES: FROM GENOME ASSEMBLIES TO REMOTE CONTROLLERS OF GENE TRANSCRIPTION

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The vast majority of an organism’s hereditary information in eukaryotes is stored in the cell nucleus. Here the genome is folded at multiple levels to be accommodated in a limited space and with a high degree of flexibility to allow structural metamorphoses during the cell cycle. Moreover, the chromatin arrangement ensures that the correct gene expression programs are executed at the right time and in the right cell type. This makes investigation of 3D chromatin arrangement to an attractive area of research.

Deep insights into the 3D genome organization have been obtained by emerging molecular biology techniques - Chromatin Conformation Capture (3C)-based methods. These utilize proximity ligation, joining pairs of genomic fragments located nearby in the 3D space into chimeric DNA segments, which are then sequenced by Illumina technology. The basic 3C technique – Hi-C – exploits the resulting sequence data to identify and quantify chromatin interactions genome wide. Since the frequency of contacts between particular genomic regions correlates with their distance in the linear genome, Hi-C data have been used to order sequences (contigs and scaffolds) in genome assemblies. The captured 3D contacts provide information to build models of higher-order chromatin packaging in metaphase chromosomes and track chromatin changes during the cell cycle. In the interphase nuclei, Hi-C reveals distinct genome compartments and self-interacting genomic regions - topologically associated domains. On a lower-order folding level, the genome is arranged in chromatin loops, some of which put into contact enhancers and promoters, thus increasing gene transcription. These functional contacts require high-resolution analysis, which can be achieved by coupling Hi-C with chromatin immunoprecipitation (HiChIP) or by targeting promoters (Capture-C).

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ROOT AND SHOOT PHENOTYPING IN SOIL

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Plant phenotyping is showing increasing promise in plant research because it can be a useful tool to obtain detailed parameters describing the overall performance of plant body development. Moreover, in research focused on various environmental stresses, phenotyping can provide us with markers that can more sensitively assess the stress impact in its early stages. A combination of different types of phenotyping methods provides a complex picture of plant development and fitness.

We used PlantScreen™ (developed by Photon System Instruments), a dedicated system for root and shoot phenotyping in soil. The system enables high-precision, low-throughput plant phenotyping of small and mid-size plants (e.g., *Arabidopsis thaliana* or *Brassica*) and combines high-resolution imaging of morphological and fluorescent parameters. Image analysis provides us with information about the morphology, while induced fluorescence of chlorophyll aims mainly at evaluating the condition of the photosynthetic apparatus.

In our work, the application of these phenotyping methods will be described in a pilot experiment, in which image analysis and induced fluorescence of chlorophyll measurement were used to determine the morphological changes and the status of the photosynthetic apparatus in *Arabidopsis* plants exposed to drought. The rosette area became smaller after seven days of drought treatment when compared to control. The fluorescent parameters showed even higher sensitivity. Quantum yield and non-photochemical quenching displayed values connected to reduced activity of photosynthetic apparatus after four days of drought treatment.

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THE ASSEMBLY OF COMPLEX PLANT MITOCHONDRIAL GENOMES USING LONG READS

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Mitochondrial genomes (mitogenomes) of land plants are essentially different from plastid genomes -. they are highly variable in size and much bigger. The number of mitochondrial genes is low and quite stable, but the structure and proportion of intergenic regions vary enormously, gene order is not conserved even in the same species. These features make the plant mitogenome assembly challenging. Whereas the complete sequencing of plastid genomes becomes a routine task, no straightforward bioinformatic tool exists to recover the sequence of plant mitogenomes.

Intramolecular recombinations across abundant repeats are permanently rearranging the mitogenome structure. Unlike the nuclear genome, no single sequence represents the mitogenome of particular plant individual, or even of the species. The mitogenome sequence varies in time and across tissues and organs. Individual cells and mitochondria may contain incomplete (or no !) mitogenome. However, we should be able to find the assembly, which contains complete mitochondrial DNA and does not contradict to the data.

Less recombinogenic or fluidic plant mitogenomes could be assembled using HiFi or standard PacBio long reads, or Oxford Nanopore Technology (ONT) data. The reads should be abundant enough to allow reliable sequence correction. The data collected during nuclear genome sequencing projects can be utilized. Mitochondrial reads shall be at first retrieved using blastn search with mtDNA sequences of the close relative and then assembled using flye with optimum read length threshold.

More complex mitogenomes require the combination of long reads and short Illumina reads for the assembly. Because short reads make possible to correct less accurate long reads, the cheapest ONT method using consumable flow cell Flongle (output about 0.8Gbp) can be adopted. Newbler assembly of short reads is used to identify repeat and inter-repeat regions. It is advantageous to use plant tissues rich in mitochondria, but with less plastids, for example young flower buds. Extensive manual editing is necessary.

CATSNAP – A MACHINE-LEARNING TOOL REVEALING THE PLASTICITY OF ALTERNATIVE SPLICING IN PLANTS AND ANIMALS

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Alternative splicing (AS) expands transcriptome complexity and regulates gene expression. Many alternative isoforms show no obvious function. The evolutionary conservation of an alternative isoform implies its functional importance. We developed a tool called Catsnap (Conserved AlTerntaitive SpliNg in Animals and Plants) which assesses the conservation of alternative isoforms at the amino acid level. By applying Catsnap to experimentally validated protein isoforms we found that most of them were conserved. Surprisingly, in plants, we observed that the alternative protein isoforms arise from various types of AS events across different species. In contrast, most (but not all) animal alternative proteins displayed more stable patterns of AS types. We link this phenomenon with the more frequent duplication events occurring in plants. We conclude that the emergence of similar protein changes resulting from various underlying mechanisms at the RNA level may suggest the existence of specific hotspots that are more likely to produce advantageous traits through AS. Compared to the typical algorithms viewing conserved AS as the same type of AS events occurring in homologous introns, Catsnap yields results consistent with those obtained from other algorithms, but also identifies additional hits where similar alternative proteins arise from different types of AS events. Thus, Catsnap offers a more comprehensive perspective on the evolutionary dynamics of AS.

INFLUENCE OF PIPETTE TECHNICAL SPECIFICATION TO YOUR PIPETTING RESULTS

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Pipetting liquids is one of the basic tasks in experiments. The main goal is to find a pipetting procedure that ensures that the maximum allowable error is not exceeded. The choice of pipette is usually based on the sample volume, number of samples and the time you want to devote to the task, or simply copying the choices made by others. A poor choice among available pipettes of different designs such as single- or multi-channel, motorized with repeat function, dispensers, etc. can ruin your results as each design has its advantages and weaknesses. Usually users only see the obvious advantages, e.g. it takes much less time to fill a micrtiter plate with a multi-channel pipette, and with a motorized pipette you can dispense several aliquots in a single aspiration. The choice of pipette should be an optimal compromise. The technical specification of the pipette can highlight some weak points and help to estimate pipetting performance.

The performance parameters of a pipette consist of the range of the pipette and the maximum allowable error within that range. Pipette errors have two components - systematic error (accuracy) and random error (precision). Both are given in the technical specification and are based on rigorous laboratory testing by pipetting demi-water using original tips in a controlled environment and by an experienced user. The systematic error is the difference between the mean of the results and the specified volume. It can be easily interpreted and compensated for, e.g. by adjusting the set volume. Random errors are expressed as a standard deviation because the pipetting results are expected to have a Gaussian distribution. This means that the value of the standard deviation given in the technical specification should be multiplied by 3 to cover the range of possible results around the mean value. Unfortunately, there is no way to compensate for this.

The presentation will compare possible errors arising from the use of single-channel, multi-channel, motorized and dosing pipettes.

VOLUME ELECTRON MICROSCOPY OF THE COMPLEX BIOLOGICAL STRUCTURES: CURRENT DEVELOPMENTS, AVAILABLE TECHNIQUES, AND FUTURE OUTLOOK

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In the last decade, three new imaging technologies that provide high-resolution 3D structural information about cells and tissues at the nanometre scale have been developed. These techniques use high-resolution scanning electron microscopy (SEM) to image the face of a block of a resin-embedded material or serial resin sections using backscattered electrons. These techniques (focused ion beam SEM, serial section SEM, and serial block SEM) are referred to as volume electron microscopy or 3D electron microscopy in addition to older techniques such as serial section transmission electron microscopy and electron tomography. Thanks to some significant advances in recent years, the automated data acquisition is now possible. This allows continuous operation of the instruments throughout the experiment which generally increases the data acquisiton rate and speeds up the work immensely. Moreover, accessible are various software tools for image autocorrelation for alignment purposes, tools for 3D reconstruction of data sets, and creation of 3D models using semi-automatic or automatic segmentation tools made possible by developments in machine and deep learning. These advancements facilitate efficient exploration of the complexity of biological samples ranging from unicellular microorganisms to complicated animal and plant tissues. Volume electron microscopy thus provide not only the colourful models but also allows excellent, straightforward quantification, including measurement of volumes, lengths and counts of the studied structures.

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GENE EDITING IN BARLEY

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Barley represents an important staple crop for the brewing industry or as feeding for animal production being the fourth most important crop in the world. Barley has also an indispensable place in research as it serves as a model plant for wheat. Barley genome is less complex than the wheat genome, which makes it a perfect target for gene editing to verify gene function. Genome databases provide basic sequence information necessary for the design of protospacer sequences that guide Cas nuclease to the target site. Evaluation of gene off-targets in homologous sequences within the genome is useful to avoid unintended targeting. For efficient gene editing, high transformation efficiency should be achieved accompanied by high editing efficiency. Hence, a reliable transformation technique that provides sufficiently and repeatedly transgenic material is necessary. *Agrobacterium*-mediated transformation of immature embryos is still the most reliable method of stable barley transformation. Selection of the expression vector is crucial for successful plant transformation. Currently, expression vectors carrying various transcription factors improving *in vitro* regeneration have been developed. The use of such vectors has the potential to enable direct editing of modern high-yielding varieties. Various mutations could be obtained from single base pair indels to larger deletions. Optimization of genotyping before obtaining primary regenerants is favourable. Sequencing of the target sequence states the golden standard for detecting the induced mutations especially when single base pair indels were induced.

ELIXIR AND „PLANT SCIENCE COMMUNITY“ - A CARRIAGE TO TACKLE BIOLOGICAL COMPLEXITY VIA DATA

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The need to cope with population growth and climate change adaptation is a major challenge. ELIXIR as a large infrastructure for biological data can help address this challenge by filling the gaps in managing the research data life cycle, and help plant scientists integrate data to extract biological knowledge.

The Plant Sciences Community is an interdisciplinary group of researchers with diverse backgrounds from computer science to different fields of plant biology. We answer the needs of both bioinformaticians and plant biologists.

The Community objective is to develop an infrastructure supporting the integration and linking of different plant data types. These data types range from phenomics and genomics to transcriptomics, metabolomics, modelling and bibliographic data.

The underlying scientific use cases encompass genetics and genomics approaches in plant sciences, with an extension of the scope towards system biology. The Community aims at promoting tools, databases, standards and best practices for plant research.

THE LIVE IMAGING OF ANTHER OPENING

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Roots, root hairs, pollen tubes..., these are systems where observation of their development over time is not a major problem. They grow continuously, in an aqueous environment and over the long term. But what if we wanted to explore the processes taking place in the anther as it opens? The dehiscence of anthers, an unpredictable and irreversible process lasting at most small tens of minutes and taking place in the early hours of the morning, a process consisting in the desiccation of the tissue, therefore a process requiring exclusively a non-aqueous environment, a process taking place inside the flower after several weeks of cultivation of the plant – at first sight, a process that contradicts all the requirements of a practical experimental system, yet it is a process that requires urgent scientific attention, since it is a crucial step in the life cycle of the plant, the precise timing of which with respect to environmental conditions is essential for its fitness. It is an essentially uncharacterized process, and its study may perhaps shed entirely new light on the EVO-DEVO study of plants – after all, the desiccating sporangium was the first essential organ of land plants, and the anther is its direct evolutionary descendant!

Despite all the difficulties, however, we have managed to penetrate the mystery of anther opening using the vertical spinning disk microscope with all the benefits of confocal microscopy, and we can even try to observe in real time even such sophisticated processes as calcium signalling.

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PLANT SYNTHETIC AND RECONSTRUCTION BIOLOGY APPROACHES FOR THE STUDY AND CONTROL OF CELLULAR PROCESSES IN PLANTS – OPTOGENETICS AS AN ENABLING TECHNOLOGY

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Our synthetic biology research focuses on applying reconstruction biology approaches in orthogonal cellular systems to the study of plant regulatory and metabolic networks. This approach helps overcoming the experimental constraints posed by the combinatorial genetic complexity and multifactorial dynamic interactions of signaling networks and yields a quantitative understanding of mechanistic and regulatory principles. We combine these strategies with the engineering of molecular photoswitches implementing bacterial and plant photoreceptors with effector output modules mediating the precise control of cellular processes. There is a broad range of optogenetic tools responsive to different wavelengths of the white light spectrum (UV-B, blue, green, red/far-red) and they are designed to control various molecular processes with high precision, quantitative and high spatio-temporal resolution, in a non-invasive way and with minimized toxicity. We implement these molecular tools into microbial, mammalian and plant cells, and *in vivo* in animals and plants for selectively manipulating signaling networks and metabolic pathways. However, the need of light by plants for growth and development imposes an intrinsic experimental constrain to the use of optogenetic tools under normal plant culture conditions, i.e. cycles of dark/white light. Therefore, molecular engineering strategies have to be employed to overcome this limitation. Selected applications of the synthetic biology approach will be discussed and examples for the light control of CRISPR/Cas9 technologies and the study of signalling networks shown.

Posters

(alphabetical order)

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MODERN METHODS FOR TRANSGENE DELIVERY AND GENOME EDITING OF HOP (*HUMULUS LUPULUS* L.)

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Hop (*Humulus lupulus* L.) is a perennial herbaceous plant that belongs to the *Cannabaceae* family. Hop is an important and widely known crop in Czech agricultural production and its cultivation and research have a long tradition. It is actively used in the brewing industry. Female hop flowers impart bitterness, aroma, and flavor to beer.

To study the function of genes related to commercially significant traits in hop, we have previously established the hop genetic transformation procedure. Next, we plan to increase the efficiency of agroinfection and *in vitro* regeneration of transgenic plants using novel genetic engineering approaches. This will allow us to use CRISPR/Cas9 gene editing and other methods of genetic engineering on a large scale.

The use of hop as a model system for studying the genetic basis of biological processes is limited by the length of the annual life cycle. In general, model plants can complete several generations of offspring in a year, but for the hop, it is usually only one generation. Accelerated flowering can be induced by the overexpression of genes involved in the vegetative-to-generative transition. Therefore, another objective of this study is to develop hop lines with early flowering, which will greatly improve the efficiency of hop research.

In this project, we will combine state-of-the-art genetic engineering methods to provide a tool for functional genomics and the improvement of hop cultivars.

AT THE HEART OF THE BALANCE BETWEEN IMMUNITY AND GROWTH IN PLANTS: EFFECT OF SALICYLIC ACID ON PLANT METABOLISM ENZYMES

Lucas Amokrane[a], Eric Ruelland[a]
The abstract is available in the abstract book of Student conference.

POLYPHENOL OXIDASE FUNCTION IS ASSOCIATED WITH SEED HILUM PIGMENTATION

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Seeds as means of species survival and dispersal are often deposited in unfavourable environments. While in soil, they need to protect the vulnerable embryo from various biotic and abiotic stresses. During evolution, seeds have developed protection by physical, chemical and biochemical means. It is mainly the seed coat that provides this protection. Wild and crop plant seeds differ in their seed coat properties as a result of the domestication process.

We used a range of genetic, transcriptomic, proteomic and metabolomic approaches to determine the function of the pea seed polyphenol oxidase (*PPO*) gene during the development of the seed coat of the wild in comparison to cultivated pea genotypes. We show that while in wild pea seeds, the *PPO* gene is functional and highly expressed, in cultivated pea genotypes the *PPO* is truncated. The majority of domesticated peas have an allele associated with the loss of hilum pigmentation indicating selection during domestication. The functionality of the *PPO* gene relates to the oxidation and polymerization of phenolics (melanin formation) in the seed coat. As mentioned, the seed coat represents a barrier preventing seeds from pathogen invasion and it seems that the hilum is the weakest part (the “Achilles heel”) of this barrier. We hypothesize that phenolic oxidation in the hilum region provided by PPO and its activity is crucial for seed defence against microbial and fungal pathogens.

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ANALYSIS OF THE B CHROMOSOME IN A COLLECTION OF MAIZE LANDRACES

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Maize (*Zea mays* L.) is one of the most important crops and serves as a well-established model. B chromosomes occur in thousands of plant and animal genomes and persist in populations despite being non-essential. The maize B chromosome is therefore one of the most thoroughly studied supernumerary chromosomes. However, the diversity of the maize B chromosome and its distribution within maize landraces are still unknown. Here, we present the results of screening various maize accessions from the CIMMYT germplasm bank. We introduce a methodology for presence/absence screening using direct PCR and a rapid and simple method for scoring a number of B chromosomes in droplet digital PCR. B-chromosome diversity was studied using cytogenetic techniques and SNPs detected after resequencing of one hundred B-chromosome possessing lines.

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INVESTIGATING THE MOLECULAR MECHANISM OF THE PIVOTAL AUXIN SIGNALING COMPONENT AUXIN RESPONSE FACTOR 5 IN EMBRYOGENEIC TRANSITION IN ARABIDOPSIS THALIANA

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In *Arabidopsis thaliana*, the *in vitro* culture of immature zygotic embryos at a late stage of development on a solid medium containing synthetic auxin 2,4-D leads to the formation of somatic embryos via direct somatic embryogenesis (SE). It exemplifies the unique capacity of pluripotency of somatic cells. This developmental process provides an appealing model system for studying embryogenesis and understanding the molecular mechanisms controlling totipotency and pluripotency in plant somatic cells. It is widely exploited to regenerate plants *in vitro*, for plant mass micropropagation, for the protection of plant biodiversity, and for the production of transgenic plants.

Previous research and preliminary results suggest that the phytohormone auxin via one of the key auxin response factors, AUXIN RESPONSE FACTOR 5 (ARF5), is essential for SE induction, most probably by controlling genes involved in auxin production (YUCCA) and signaling (MIR390). Those regulators play a significant role in SE by regulating auxin homeostasis during the embryogenic reprogramming of Arabidopsis somatic cells.

The project aims to unravel the nature of the regulatory relationship between ARF5, the auxin production via the transcriptional regulation of *YUCCA* genes, and auxin signaling via MIR390 in controlling the SE process based on the use of various methodologies, including CRISPR-induced mutagenesis and plant transformation, MIR390 sensors design and development, somatic embryogenesis induction, dual-luciferase reporter assays, hormonal profiling, gene expression analysis, greenCUT&RUN method, etc.

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OPTIMISATION OF PLATE METHODS TO ACCESS ARABIDOPSIS ROOT[1] AHAS AND FERRIC CHELATE REDUCTASE ACTIVITIES

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The activation of plasma membrane proton pumps (AHA) and ferric chelate reductase (FCR) in plant roots is a crucial characteristic that defines iron deficiency syndrome in dicotyledon plants. The conventional spectrometric method involves immersing treated plant roots in a solution of bromocresol purple or Ferrozine, which bind to protons or ferrous iron respectively, followed by colorimetric detection of the solution. However, this approach suffers from limited accuracy due to normalization based on the number

of roots, main root length, or root weight in each sample. Additionally, the biomass of roots can vary significantly under different iron deficiency conditions, and these methods do not provide information about the impact of root zones on overall rhizosphere acidification.

A more precise method that allows for spatial localization of the signal involves transplanting plants onto test plates containing a coloured indicator. Unfortunately, the resulting data are typically presented as images of plates with seedlings, and the quantitative determination of AHAs and FCR activities on the plates remains unclear.

The objective of our work was to optimize the method for determining AHAs and FCR activity in Arabidopsis seedling roots on Petri plates. Regarding the activity of proton pumps, optimization focuses on the preparation of test plates, as the thickness and surface roughness of the solidified agar with dye significantly impact its optical properties and the colour analysis of images in ImageJ after scanning plates with plants. To determine the induction of FCR in plant roots following iron deficiency treatment, we introduced an increased amount of the ferric iron chelate and Ferrozine simultaneously into the nutrient medium. This enhanced the colour reaction of the Ferrozine-ferrous iron complex and enabled colour analysis.

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CROSSTALK BETWEEN LIGHT AND ABA SIGNALING PATHWAYS IN PLANT RESPONSES TO ABIOTIC STRESSES

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In recent years, research focused on plant stress is becoming more and more popular due to climate change on Earth. Environmental stresses such as drought, extreme temperatures and high salinity are the major factors reducing productivity of crop plants. Negative effects of abiotic stress on plants lead to changes in its metabolism, to reduction of the growth or finally to death of the plant. Scientists suppose that exposure to abiotic stress factors can reduce crop production by up to 70%. In the future, it will be necessary to breed tolerant plants able to adapt to new external conditions. Therefore, understanding of mechanisms involved in plant responses to environmental stresses is very important to solve the agronomic problems.

Plant hormones, especially abscisic acid (ABA), play a critical role in adapting plants to environmental conditions. When exposed to abiotic stress, plants accumulate ABA. At the same time, the level of ABA in plant is altered by light conditions, especially by blue light. This fact raises the question what role does blue light play in plant responses to abiotic stress. In our project, we investigate molecular mechanisms by which blue light crosstalks with ABA during responses to abiotic stress factors. We apply genetic approach consisting in analysis of tomato mutants (*Solanum lycopersicum*) with defects in various stages of ABA biosynthesis and/or in genes coding for blue light photoreceptors. To find out the role of light and ABA in plant responses to abiotic stress, we study growth responses of the mutants to various effectors (salt, mannitol, exogenous ABA) and under various light conditions.

It is hypothesized that salt stress leads to decreased seed germination concomitantly with higher ABA production by seeds. But the results are contradictory. In ABA-deficient mutant (*flacca*, *sitiens)* and mutant *hp1* (*high pigment 1*) the responses to salt stress were not the same. The tomato mutant *hp1* shows hypersensitive responses to blue light and red light and according to our results the mutation lead to reduction of ABA in seeds. Similarly like *sitiens*, *hp1* was less sensitive to NaCl than corresponding cultivars in the dark, in blue light or red light. On the contrary, mutant *flacca* seems to be more sensitive to NaCl than corresponding cv. Rheilands Ruhm, in all light conditions. Furthermore, we discuss the possibility that ABA can interact with other hormones, such as ethylene, gibberellin in plant responses to salt stress.

VISUALIZATION OF THE CUTICLE ARCHITECTURE IN LIVING PLANT CELLS

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The hydrophobic cuticle layer is deposited on the outer surface of the epidermal cells. The cuticle covers all aboveground plant organs. Cuticle is one of the evolutionary adaptations of plants to the constantly changing environment, and as such, it facilitated plant terrestrialisation. The visualisation techniques to observe the plant cuticle architecture include primarily electron microscopy or anatomical staining of fixed transversal leaf and stem sections. In both cases, the non-living plant pavement cells are observed. Here, we established visualisation of the cuticle in living plant cells using specific fluorescent dyes. With our methodology, we could compare the cuticle architecture of different plants, plants from different cultivation conditions or distinct *Arabidopsis thaliana* cuticle synthesis and transport mutants. Finally, we described the root cuticle architecture. We found that the cuticle layer covers not only root cap cells but also root pavement cells in distinct patterns.

The ability to visualize the plant cuticle layer of living cells within a few minutes is an essential tool for studying the effects of various stresses to which plants are exposed.

IMMUNOFLUORESCENCE IMAGING OF ATG8 COUPLED WITH STEREOLOGICAL METHODS FOR UNBIASED QUANTIFICATION OF AUTOPHAGOSOMES IN *ARABIDOPSIS THALIANA* ROOT

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Macroautophagy is an essential biological process characteristic with specific double membrane compartment called autophagosome in which cellular material destined for degradation is enclosed. ATG8 protein is present at the autophagosome membrane from its early development stage up to the degradation of autophagic body inside the vacuole, which makes it a powerful tool for studying autophagy. In *Arabidopsis thaliana*, 9 isoforms of ATG8 were identified, i. e. *At*ATGa – *At*ATG8i, some of them – when fused with fluorescent proteins (FT-ATG8) – are commonly used as markers of autophagosome in live imaging where the intensity of autophagy is estimated by the number of autophagosomes. This approach requires the introduction of FT-ATG8 marker into plant material of interest by crossing or transformation. Time-consuming generation of suitable lines can be inconvenient e. g. in case of screening for autophagy-deficient phenotype in a large collection of mutants.

To circumvent this hurdle, we tested the feasibility of immunofluorescence imaging for the purpose of autophagosome visualization by probing *At*ATG8 with anti-ATG8 antibody. To precisely quantify the number of autophagosomes in epidermal root tissue in *A. thaliana*, we applied optical disector and volume estimation by Calavieri principle, i. e. stereological methods suitable for quantitative assessment in 3D.

Our immunolabelling protocol specifically recognized autophagosomes in both the elongation and meristematic zone of the root. A higher number of autophagosomes was observed by immunolabelling than with the use of FT-*At*ATG8e marker, suggesting that single *At*ATG8 isoform markers do not detect all autophagosomes in a cell. Immunolabelling thus provide more precise information on number of total autophagosomes present in a cell as the anti-ATG8 antibody recognizes virtually all *At*ATG8 isoforms. The number of autophagosomes per volume of tissue determined by stereological methods correlated with the intensity of autophagy and provided reproducible and robust results.

Immunolabelling coupled with stereological methods thus constitute a powerful toolbox for unbiased quantification of autophagosome number which makes it a convenient alternative to live imaging gold standard use of FP-ATG8 markers.

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EPIGENETIC REGULATION OF PIN1

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The auxin efflux carrier PIN is the key mediator for polar auxin transport in developing plant tissues. To establish polar auxin transport, a narrow PIN-positive channel needs to be established. This coordination requires an intricate interplay of many proteins. The PIN expression itself is regulated by auxin, however, we have a very limited understanding of how mechanistically it is manifested. Several studies have characterized a long non-coding RNA, APOLO, that plays a role in this process. The APOLO lncRNA functions as a scaffolding RNA and takes a role in the creation of chromatin loops. APOLO uses chromatin folding, loop creation, and promoter methylation to mediate the silencing of PINOID (PID). PID is the auxin-responsive gene and it is essential for proper cotyledon positioning and development, for maintenance of the inflorescence meristem, for whorl definition during flower development and it is important for wild-type root growth.

A novel long non-coding RNA (lncRNA) was identified within the PIN1 promoter region. Our investigation of their function in the PIN1 promoter revealed significant differences between transcriptional reporter pPIN1::NLS-GFP-GUS transgenic line with and without lncRNA deletion. Notably, when the long non-coding RNA (lncRNA) is absent, PIN1 expression is significantly upregulated in the leaves and primary roots, but not in the lateral roots. This tissue-specific epigenetic mechanism could shed new light on the non-coding fine-tuning of PIN1 expression in the development of distinct plant tissues.

Keywords: lncRNA, PIN1, Auxin

NEW PHYTOHORMONE DERIVATIVES AS A MODERN TOOL FOR BASIC AND APPLIED PLANT RESEARCH

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Plant hormones cytokinins regulate many growth and developmental processes in plants (Skoog et al., 1965). 6-benzylaminopurine (BAP) is most frequently exogenously used CK in plant biotechnology. However, its fast *in-situ* N9-glucosylation can also induce negative effects, which complicate micropropagation processes, especially in rare and susceptible medicinal plants (Bairu et al., 2009). To solve this problem, N9-glucosylation could be suppressed by appropriate N9 purine substitution of BAP or hydroxylation of its benzyl ring. Series of CK derivatives substituted at N9-position by various sugars and tetrahydropyranyl protective groups have been recently prepared to improve CK specific biological activity and are already routinely used in plant micropropagation (Plíhalová et at., 2016). Additionally, the replacement of the 2′ or 3′ hydroxyl groups of a nucleoside with a fluorine atom has also showed promising results in enhancing biological activity and increasing chemical or metabolic stability (Murvanidze et al., 2019). Moreover, by small change in cytokinin structure, a potent cytokinin antagonists and/or inhibitors of their inactivation have been obtained, including their isotopically and fluorescently labelled analogues (Plíhalová et at., 2016). Here, recent results of synthesis, characterization and biological activity testing of several new phytohormone derivatives will be presented and demonstrated that they can be used as an interesting new tool for plant biotechnology and agriculture.

References

Bairu et al. (2009). South African Journal of Botany **75**: 122-127.

Murvanidze et al. (2019). Propagation Of Ornamental Plants **19** (2): 48-51.

Plíhalová et al. (2016). New Biotechnology **33** (5): 614-624.

Skoog et al. (1965) Science **148** (3669): 532- 533.

ROLE OF *KUP9* GENE IN ADAPTATION OF *ARABIDOPSIS ARENOSA* TO SERPENTINE SOILS

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Serpentine soils represent powerful models to understand adaptation to local conditions because their extreme chemical and physical properties act as strong selective pressures. They are generally toxic for the plants due to high magnesium to calcium ratio and high concentration of nickel and other heavy metals. Despite these challenges, some plants, including several *Arabidopsis* species, adapted to this environment. Furthermore, the island-like distribution of serpentines across landscapes leads to repeated adaptation, making it a good model to study repeatability of evolution and various ways the plants can adapt to toxic soils.

Our genomic comparison of wild populations of *Arabidopsis arenosa* adapted and non-adapted to serpentine soils resulted in the list of candidate genes for adaptation. One of the main candidates with very frequent differences between adapted and non-adapted populations is *KUP9* gene, belonging to KUP/HAK/KT family of K^+ transporters. Here, we compare protein sequences of serpentine and non-serpentine alleles of *KUP9* gene in relation to the structure of the protein and the conserved regions of the protein. We show the potential functional implications of the differences, which will be tested by insertion of the respective alleles into *Arabidopsis thaliana* plants.

STREAMLINED DETECTION OF RNA PLANT VIRUSES: SINGLE-ENZYME RT-PCR ASSAYS SIMPLIFY PATHOGEN IDENTIFICATION.

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RT-PCR single-enzyme assays offer a simplified and efficient approach for the detection and amplification of specific RNA sequences. Unlike traditional RT-PCR assays that involve separate enzymes for reverse transcription and DNA amplification, single-enzyme assays combine both activities into a single enzyme, reducing the complexity of the experimental procedure. This streamlined process not only saves time but also minimizes the risk of contamination during the assay. In the context of pathogen identification and monitoring, single-enzyme RT-PCR assays have proven to be valuable tools. These assays can be utilized to detect and characterize RNA-based pathogens, including plant viruses. We developed our own single-enzyme RT-PCR assays in the laboratory. The RTX enzyme was expressed and purified from *E. coli*, and it was then successfully employed in the RT-PCR assay for the detection of several RNA plant viruses (such as ALSV, TMV, ToMV, PVX, and PVY) from plant tissue and seeds.

SUBCELULAR AND TISSUE SPECIFIC LOCALIZATION OF ARABIDOPSIS IRON SUPEROXIDE DISMUTASE 1 AND ITS COPPER-DEPENDENT REGULATION

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Superoxide dismutases (SODs) are key antioxidant enzymes responsible for the deactivation of superoxide radical by catalyzing its dismutation to hydrogen peroxide and oxygen. It is known that SODs determine plant abiotic stress tolerance, but the knowledge about their *in vivo* developmental expression and *in vivo* subcellular localization is still elusive. Here we address the organ- and tissue- specific developmental expression patterns, as well as subcellular localization of iron SOD1 (FSD1) using advanced fluorescence microscopy methods in Arabidopsis. FSD1-GFP temporarily accumulated at the site of endosperm rupture during seed germination. In emerged roots, it showed the highest abundance in cells of the lateral root cap, columella, and endodermis/cortex initials. The largest subcellular pool of FSD1-GFP was localized in the plastid stroma, while it was also located in the nuclei and cytosol. Mutant analysis also revealed salt sensitivity and requirement for FSD1 in seed germination during salt stress. In accordance with the known regulation of *FSD1* expression, and abundance, our findings demonstrated that the activity of FSD1 depends on the availability of Cu^{2+} in growth media. Additionally, we describe the Cu^{2+}- dependent regulation of SOD isoforms in *Medicago sativa*, an important crop species. In summary, our study suggests new developmental and osmoprotective functions of SODs in plants.

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VISUALISATION OF AUTOPHAGOSOMES IN SOMATIC EMBRYOS OF NORWAY SPRUCE

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Autophagy is an evolutionary conserved catabolic process to maintain or restore cellular and organismal homeostasis. In plants, basal autophagy is essential for growth and development; it is required for nutrient remobilisation during senescence and nutrient deficiency and to remove organelles and macromolecules formed during plant development or damaged by environmental stresses. The material to be degraded is delivered to the vacuole within the double–membrane vesicles - autophagosomes that are generated in the cytoplasm when macroautophagy is induced.

We studied autophagy in Norway spruce somatic embryos. Embryogenic culture consists of early embryos composed of meristematic cells and long vacuolated suspensor cells. Somatic embryo maturation is triggered by the addition of phytohormone abscisic acid to the cultivation medium. Meristems then develop into embryos proper, while suspensor cells are gradually eliminated by programmed cell death, in which autophagy plays a crucial role.

We followed up autophagy-related protein ATG8, which can be located in both autophagosomal membranes and is, therefore, a convenient marker for monitoring macroautophagy. We optimised a protocol for whole-mount indirect immunofluorescent labelling of ATG8 in early embryos and in embryos after one week of maturation carried out in the automated system InSituPro VS (INTAVIS, Biological Instruments, Germany). As a primary antibody, we used anti-ATG8 (Agrisera, Sweden), and as a secondary antibody, Goat anti-Rabbit IgG (H&L), DyLight® 488 (Agrisera) or AlexaFluor 555 Goat anti-Rabbit IgG (Invitrogen, Molecular Probes, USA. Cell walls were counterstained with Fluorescent Brightener 28 (Calcofluor White M2R). Embryos were mounted to the antifading Roti®- Mount FluorCare mounting medium (Roth, Germany) containing nuclear stain DAPI.

We detected autophagosomes in both meristems and suspensors, where they were more abundant. Secondary antibody AlexaFluor 555 helped to avoid autofluorescence of embryos that interfered with the signal of DyLight® 488.

TELOMERASE RNA: PHYLOGENY ASSISTED COMPUTATIONAL IDENTIFICATION OF STRUCTURAL RNAS IN "AUTOMATED" WAY

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Telomerase RNA (TR) is a crucial non-coding RNA that serves as a template for telomere repeat synthesis through the telomerase ribonucleoprotein (RNP) complex and also acts as a scaffold for the telomerase RNP assembly. Unfortunately, the highly variable nature of TRs makes their characterization extremely challenging.

Understanding the biogenesis of TRs in diverse organisms is key to elucidate telomerase regulation and correct spatio-temporal assembly. Our recent characterisation of TRs in plants, algae + other protists, and the insect order Hymenoptera (Fajkus NAR 2019, 2021, 2023) substantially expanded knowledge in this this field.

While identifying TR homologs using blast-based searches is inefficient due to their low sequence conservation, the implementation of sequence-structure homology searches using the Infernal tool significantly improved such identification in a wider evolutionary scale. Nevertheless, compared to tools like blast, which usually offer user-friendly web-based implementations and provide results in a clear format, using Infernal requires more demanding procedures, particularly for researchers who are not proficient in programming and command line operations.

To address this issue, we developed a user-friendly pipeline to perform Infernal analyses. This pipeline requires only two user defined inputs: i) query sequences (in Stockholm format), ii) subset of NCBI genome assemblies as a database. The results are automatically processed into a comprehensive table, providing information about significant hits, along with the organisms' taxonomy. Since Infernal lies at the core of this analysis, such searches are particularly suitable for identifying homologs of structural non-coding RNAs in a broad evolutionary context. In line with T. Dobzhansky's statement, "Nothing in Biology Makes Sense Except in the Light of Evolution" we present a computational pipeline to facilitate searches of non-coding RNAs.

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TRANSFORMATION OF *CANNABIS SATIVA*

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In recent decades, *Cannabis sativa* (L.) has been the subject of extensive research namely due to its pharmaceutical value. Modern genome editing tools open up the possibility of altering the metabolic pattern of *Cannabis.* A prerequisite is the establishment of reliable transformation and regeneration protocols, as *Cannabis* is highly recalcitrant to both.

We have optimized protocols for agroinfiltration of young plants and *in vitro* transformation of different types of primary explant. In fiber-type cultivars, we have successfully transformed hypocotyl and leaves explants from in-vitro germinated seedlings, while in drug-type cultivars we transformed leaves segments either from *in vitro* grown lateral buds or from surface-sterilized leaves of greenhouse plants.

To overcome the recalcitrance of *Cannabis* to regeneration, we use different approaches. We conducted a comparative study in which we tested different media compositions to induce plant regeneration. We compared the effect of selected cytokinins (mT, zeatin, TDZ, 2iP) in combination with auxin NAA and described the changes in organogenesis after the application of novel cytokinin oxidase/dehydrogenase inhibitors. As a second approach, we have focused on overexpressing morphogenic regulators WUS (WUSCHEL) and STM (SHOOT MERISTEMLESS). Even though both approaches affected the response of primary explants to transformation, they did not lead to the formation of transformed organs. Newly we are implementing the third approach which involves the use of the chimeric GRF-GIF (GROWTH-REGULATING FACTOR and GRF-INTERACTING FACTOR) fusion protein.

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FEEDME, COMPLEMENTARY PEPTIDE MEDIATED GENE KNOCKDOWN IN POLLEN TUBES

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Gene knockdown is a powerful forward genetic workflow that is when weldone can pinpoint a gene function at the molecular level. T-DNA collections are abundant but even in Arabidopsis thaliana covers <50% of the annotated gene. CRISPR-Cas9 has nicely filled this gap and expanding, nevertheless, it's laborious as it needs screening multiple generations to confirm the phenotype and it can be exposed by gene redundancy or gene lethality.

Here, we have attempted and show that complementary synthetic peptides (csPEPs) designed against protein of interest can lead to protein levels manipulation revealing a gene function. Uncoated label (FAM and FITC, TAMRA, Cyanine dyes, STAR635 for STED and other labelling) or unlabelled csPEPs are fed to growing pollen tubes and endocytotically internalized leading to target protein levels alteration either via knockdown or overexpression. This has a potential as a super fast-efficient tool for large scale functional screen prior to indulging into generation of CRISPR-Cas9 stable lines.

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USING THE LUMINESCENCE-BASED METHOD FOR QUANTIFYING BACTERIAL INFECTION IN ARABIDOPSIS

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The quantification of bacterial infection in *Arabidopsis thaliana* has always been a practical tool to analyze how plants respond under different conditions, treatments with chemical compounds, or genetic modifications against bacterial infection. For such purpose model, pathosystems *A. thaliana* against *Pseudomonas syringae* pv *tomato* DC3000 was mainly used. However, the classic bacteria quantification method has several limitations, including being time-consuming and labor-intensive, which limits its usage for detail spatio-temporal analyses.

Nowadays, the development of versatile tools for the quantitative and spatial detection of bacteria in plants uses various approaches (López et al., 2003). One of the most prominent approaches is the luminescence-based method, which involves the integration of the vectors *luxCDABE* luciferase operon into the plant pathogenic bacteria genomes. The Tn7 transposon system mediates this integration, allowing for stable and selective genomic integration across bacterial phyla (Matsumoto et al., 2022). The vectors were effectively employed by Matsumoto et al.,(2022) to create bioluminescent *Pseudomonas*, *Rhizobium* (Agrobacterium), and *Ralstonia* transformants.

In this poster we would like to present you the luminescent based quantification technique of A. thaliana infected with *Pseudomonas syringae pv. tomato DC3000* (*Pto-lux*). This method was developed and describe in Matsumoto et al. (2022) and we would like to compare the results from the paper with our results related to establishing of the method in our lab. This innovation in detection technologies has the potential to simplify plant pathology research and lead to a better understanding of host-pathogen interactions.

Reference: Matsumoto A, Schlüter T, Melkonian K, Takeda A, Nakagami H, Mine A. A versatile Tn7 transposon-based bioluminescence tagging tool for quantitative and spatial detection of bacteria in plants. Plant Commun. 2021 Jul 20;3(1):100227. doi: 10.1016/j.xplc.2021.100227. PMID: 35059625; PMCID: PMC8760037.

ADVANCED LIGHT-SHEET AND SUPER-RESOLUTION MICROSCOPY IN ALFALFA RESEARCH

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Medicago sativa (alfalfa) is recognized as one of the most widely grown agronomic crops with fundamental importance to the ecosystem and sustainable agriculture worldwide. The interest is motivated by its ability to fix atmospheric nitrogen in symbiosis with beneficial soil bacteria in root nodules and by its extraordinary biological and agronomical potential. Therefore, microscopy approaches usable in biotechnologically oriented crop research allowing detailed investigation of crops anatomical, structural

and physiological characteristics are highly desired. However, accommodation of robust crop samples for imaging in conventional microscopy platforms is highly challenging. We have developed two innovative methods for crops advanced microscopy imaging. First, we significantly improved immunolabeling protocols well adapted for super-resolution imaging of alfalfa roots that might serve as a powerful tool improving cell biological structural studies (Tichá et al., 2020). The second one aims the volumetric imaging by light-sheet fluorescence microscopy (LSFM) allowing to study early symbiotic interaction of alfalfa with beneficial soil bacterium *Ensifer meliloti* (Hlaváčková et al., 2023). Considering the robustness of alfalfa roots, LSFM is essential to secure unique live-cell imaging conditions for alfalfa–*E. meliloti* interaction that are highly compromised in conventional microscopy systems. These innovative approaches open up new opportunities for studying plant–microbe interactions in real time and for the long term, which is indispensable to understand the regulation of the complex developmental and nodulation mechanisms.

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CHEMICAL PRIMING OF THE RESPONSE TO STRESS IN PLANTS BY AROMATIC CYTOKININ ARABINOSIDES

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Two aromatic cytokinin (CK) derivatives, 6-benzylaminopurine-9-arabinosides (BAPAs), namely 3-methoxy-BAPA and 3-hydroxy-BAPA, are able to trigger pathogen-associated molecular pattern-triggered immunity (PTI) in *Arabidopsis thaliana*. In this study, we focus on induced resistance by these cytokinin arabinosides (CK-A) in more detail and aim to elucidate the underlying molecular mechanisms.

Foliar application of CK-A on four-week-old soil-grown Arabidopsis plants induced resistance against *Pseudomonas syringae* pv. *tomato DC3000* (*Pst*) and *Botrytis cinerea*. This effect is caused by the ability of CK-A to enhance the defense capacity of treated plants rather than by direct interaction of the compounds with *Pst* or *B. cinerea*. Defense signaling, including pathogen-associated molecular patterns (PAMPs), was found to be upregulated after foliar application of CK-A. Moreover, after *Pst* inoculation, both *FRK1* and *PR1* genes were strongly upregulated in CK-A-treated plants in comparison to untreated control plants, indicating that CK-A triggers improved plant immunity. This was reflected in the decreased number of *Pst* colony-forming units occurring in CK-A-treated plants compared to untreated controls. Moreover, pretreatment of Arabidopsis by CK-A also reduced the formation of lesions after *B. cinerea* infection.

It was observed that CK receptor mutants are not primable by either CK-A or *t*-zeatin. The primability for *Pst* infection by CK-A has been tested in several mutants of the salicylic acid (SA) and jasmonic acid (JA) signaling pathways. Results indicate that the SA pathway is important for the CK-A priming effect. Together, CK-A can induce resistance in Arabidopsis plants for future infection by *Pst* and *B. cinerea*. Results indicate that a priming mechanism is involved in resistance induced by CK-A, but the underlying mechanisms are still under investigation.

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CHANGES OF THE IAA CATABOLIC NETWORK IN AUXIN-STARVED BY-2 CELLS SHOWN BY COMPUTATIONAL MODELLING

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Auxin metabolism is a key regulatory process responsible, together with auxin transport, for the modulation of auxin concentration and hence also for the intensity of the respective hormonal signal. A wide spectrum of metabolites of the natural auxin IAA have been described, forming a complex network with numerous deactivation pathways. In order to elucidate how flexible this network is in its response to varying auxin availability, BY-2 tobacco cells grown in suspension with regular auxin-supplemented media as well as after a short period of auxin starvation were exogenously treated with IAA and the resulting metabolite levels were assessed using HPLC/MS in the cell contents and cultivation media. Subsequently, a mathematical model simulating the metabolic conversions together with mutual cell-media metabolite transport was developed in the MATLAB computing environment. This model was then fitted into the experimental data, thus obtaining estimates of the reaction rates from the kinetic parameters of the model. Comparison of these independent parameter estimates showed that auxin starvation resulted in a substantially decreased rate of both amino acid conjugation with IAA and the following oxidation of the conjugates on the one hand, with a simultaneous increase in the production of auxin decarboxylation metabolites indole-3-carbinol and oxindole-3-carbinol on the other. This shows a considerable flexibility of the metabolic regulation of auxin levels, which thus needs to be considered in a number of physiological and developmental situations.

TOUCH (IN)SENSITIVITY OF ANAESTHETIZED PLANT LEAVES AND HOW TO MEASURE IT

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Nowadays, it is well-known that plants sense their environment through many different receptors and downstream signalling pathways. Much has been written to date about the perception of light, temperature, water, chemicals, or various biotic factors such as pathogens or wounding caused by herbivores. Recent research has shown that plants also sense touch, and interestingly plants can react to touch as to stress factor. Touch signalling in plants can work by at least two independent pathways: one dependent on jasmonates (JAs) and the JAs-independent pathway regulated by specific calmodulin binding transcription factors and therefore by Ca^{2+}. Recent findings from our laboratory have shown that general volatile anaesthetic (GVA) diethyl ether can effectively inhibit JAs and Ca^{2+} systemic signalling in response to wounding in Arabidopsis thaliana. Here we investigated the effect of diethyl ether anaesthesia on cytosolic Ca^{2+} concentration, phytohormone levels and touch responsive genes expression in response to gentle brush touching on Arabidopsis thaliana leaves.

DYNAMICS OF PROTEIN-MEMBRANE INTERFACES DURING PLANT CELL DIVISION

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Cell division is an essential process for any living cell on Earth. Cell division is finalized by cytokinesis, which results in two daughter cells. Plant cytokinesis is fundamentally different from that of animal and fungal cells. Plant cells evolved a unique membranous compartment, the cell plate. The cell plate grows in an inside-out manner by which it separates two daughter nuclei. The formation and growth of the cell plate require a highly orchestrated interplay between various cellular processes, including exocytosis and endocytosis, and polysaccharide synthesis processes catalyzed by the callose synthase complex and the cellulose synthase complex. Work in our laboratory focuses on the dynamic interaction between proteins and membranes, including biocondensate formation.

To follow cell plate development, we mainly utilize plant cell cultures with great synchronization ability and morphological characteristics suitable for studying plant cytokinesis. We also employ various other plant model tissues, including Arabidopsis roots, tobacco leaves and pollen tubes. We combine different microscopy techniques, such as confocal laser scanning or spinning disc microscopy, AiryScan imaging, and super-resolution techniques, together with an integrative structural approach to study the intricate process of plant cell division. Our unique methodology allows us to visualize and track the dynamic associations of protein complexes with membranes, individual lipid-protein interactions, and biocondensate formation. Our laboratory aims to describe plant cytokinesis in unprecedented detail and create a 4D map of cell plate development.

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EFFECTIVE APPLICATION OF RGB IMAGING SYSTEM IN WINTER WHEAT FROST RESISTANCE SELECTION PROCESS

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Winter hardiness is an important feature of winter wheat varieties grown in a significant part of the Northern Hemisphere. The most important factor of winter hardiness is the frost resistance of seedling plants. The required level of frost resistance widely differs in different growing conditions and frequency of occurrence. Since the absence of natural selection factors of winter leads to the accumulation of low frost-resistant genotypes in the breeding program, a number of tests have been developed that create winter stress factors artificially. These tests are labor intensive, including evaluation. Simple imaging station employing Raspberry Pi microcomputer with camera and new algorithm in Python was developed to evaluation of green leaf area in tested seedlings. A total of 28 winter wheat genotypes were tested in four seasons by artificial freezing tests to estimate overwintering ability. Plants were imaged once before and 21th regeneration day after freezing period and also analysed by human scoring for validation of imaging method. Correlations between RGB imaging and manual scoring varied from 0.62 to 0.82 in individual years. The method is sufficiently accurate for determining the level of frost resistance of genotypes and their selection in breeding. More accurate results may be possibly improved by optimization of growing design and minimizing overlaps of seedlings. The presented method has a considerable advantage in the speed of measurement and non-destructiveness, which makes it very suitable for use in breeding, when it is necessary to test a large number of genotypes.

SMART TOOL FOR THE MEASUREMENT OF FLUORESCENCE LIFETIME OF PLANT LEAVES WITH SUB-NANOSECOND RESOLUTION

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The work focused on the realization of measuring head for *in vivo* Chl fluorescence decay measurements of plants using Time Correlated Single Photon Counting (TCSPC) method. In this method sub-nanosecond laser pulses with 10 MHz repetition rate excites the sample, and the arrival times of the emitted fluorescence photons are analysed. Photon statistics are generated by iteratively fitting the sum of 2 or 3 exponential functions.

Our tool was tested on both plastids and *in vivo* leaf samples of savoy cabbage (*Brassica oleracea* var. *sabauda* L.). 3-4 subsequent leaves give a complete sample coverage; thus we separated the leaves into coverage layers numbered from the outermost. The Chl fluorescence lifetime gradually increased in the isolated plastid suspensions as well as in *in vivo* leaf samples towards the innermost leaf layers explained by an increase of natural absence of light (etiolation syndrome).

We also studied cadmium stress as well as iron deficiency examined by *in vivo* TCSPS measurements. The reduced fluorescence quenching caused an increase of fluorescence lifetime. Finally, a long-term (10 week) testing of our setup was carried out on Chl-retaining resurrection *Haberlea rhodopensis* plants, which protected itself by an elevated non-photochemical quenching yielding a decrease of fluorescence lifetime.

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CHARACTERIZATION OF SEX-LINKED GENES IN *SILENE LATIFOLIA*

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The abstract is available in the abstract book of Student conference.

THE ROLE OF ELEVATED AUXIN PERCEPTION IN THE PROLIFERATION OF AUXIN-AUTONOMOUS CELL LINES

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The abstract is available in the abstract book of Student conference.

VISUALISING AND ANALYSING EVENTS NEAR THE PLASMATIC MEMBRANE

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The plasmatic membrane (PM) in plants is a vital location where numerous dynamic events occur, playing crucial roles in various cellular processes. Total Internal Reflection Fluorescence (TIRF) and Variable Angle Epifluorescence (VAEM) microscopy approaches have emerged as powerful tools for visualising these dynamic events near the PM with exceptional resolution. However, there are specific considerations when applying TIRF microscopy to plants, as they possess cell walls, unlike animal cells.

Analysing TIRF microscopy data poses challenges due to the detection and quantification of fluorescent signals, especially when dealing with a high abundance of fluorescent foci. To address this issue, TrackMate, a popular tracking and detection software, offers a solution by providing automated algorithms for detecting and tracking fluorescent objects in TIRF microscopy datasets.

In conclusion, TIRF microscopy is a valuable tool for studying dynamic events near the PM in plants. Although there are specific considerations due to the cell walls, TIRF microscopy offers exceptional resolution for visualising these events. Additionally, TrackMate software facilitates the detection and analysis of fluorescent signals in TIRF microscopy datasets, enabling researchers to statistically analyse the data and gain quantitative insights into the dynamics of processes occurring near the PM in plants.

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PET OR PEST: PLAYGROUNG FOR BUGS HELPS TO DECIPHER PLANT-HERBIVORE-PATHOGEN INTERACTIONS

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Plants in natural conditions have to interact with other inhabitants of their environment forming both mutualistic and parasitic interactions. Besides beneficial microorganisms, plants combat a wide range of phytopathogens and insect pests. It is therefore necessary to develop a system for testing mutualistic interactions.

In many studies, leaf discs are used in Petri dishes to carry out choice test. Cutting leaf discs causes mechanical damage and the production of large amounts of volatile compounds (VOCs) associated with mechanical stress, which may affect pest attraction. We have established a vial system for a feeding choice test for oilseed rape (*Brassica napus*) - *Leptosphaeria maculans* - diamond-back moth (*Plutella xylostella*). In a developed vial system, consisting of two 100 ml plastic vials connected by a plastic T-connector, the feeding choice test for *P. xylostella* caterpillars was optimised. The reliability of the vial system was confirmed using known natural insecticides: escin and neem oil.

As mentioned above, VOCs is an essential part of insect behaviour and their detection in plants helps us to understand insect decision making. VOCs from oilseed rape leaves were collected using Tenax TA sorbent tubes and analysed using a LECO Pegasus 4D GC×GC-TOFMS system. A wide variety of VOCs was detected in the headspace of oilseed rape leaves. However, a group of 55 VOCs with the highest variability between inoculated and control treatments was identified.

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EPICUTICULAR WAX OF *ARABIDOPSIS THALIANA* LEAVES AND ITS RENEWAL DYNAMICS

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The cuticle, a thin extracellular layer on the aboveground surface of terrestrial plants, is the first physical barrier between the plant and the outer environment. The cuticle and its upper part called epicuticular waxes are indispensable in protecting a plant against water loss and ultraviolet radiation (UV). It serves as one of the first defence layers against pathogens. In addition to that, the cuticle also influences the growth and development of plant organs. Its composition and renewal dynamics are presumably highly regulated because the biosynthesis of the cuticle is highly energy-consuming. Although direct evidence of cuticle renewal during leaf ontogeny is still missing.

Arabidopsis thaliana is a great model plant for plant cell biology or molecular biology studies. However, *A. thaliana* is not so convenient plant for cuticle research. To find the best way how to study *A. thaliana* leaf cuticle and epicuticular waxes, we are comparing different approaches to cuticle or epicuticular waxes isolation. The isolation of epicuticular waxes was followed by the study of its renewal dynamics. We use our newly developed sensitive method: the $^{13}CO_2$ pulse-chase labelling approach (followed by sampling for four weeks after labelling) to find dynamics in particular compound classes of epicuticular waxes. We performed compound-specific GC-IRMS identification and quantification of dominant epicuticular wax compounds from mature *A. thaliana* leaves. We provide evidence that epicuticular waxes are renewed throughout leaf ontogeny.

REAL-TIME VISUALIZATION OF MOLECULAR EVENTS USING AUTONOMOUS BIOLUMINESCENCE IN ENGINEERED ARABIDOPSIS GROWING IN NATURAL CONDITIONS

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The visualization of molecular events, like transcriptional activity, in living plants is of paramount interest in exploring their development and interactions with the environment. The current methodologies utilize fluorescent proteins or biochemical assays and are restricted to *in vitro* settings or fragmental snapshots of plant life in their natural habitats, like soil substrates. Autonomous bioluminescence relies on the concurrent biosynthesis and oxidation of the fungal luciferin, and it can be genetically transferred and manipulated in plants. We are interested in the real-time, uninterrupted visualization of the distribution of the plant hormone auxin in Arabidopsis thaliana plants growing either in *in vitro* conditions or soil. To this end, we genetically transformed arabidopsis to stably express the biosynthetic pathway for the fungal luciferin under constitutive, viral promoters, and the fungal luciferase under the auxin-responsive (DR5) promoter. Using a commercial camera and lens, we record stable, robust autonomous luminescence in subsequent generations of Arabidopsis grown in *in vitro* media or soil. Given the essential morphogenetic role of auxin in the roots, we sought to reveal its distribution in the root system growing in soil. Applying simple design and materials, we constructed mini rhizotron devices, filled with the soil substrate, and grew bioluminescent plants in slight inclination. This allowed us to visualize the real-time auxin distribution in roots in their natural habitat, the soil, without any intervention or treatment. In the future, we intend to study further basic auxin concepts in plants grown in rhizotrons, and the alteration of auxin abundance and redistribution in the course of plant responses to environmental stress. This poster brings Autonomous Bioluminescence as the new means of visualization of transcriptional activities, together with the simple, do-it-yourself design and application of rhizotrons to visualize roots in nature.

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ARABIDOPSIS THALIANA MRNA STORAGE AND REGULATION

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Seed dormancy, germination, and longevity are important agricultural traits subjected to precise regulation of translation. After activation of germination, stored mRNA bonded to monosomes in the dry stage, attach to polysomes, undergoes strong oxidation, and is translated very fast. It is unknown how this storage and translational burst are regulated.

Arabidopsis thaliana genotypes Columbia (Col) and Capo Verde (Cvi), with non-dormant and dormant seeds respectively, were used for the isolation of mono- and polysomal fractions. The fractions were collected from freshly-harvested (FH), after-ripened (AR, 3 months after harvesting), and imbibed seeds; and were subjected to RNA-seq and LC-MS/MS protein analysis.

Transcripts unique for mono- and polysomal fractions of given stages were found. Altogether, 14,488 proteins were identified, with 430 and 589 proteins significantly up-regulated in polysomes compared to monosomes in Col and Cvi, respectively.

Analysis of epitranscriptomic RNA modifications revealed the predominant occurrence of 1-methyladenosine followed by methyl-6-adenosine. The quantity of m6A modifications increased with a maximum at 3 months of post-harvest storage followed by a decline.

Using immunopurification, the m6A RNA fractions were enriched and sequenced, providing 2,841 and 5,394 genes for the dry and imbibed Col seeds, respectively. In the dry stage, the m6A modifications were found mostly on genes belonging to the “nuclear export signal receptor”, while from the imbibed stage were classified into “snoRNA binding” GO terms.

Our findings provide new insight into mRNA storage and regulation in connection to dormancy and germination of Arabidopsis seeds.

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IMPROVING THE ACCURACY OF MEASUREMENT OF LIGHT AND TEMPERATURE CONDITIONS DURING PLANT CULTIVATION

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The reproducibility of the published results is aften hindered by insufficient description of temperature, light intensity and spectra occurring in climate chambers. All these parameters play a critical role, especially under stress conditions. Here, we would like to highlight the importance of precise specification of plant and climate chamber temperatures, which can be determined using the thermal camera FLIR A500. The camera has been used for the evaluation of the ability of plants to tolerate heat stress. The results can be correlated, e.g., with the changes in hormonal levels.

The problem with specification of light intensity and spectrum composition is mostly caused by the use of non-specified types of light sources in climate chambers. Due to the high variability of spectra, the information about light intensity can be misleading. Moreover, often used units - Photosynthetic Photon Flux Density (PPFD), indicating the concentration of photons ($\mu mol\ m^{-2}\ s^{-1}$), reflect only a narrow wavelength band of 400-700 nm. PPFD does not sufficiently describe the amount of energy that hits the plant, because photons of different wavelengths have different energy.

The presented data will illustrate the use of the devices for precise measurement of intensity in each wavelength and temperature to characterize temperature and light conditions, which strongly affect behaviour of rice plants.

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USER-FRIENDLY TOOLKIT FOR CRISPR/CAS9 MUTAGENESIS IN TOBACCO BY-2 CELL LINE: INTRODUCING INDUCIBLE SELF-EXCISION SYSTEM

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The abstract is available in the abstract book of Student conference.

VISUALISATION OF RAPID LONG-RANGE SIGNALS IN *ARABIDOPSIS THALIANA* AND THEIR EFFECT TO DOWNSTREAM REACTIONS UNDER DIETHYL ETHER ANAESTHESIA

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Electrical and calcium signals play an important role in the induced plant defense reactions after wounding. General volatile anaesthetics (GVA) inhibit electrical signal propagation in animal neurons. Although plants do not have neurons, they generate and propagate electrical signals systemically from a local damaged leaf to neighbouring leaves. This systemic electrical signal propagation is mediated by ligand-gated glutamate receptor-like (GLR) channels. We investigated the effect of GVA diethyl ether on the systemic electrical and further downstream responses in Arabidopsis thaliana. We monitored electrical signals, cytoplasmic Ca^{2+} level ($[Ca^{2+}]_{cyt}$), amino acid contents, phytohormone response as well as gene expression in response to heat wounding during diethyl ether anaesthesia.

PROFILING HISTONE POST-TRANSLATIONAL MODIFICATIONS IN THREE GREEN ALGAE SPECIES

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Epigenetic regulation is mediating transcriptional activity in response to environmental changes. Because of complex growth patterns and requirements, understanding the epigenetic machinery and targets is crucial for optimizing the growth of green algae. Mass spectroscopy allows to quantitative examine the presence and combination of histone epigenetic modifications, while ChIP-qPCR/-Seq are powerful tools to assess gene targets of these epigenetic marks. We present an approach to quantitatively and qualitatively profiling histone PTMs in three different green algae species (*Chlamydomonas reinhardtii*, *Scenedesmus quadricauda,* and *Chlorella sorokiniana)*.

We applied an unbiased MS to identify and characterize histone H3 variants and histone PTMs in three algae species. Our results show a high diversity of composition and relative quantities of modifications in different species, with Chlamydomonas lacking the facultative heterochromatin mark - H3K27me3.To describe the gene targets of PTM histone modifications, we adapted ChIP to use in two previously not examined green algae species. We optimized crucial steps such as cell harvesting, crosslinking of histone-DNA complex, fragmentation, co-immunoprecipitation, and the optimization of final ChIP steps: qPCR and libraries preparation for sequencing. We showed the same method with slight changes could be used to examine epigenetic modifications in distinct species, with different cell wall compositions and number of connected cells.

Our straightforward approach allows wider utilization of epigenetic studies using ChIP method in algae research. A new tool to examine epigenetic changes in organisms with dynamic adaptation to environmental changes would lead to the discovery of genes important for the growth and production of ingredients.

A NOVEL GENETICALLY ENCODED CELL WALL PH SENSOR

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Accurate determination of cell wall pH is of great importance for understanding plant tropisms and diverse developmental processes, among others root cell elongation, pavement cell lobing, and pollen tube growth. Although numerous dyes and sensors have been used to estimate cell wall pH, these methodologies suffer from significant limitations. Here, we introduce a novel assemblage of pH sensors comprising two fluorescent proteins and a cell wall binding module. Our approach capitalizes on the distinct sensitivity of diverse fluorophores to different pH. The cell wall binding modules originate from distinct cellulolytic bacteria and exhibit specific affinities for various cell wall components, such as cellulose or xylan. Collectively, the new cell wall-binding pH sensors enable ratiometric determination of cell wall relative pH *in vivo*, and when combined with live cell imaging, they furnish a helpful tool for investigating a wide array of plant developmental processes.

MORPHOGENIC AND BIOCHEMICAL REACTIONS OF EXPLANTS TREATED WITH NANOPARTICLES DURING CRYOPRESERVATION PROCEDURE

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Nanoparticles (NPs) have emerged as a promising tool in plant biotechnology due to their unique physicochemical properties, which make them useful for various applications such as improving plant growth and development, enhancing plant resistance to biotic and abiotic stresses, and delivering biomolecules to targeted plant cells. Recently, NPs have been used as a new type of cryoprotectant agent useful in cryopreservation studies. Plant cryopreservation, i.e. storage of tissues in liquid nitrogen (LN) at -196°C, is important because it enables the long-term storage of valuable plant genetic resources, allowing for their conservation and future use in research and plant breeding programs. The aim of this study was to verify the morphogenic response and biochemical activity of LN-stored explants treated with nanoparticles. Shoot apices of two cultivars of bleeding heart (*Lamprocapnos spectabilis* Fukuhara) were used as the plant material. Gold, silver, and zinc nanoparticles were added at various concentrations either in the preculture medium or the alginate bead matrix in the encapsulation-vitrification cryopreservation procedure. After LN storage and rewarming, the morphogenetic and biochemical response of the explants was analysed. The effect of NPs was cultivar-dependent. As for bleeding heart 'Valentine', the addition of 5 ppm AgNPs and 15 ppm AuNPs into the alginate bead matrix increased the cryopreservation efficiency by even 13%. As for 'Gold Heart' cultivar, supplementation of alginate with AgNPs and ZnONPs increased the survival rate of the shoot tips by 30%. The addition of NPs into the preculture medium was less effective or even hampered the cryopreservation efficiency, although such treated explants produced more shoots and of greater length compared with the non-treated control. Moreover, nanoparticles induced oxidative stress, which in turn affected the biosynthesis of plant pigments. Nonetheless, the beneficial action of nanoparticles during cryopreservation of plant tissues was confirmed.

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STRUCTURAL INSIGHT INTO EIF3E FUNCTION IN THE GAMETOPHYTE

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The abstract is available in the abstract book of Student conference.

EXTENSIVE OUTDOOR INSTRUMENTAL MONITORING OF EUCALYPTUS PLANTS EXPOSED TO WATER DEFICIENCY IN BRASIL

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We carried out water deficiency tests on Eucalyptus (of *Eucalyptus urophylla* x *Eucalyptus grandis,* commercial clones I144) plants in an open-air area, located in the Department of Forest and Wood Sciences of the Federal University of Espírito Santo (DCFM-CCA-UFES) in Jerônimo Monteiro, Espírito Santo, Brazil. A group of plants received daily irrigation leaving the substrate with moisture close to its field capacity (control) and two other groups (moderate and severe stressed) kept without irrigation starting from different days.

The plant physiological conditions were extensively monitored by portable infrared gas analyser, thermography, leaf water potential measurement in pressure chamber and chlorophyll fluorescence measurements. The paper is focused on the preliminary results of fluorescence parameters. Among them, the PSII operating efficiency (the quantum yield of PSII photochemistry during actinic light) was found to be most promising indicator of water deficiency.

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INVESTIGATING THE DOWNSTREAM SIGNALING MECHANISMS OF PHYTOCHROME DIMERS USING SINGLE-MOLECULE IMAGING

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In the model plant *Arabidopsis thaliana* five phytochromes [phys] exist, among which phyA and phyB are the most abundant and considered the most important for sensing changes in the environment. phyB-E act as red-light receptors, whereas phyA is considered the only far-red light receptor. All phys form obligatory dimers, a fact that has been mostly neglected in phy research.

Although phyB is considered the most important red-light receptor, heterodimers of phyB with other type II phys may mediate specific responses within the plant. Which dimers form *in planta* and what role they play is still unclear.

Combining single-molecule imaging and biochemical techniques with physiological experiments, we aim to decipher the dimerization profile of type II phytochromes. Our primary question is whether type II phytochromes only dimerize with phyB or also exist as homodimers *in planta*. In addition, we investigate the formation of higher order phytochrome oligomers.

Our initial results for phyC indicate that it forms homodimers when expressed in HEK293T cells and heterodimerizes when expressed with phyB. Using *A. thaliana* lysates, we investigate the dimerization of phyB with phyE using single-molecule imaging. Understanding the interaction profile of the type II phytochromes will help elucidate their specific role in plant development.

ALPS IN THE BOX: ENVIRONMENTAL CHAMBER FOR STUDYING ALPINE ADAPTATIONS IN *ARABIDOPSIS* POLLEN TUBES

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The primary aim of this project is to uncover the molecular mechanism behind the MAP18 gene allele, which shows signs of repeated positive selection in high-altitude populations of *Arabidopsis arenosa*. MAP18 is actively expressed in both pollen tubes and root hairs, functioning as a key regulator of actin polymerization, a process crucial for apical growth. Our hypothesis suggests that a lysine-to-arginine substitution at position 14, identified during the initial population-genomic screen, may be linked to adaptation to thermal stress, a challenge faced by high-altitude plants.

Within this project, we employed a diverse set of methods to investigate pollen tubes of WT and *Arabidopsis thaliana* harboring the selected mutation in the MAP18 gene across various temperature conditions. Utilizing a specially designed cultivation chamber equipped with a Peltier module controlled by an Arduino, we achieved a precise system for in vitro and semi–in vitro cultivation of pollen tubes under temperature fluctuations mirroring realistic environmental conditions. By cultivating the tubes directly on glass slides, we facilitated a straightforward microscopic analysis of their growth and morphology. Furthermore, this approach allowed for easy histological and immunological staining to explore the structure of the cytoskeleton and the composition of the cell wall.

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ARABIDOPSIS IRON SUPEROXIDE DISMUTASE FSD1 PROTECTS AGAINST METHYL VIOLOGEN-INDUCED OXIDATIVE STRESS IN A COPPER-DEPENDENT MANNER

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Iron superoxide dismutase 1 (FSD1) is a plastidial, cytoplasmic, and nuclear enzyme with antioxidant and osmoprotective functions. In our study, we attempted to characterize the role of FSD1 in response to methyl viologen (MV)-induced oxidative stress in *Arabidopsis thaliana*. The results demonstrated that the antioxidant function of FSD1 depends on the availability of Cu^{2+} in growth media. Arabidopsis *fsd1* mutants showed lower capacity to decompose superoxide at low Cu^{2+} concentrations and prolonged exposure to MV led to reduced ascorbate levels and higher protein carbonylation in plants lacking a plastid FSD1 pool as compared to the wild type. MV induced a rapid changes in FSD1 activity and genetic disruption of FSD1 negatively affected the hydrogen peroxide-decomposing ascorbate peroxidase as well. It was shown, that chloroplastic localization of FSD1 is crucial to maintain redox homeostasis. The study provides evidence for the conditional antioxidative function of FSD1.

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AN L-TYPE LECTIN RECEPTOR-LIKE KINASE IS ESSENTIAL FOR EMBRYO AND ENDOSPERM DEVELOPMENT IN *ARABIDOPSIS*.

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In angiosperms, seed acts as a functional unit which is accountable for generating the plant's offspring characterized by the embryo enclosed by the seed. The endosperm not only performs a vital role primarily as a nurse tissue to assist embryo development, but also in communicating signals between the embryo and maternal tissues. Lectin receptor-like kinases (LecRLKs) are a large family of receptor-like kinases (RLKs). In *Arabidopsis*, among 75 LecRLKs, 42 belong to L-type with an extracellular legume lectin-like domain. Recent studies suggest that LecRLKs act as sensors to facilitate the cellular response toward various stress and hormonal signals but the molecular mechanisms of L-type LecRLKs in plant growth and development is relatively less studied. We characterized a gene that encodes an L-type LecRLK involved in embryo and endosperm development in *Arabidopsis*. The phenotypic analysis suggests that the mutation in one L-type *LecRLK* develops aborted seeds as well as regulates the seed size. Interestingly, the gene promotes the rosette, root, and stem growth by coordinating the source-sink relationship. We also observed delayed and arrested embryo development in the mutants mostly at the heart stage which may lead to seed size and seed abortion. We proposed that the L-type LecRLK may play an important function in seed size determination. Further analysis will be required to elucidate the molecular mechanisms.

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COMPUTATIONAL ASSESSMENT OF INTERACTIONS BETWEEN CYTOKININ DEHYDROGENASE AND ITS SUBSTRATES

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Interactions between proteins and small molecules occur indispensably during enzyme reactions, membrane transport or signalling. Several computational algorithms have been developed to allow researchers to study protein-ligand interactions with little material expense and optimize experiments conducted in the wet lab. We present a methodological pipeline consisting of protein structural modelling, molecular docking, unbiased molecular dynamic simulations, and alchemical calculations to assess free binding energies between enzyme cytokinin dehydrogenase (EC 1.5.99.12) and its physiological substrates – plant hormones cytokinins. We report the effects of the enzyme's active site amino acid composition and the substrates' atomic structures on their mutual affinities and compare them to previous experimental findings.

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MOLECULAR DYNAMICS SIMULATIONS AS A TOOL TO STUDY PROTEIN-LIPID INTERACTIONS IN PLANTS

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Nowadays, with advanced protein structure prediction tools such as AlphaFold2, obtaining structural information about any protein of interest is relatively straightforward. However, these tools do not provide insight into the protein dynamics and its possible interactions with other (bio)molecules which occur within the cell. This gap can be bridged by molecular dynamics (MD) simulations.

Based on applying Newton's equation of motion to the system of our interest, MD simulations can, in atomistic detail, simulate how every atom in a protein or other (bio)molecule moves over time. Thus, MD simulations can be seen as a powerful computational microscope enabling the study of biological systems in unprecedented detail. Nevertheless, despite their obvious potential, MD simulations are still not widely used in the plant field.

Here, we present how MD simulations can be used to study plant protein-lipid interactions, particularly hand-in-hand with wet lab experiments.

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ROLE OF CYTOKININS AND CYTOKININ SIGNALING IN COLD RESPONSE OF ARABIDOPSIS

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Cytokinins were discovered as hormones regulating growth and development processes, but recently they are also known as regulators of abiotic stress responses. Here, we have focused on the role of cytokinins in plant response to low temperatures. Cytokinin-responsive genes *ARR-A* were previously shown to increase their expression in response to cold treatment with similar kinetics as the key cold transcription regulators *CBF* genes. In contrast to previous studies, we show that long-term cold treatment is followed by a decrease in the expression of *ARR-A* that is correlated with a decrease in the level of active cytokinins and other cytokinin metabolites. Moreover, cold treatment is followed by a decrease in the expression of *AHP* genes. Analysis of the triple mutants in cytokinin signaling *AHP* genes showed that cold-regulated repression of *ARR-A* could be mediated by AHP2, AHP3 and AHP5 but not by AHP4. Interestingly, *ahp2ahp3ahp5* triple mutant showed a higher increase in *CBF* expression after cold treatment when compared to wild-type plants. Our data suggest that cold regulates cytokinin levels and *AHP* genes to inhibit plant growth under low temperatures, inhibit cytokinin-responsive genes *ARR-A* and promote *CBF* expression.

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LC/MS PROFILING OF MAMMALIAN STEROIDS IN DIFFERENT TYPES OF PLANT MATERIAL

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Mammalian steroids, a diverse group of bioactive compounds with essential physiological functions in animals, have traditionally been associated exclusively with animal tissues. However, recent studies have provided compelling evidence for the presence of mammalian steroids in various plant species. The distribution of mammalian steroids within plant tissues has been investigated in various plant species. Steroids such as, pregnenolone, progesterone, testosterone and estrogens have been detected in different parts of plants, including leaves, stems, roots, and reproductive organs. Their presence and localization within specific tissues suggest potential roles in plant development and reproductive processes[1].

Furthermore, studies have indicated that mammalian steroids in plants may play a role in plant-environment interactions. For instance, certain steroids have been found to modulate plant defense mechanisms against pathogens and herbivores, suggesting their involvement in plant immune responses. In recent years, extensive research has been conducted to explore the potential medical applications of plant steroids, particularly in medicinal plants.

The aim of this work was to determine the profile of steroids (mammalin sex hormones) in medicinal plants (flowers, leaves, buds). For the determination of steroid compounds, combination of SPE and ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) was used.

Reference: 1. Janeczko A., Skoczowski A. Mammalian sex hormones in plants. Folia Histochemica et Cytobiologica. 2005, 43(2), 71-79.

HIGH-TEMPORAL RESOLUTION IMAGING OF ROOTS USING CLOSABLE MICROFLUIDIC CHIPS

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Plants constantly fine-tune their metabolism and growth to the environment. Fast growing organs such as roots need to do so in a very rapid manner, making detection of such reactions challenging. Recently, microfluidic platforms have enabled continuous microscopic imaging of growing roots in controlled perfusion with a possibility to capture initial seconds of ultrafast responses to various treatments.

We introduce an optimized microfluidic platform connected to a vertical stage spinning disk microscope. Our tailored closable single-layer PDMS silicone chips provide easy handling of experimental plants. Besides a basic system setup, we present an advanced pressure-controlled chip facilitating a continuous flow of both control and treatment media, thus avoiding unspecific "dead volume effect" responses. We also provide ideas for further custom optimization regarding chip material choice and design.

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IDENTIFICATION AND ANALYSIS OF CIS-REGULATORY ELEMENTS IN BARLEY GENOME

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Despite advances in sequence assemblies and genome annotations, identification of distal cis-regulatory elements (CREs) – silencers and enhancers – and understanding their interplay with promoters, transcription factors and non-coding RNAs in regulating gene transcription in plant species lag significantly behind.

We identified and characterized promoters and putative enhancers in three stages of barley embryo development. With the aim to characterize core promoters and their architectures, we opted for Cap Analysis of Gene Expression (CAGE) technique, which allows promoter analysis and single-base localization of transcription start sites (TSSs) and provides a measure of gene expression level in large plant genomes at costs comparable to RNA-seq. CAGE was complemented with epigenome and interactome profiling, which also allowed prediction of the distal CREs and their target genes.

To localize transcription factor-bound regions, ATAC-seq data were generated to identify open chromatin regions. These are usually significantly hypomethylated. The information about cytosine methylation level was extracted from bisulfite sequencing data. Besides, histone post-translational modifications typically associated with active CREs, such as H3K9 acetylation and H3K4 trimethylation were profiled by ChIP-seq. Finally, spatial contacts between potential distal elements and promoters were identified using chromosome conformation capture assays combined with ChIP (HiChIP technique).

All the data have been mapped to MorexV3 barley reference genome and integrated with previously generated and published transcriptomic data in our internal JBrowse-based epigenome browser for viewing and detailed analysis.

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RECENT ADVANCES IN PLANT HORMONES PROFILING

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Auxins are a group of plant hormones that affect a large part of the processes taking place in plant growth and development. The most important natural auxin indole-3-acetic acid (IAA) is involved in large part of the processes taking place in plant growth. The establishment and maintenance of auxin gradients within plant organs and tissues are coordinated by local IAA biosynthesis,

metabolism, and transport. The interacting network of these mechanisms regulates auxin levels and distributions in plant tissues. This is important for driving of developmental stages or inducing appropriate responses to environmental changes.

To improve our understanding of these mechanisms, information on levels of the free hormones as well as their metabolites is highly important. However, analysing plant hormones is demanding due to their very low concentrations and the tremendous complexity of plant samples. A sensitive analytical method with the highest possible resolution is necessary for this purpose. Modern methods provide rapid and effective separation of several classes of phytohormones. Ultra-high-performance liquid chromatography coupled with high-sensitivity tandem mass spectrometry (UHPLC-MS/MS) is the most widely used approach in phytohormone analysis. As conventional separation methods sometimes suffer from limitations in sensitivity and selectivity, there is a strong need for better separation techniques. We exploited recent advances in supercritical fluid chromatography (SFC) to take advantage of this highly efficient technique that overcomes the limits of other chromatographic methods. We have developed the SFC-MS/MS method for the determination of IAA metabolites and stress-related phytohormones that will be used for studying stress responses in various plant species.

DECODING THE BIOSYNTHETIC PATHWAY OF THE ANTICANCER ALKALOID PIPERLONGUMINE IN PIPERACEAE PLANTS

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Piperaceae plants have been traditionally used to alleviate cancer-like symptoms.[1] Piperlongumine and its analogs are bioactive alkaloids produced by some plant species of the genus Piper. Piperlongumine selectively induces apoptotic and autophagic cell death pathways in primary myeloid leukemia cells[2] and glioblastoma multiforme cells[3] via inhibition of GSTP1 and subsequent accumulation of ROS.[4] Because of ROS induction, the compound also exhibited selective SARS-CoV-2 antiviral activity in a mouse model.[5] We used mass spectrometry analysis and molecular networking to analyze fifteen Piper plant species to obtain insights into the diversity of piperlongumine-related compounds. The analysis revealed the presence of a high amount of piperlongumine in *P. fimbriulatum* species. Using computational mass spectrometry tools MZmine, GNPS and SIRIUS, we obtained clusters of Piper "chemical space" and focused on the piperlongumine cluster. We combined fragmentation spectra comparison and SIRIUS prediction to annotate some of the piperlongumine analogues which could be potential candidates for drug development. Using metabolomics, we selected *P. fimbriulatum* because this Piper plant accumulates more piperlongumine than the fifteen other species we tested. Furthermore, in order to understand the biosynthetic pathway of piperlongumine we have generated hypotheses that amidation of piperlongumine might be catalyzed in a similar fashion to piperine through BAHD acyltransferase-mediated catalysis, considering that piperlongumine displays the same amide moiety as piperine, another piper alkaloid for which the last enzymatic step of biosynthesis is catalyzed by a BAHD enzyme.[6] Afterwards, we approached RNA isolation to obtain the transcriptomes of three Piper species including *P. fimbriulatum*. After mRNA sequencing data was obtained, we assembled their transcriptomes *de novo* using transXpress pipeline. Through a comparative analysis of transcriptomes of different species to identify homologous proteins of BAHD, we are conducting functional characterization of these proteins using agrobacterium-mediated heterologous expression in *N. benthamiana.* The products of the transient expression will be analyzed by high-resolution mass spectrometry to confirm the function of the enzymes. Elucidating the entire pathway holds promise for the production of piperlongumine and its derivatives via metabolic engineering. Compared to traditional technologies, bioengineering provides a more sustainable, less time-consuming process and ecologically favoured strategies to synthesize high-value chemicals.

References:

1. Wang, Y.-H. et al. Anticancer principles from medicinal piper (hú jiāo) plants. Afr. J. Tradit. Complement. Altern. Med. ***4****, 8–16 (2014).*

2. Xiong, X.-X. *et al.* Piperlongumine induces apoptotic and autophagic death of the primary myeloid leukemia cells from patients via activation of ROS-p38/JNK pathways. *Acta Pharmacol. Sin.* **36**, 362–374 (2015).

3. Liu, J. M. *et al.* Piperlongumine selectively kills glioblastoma multiforme cells via reactive oxygen species accumulation dependent JNK and p38 activation. *Biochem. Biophys. Res. Commun.* **437**, 87–93 (2013).

4. Harshbarger, W. *et al.* Structural and Biochemical Analyses Reveal the Mechanism of Glutathione S-Transferase Pi 1 Inhibition by the Anti-cancer Compound Piperlongumine. *J. Biol. Chem.* **292**, 112–120 (2017).

5. Tang, C. *et al.* A Selective SARS-CoV-2 Host-Directed Antiviral Targeting Stress Response to Reactive Oxygen Species. *ACS Cent Sci* **9**, 109–121 (2023).

6. Schnabel, A. *et al.* Identification and characterization of piperine synthase from black pepper, Piper nigrum L. *Commun Biol* **4**, 445 (2021).

CYTOKININS – REGULATORS OF *DE NOVO* SHOOT ORGANOGENESIS.

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Plants, unlike animals, possess a unique developmental plasticity, that allows them to adapt to changing environmental conditions. A fundamental aspect of this plasticity is their ability to undergo postembryonic *de novo* organogenesis. This strategy requires the presence of regulators that mediate specific spatiotemporal changes in developmental programs. The phytohormones cytokinins and auxin have been known as the principal regulators of plant development for a long time. In *de novo* organogenesis, auxin is the principal morphogen required to induce new organ onset, while cytokinins modulate the type of organogenic response. In *in vitro* regeneration assays, the presence of auxin alone or high auxin-to-cytokinin concentration ratio in media induces root regeneration from various plant tissues. On the other hand, if the auxin-to-cytokinin ratio is low, shoots are formed.

The process of *de novo* shoot apical meristem initiation is accompanied by strong activation of AHK4-mediated cytokinin signalling and induction of shoot-specific homeodomain regulator WUSCHEL specifically in the regenerating organs. Exogenous but also endogenous cytokinins influence both the initiation of newly formed organs as well as the pace of organ developmental sequence, especially endogenous isopentenyladenine-type cytokinins. We propose an important role of cytokinin biosynthesis and metabolism in the control of *de novo* induced organ identity.

TELOBASE: DECODING TELOMERES ACROSS THE TREE OF LIFE - EMPOWERING DISCOVERY THROUGH USER FEEDBACK

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Delve into the depths of telomere research with TeloBase, an exciting and publicly accessible database that brings together a wealth of information spanning the entire tree of life. From historical literature (1978–present) to cutting-edge predictions derived from publicly available Next-Generation Sequencing (NGS) data, TeloBase is your gateway to unlocking the secrets of telomere motifs.

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IDENTIFICATION AND CHARACTERIZATION OF GDPDL6: A NOVEL GPI-ANCHORED PROTEIN INVOLVED IN POLLEN DEVELOPMENT AND OVULAR ATTRACTION

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Glycerophosphatidylinsitol (GPI)-anchored proteins (GAPs) are a ubiquitous feature of eukaryotic organisms. GAPs are targeted to the extracellular side of the plasma membrane via post-translational, C-terminal modifications known as GPI-anchoring. GPI-anchor synthesis mutants are embryo lethal, and have been shown to be critical for pollen tube (PT) targeting and attraction. As extracellular proteins, GAPs play a crucial role in environmental interactions and cellular perception. Consequently,

pollen-expressed GAPs are prime targets for further investigation to elucidate their role in PT development, growth, and pollen-ovule intercommunication.

Here, we identified pollen-expressed GAPs and screened selected T-DNA mutants. Our results indicate that Glycerophosphodiester phosphodiesterase-like (GDPDL) genes could play an essential role in fertilization, as *gdpdl6* and *gdpdl7* showed reduced seed sets. GDPDLs have been implicated in cell-wall deposition and PT growth. Characterization of our novel *gdpdl6* T-DNA mutants revealed that the severe loss of seed set was due to pollen abortion and loss of PT attraction in mutant ovules. Reduced allele transmission was observed through the female gamete, confirming GDPDL6's vital function in female gametophyte. GDPDL6 localization confirmed the pollen plasma membrane targeting and additionally showed ovule filiform apparatus enrichment. Complementation rescued the phenotypes and recovered homozygous *gdpdl6* plants.GDPDL6 pollen and PT co-immunoprecipitation pulldown revealed interactions with pectin modifying enzymes elucidating to the potential function in pollen development. GDPDL6 enzyme characterization confirmed loss of canonical glycerophosphodiester activity.

Thus, GDPDL6 is a pollen-enhanced GAP but also expressed in ovule, and is required for pollen development and ovular attraction of the PT.

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COMPACT THERMOREGULATORY VESSELS FOR HYDROPONICS

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We are presenting our new compact thermoregulatory vessels constructed for the analysis of temperature stresses on plants grown in hydroponics. The vessels fit Araponics system used for hydroponic cultivation and they can be inserted into commonly used climate chambers. They are primarily designed to be used for studying cold/heat stress targeted to leaves or roots, but they could be used also as compact water baths. The temperature of the medium can reach up to 15°C difference from the air temperature while maintaining highly homogenous environment (0.5°C/2.6°C under the maximum heating/cooling conditions). The temperature of the medium can be kept over a long period or it can gradually change. The vessels are smaller and lighter than available instruments, and energetically friendly to climate chambers. They can be adjusted according to the requirements.

When we used these vessels for experiments on organ-targeted stress of rice plants, significantly different responses of plants exposed to cold stress on shoots were recorded, while the stress on roots was more similar to the stress on the whole plant, indicating the key role of roots in the cold stress response. The results also revealed the impact of individual (non-) stressed organs on the cold stress sensing and growth regulation.

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AN OVERLOOKED DNA REPAIR MECHANISM IN PLANTS AND ANIMALS? 5' NUCLEOTIDE MICROHOMOLOGY-MEDIATED DNA REPAIR AFTER CRISPR/CAS9 CUT

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The CRISPR/Cas9 system is widely utilized across scientific disciplines, spanning from basic research, where it is used, for example, to generate knock-outs of specific genes to study their role, to applied research and plant breeding. This programmable target-specific nuclease induces double-strand DNA breaks, which are repaired by error-prone DNA-repair mechanisms, that may result in deletions, insertions, or a combination of both. Those repair mechanisms are quite conserved within plants and animals, but their activity differs within individual organisms, tissues, and the cell cycle stages.

In our recent study on *Nicotiana benthamiana* (Přibylová et al., 2022), we showed that a significant proportion of deletions might be directed by a 5' nucleotide at the PAM proximal end of the Cas9-induced double-strand break. To our knowledge, this type of DNA repair was not reported before. At the Methods in Plant Sciences 2023 conference, we will show to what extent this type of repair is found in other plant species and how it is represented in the animal kingdom.

Understanding the repair processes at the tissue/cellular level is crucial for enhancing the accuracy and efficiency of CRISPR/Cas applications. By predicting mutagenesis outputs, we can minimize the number of required experiments, significantly reducing time and costs to achieve the desired results. Describing the overlooked 5' mediated DNA repair after Cas9 cleavage contributes to improving the prediction of CRISPR/Cas9 mutagenesis outcomes.

SULFATION PATTERNS AS KEY DETERMINANTS OF GLYCOSAMINOGLYCAN LOCAL STRUCTURING AND PROTEIN BINDING

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Glycosaminoglycans (GAGs) are a series of complex polysaccharides found in the extracellular matrix. They are critical in regulating cellular processes in plants and animals, such as cell-cell communication or molecular recognition. A significant feature of all GAG types is their high negative charge, which is a defining factor in their ability to interact with positive regions of proteins. Most GAGs present local structural modifications in the form of sulfations, which can appear in different numbers or locations in the monosaccharides, often forming distinct characteristic patterns.

In this work, we evaluate the importance of sulfation patterns on the local structuring of GAG chains using Molecular Dynamics (MD) and the influence of such restructuring in the interaction with fibroblast growth factor 2 (FGF2) and antithrombin, two ubiquitous sulfated GAG binding proteins. We observe that certain combinations of sulfation patterns and monosaccharide sequences strongly promote the formation of kinks in the GAG chain. The nature of the kinks is related to intrachain repulsions between sulfate and carboxyl groups and the ability (or lack thereof) of a given monosaccharide to adopt different conformations (puckers) depending on the environment.

Specifically, iduronic acid (IdoA) is a very flexible monosaccharide that can adopt three distinct conformers. With the help of free energy calculations, we calculated how the most stable conformer strongly depends on the sulfation of neighboring molecules and, even more drastically, on the nature of the sulfations on the IdoA itself. The most stable conformation is determined by the combined action of all the sulfates in the pattern, and additionally the presence of sulfation in position two of the IdoA dramatically increases the energy barriers that must be overcome to change conformation. The pucker adopted by the IdoA is vital for forming the kinks.

Furthermore, we study how the polysaccharide's charges and local conformation can affect its ability to bind to proteins. So far, we have observed that a combination of correct charge placement and precise conformation is needed to recognize polysaccharides by proteins. This also points us to a model in which the sulfate distribution, an easily tunable parameter for the cells, directly affects the secondary polysaccharide structure, which is an essential player in protein-polysaccharide interactions.

INVESTIGATING THE CYTOPLASMIC PROCESSING-BODY DYNAMICS IN RESPONSE TO LIGHT BY FLUORESCENCE ACTIVATED PARTICLE SORTING (FAPS).

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Plants are sessile organisms and therefore sensing and integrating signals to adapt to changes in the environment is key to survival. Liquid-liquid phase separation (LLPS) is emerging as an important process in signalling and signal integration. Plants contain several membrane-less compartments possibly formed by LLPS. Some of those are influenced by external factors such as light. One of these compartments are processing bodies (p-bodies).

In this project we analysed the response of p-body numbers in response to light stimuli. We were able to show, that cytoplasmic phyA is necessary and sufficient to initiate p-body disassembly in response to far-red light. In a next step, we aim to understand the biochemical mechanisms that lead from a perception of light stimuli to the disassembly of a phase-separated entity. To this end, we established a pipeline to purify p-bodies from plant material by differential centrifugation coupled to Fluorescent Activated Particle Sorting (FAPS) and analyse them for their protein and RNA content. Preliminary proteomics data indicate that the protein content of p-bodies are indeed changed by light. In a next step, we aim to apply a similar approach to other phase-separated entities relevant to light signalling. For both photobodies and cytoplasmic SAPs this pipeline shall be used to understand their contents, relevance, and mechanisms of formation.

In a complimentary approach we aim to establish a bottom-up *in vitro* system for LLPS-formed organelles relevant to plant light signalling to identify minimal components of those. Those complimentary approaches aim to elucidate mechanisms by which light influences the assembly and/or destruction of non-membraneous organelles.

OCCUPATION: THE PUPPET MASTER OF PHOSPHATIDIC ACID THE TARGETED MANIPULATION OF PHOSPHOLIPID LEVELS IN CELLULAR COMPARTMENTS

Eliška Škrabálková[a,b], Přemysl Pejchar[a], Martin Potocký[a,b]

The abstract is available in the abstract book of Student conference.

USING PROTEOMICS TO INVESTIGATE OXIDATIVE STRESS IN PLANTS

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Our objective was to delineate changes in protein abundances associated with the response of Arabidopsis to methyl viologen (MV)-induced oxidative stress using shot-gun proteomic analysis. Wild type (WT) plants have been compared with oxidative stress-hypersensitive *fsd1-1* and *fsd1-2* knockout mutants of IRON SUPEROXIDE DISMUTASE (FSD1). Along with the early, 30 min long effects, proteome remodeling after 8h long MV treatment has also been followed. Proteins localized to chloroplast and cytoplasm were considerably affected in all lines treated with MV. Proteomic analysis showed that the sensitivity of *fsd1* mutants to MV coincided with decreased abundances of ferredoxin and photosystem II light-harvesting complex proteins. These mutants have higher levels of chloroplastic proteases indicating an altered protein turnover in chloroplasts. Moreover, FSD1 disruption affects the abundance of proteins involved in the defense response. In conclusion, our proteomic approach showed specific proteome remodeling in *fsd1* mutants, pointing to possible signaling roles of this antioxidant enzyme.

This contribution was supported by the UP Young Researcher Grant Competition, project n. JG_2023_018.

CHARACTERIZATION OF A NOVEL SET OF BZIP TRANSCRIPTION FACTORS IN *ARABIDOPSIS*.

Elnura Torutaeva[1,2], Anna J. Wiese[1], David Honys[1,2]

The abstract is available in the abstract book of Student conference.

OPTICAL MAPPING – A POWERFULL TOOL TO ENHANCING GENOME ASSEMBLIES AND STRUCTURAL ANALYSES.

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Optical mapping is a cytomolecular technique that labels and visualizes short sequence motives along DNA molecules of hundreds kilobases to megabases in length. Consensus optical maps resulting from *de novo* assemblies have lengths up to tens of megabases, which enables improving sequence assemblies even in the era of the third-generation sequencing technologies. The highly contiguous maps serve as a template for a hybrid scaffolding pipeline, combining information from an optical map and a sequence assembly, which facilitates extension and merging of the sequence scaffolds or correction of miss-placed or miss-oriented genome segments. For example, the hybrid scaffolding applied to assemble reference genome of diploid banana cultivar Mchare (1C = 600 Mbp) merged 3,227 sequence scaffolds with N50 of ~1,3 Mbp and resulted in a telomere-to-telomere assembly. Optical maps can also span regions of long tandem repeats and thus facilitate sizing of gaps or identification of missing parts in genome assemblies. The optical mapping is often used in analyses of structural variations. With resolution of 500 bp, the optical mapping outperforms FISH and cytogenetic techniques and provides more comprehensive structural variant profiles

FIRST SINGLE-CHROMOSOME SEQUENCE ANALYSIS USING MICROFLUIDIC PLATFORM

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Individual chromosomes of important grass crops cannot be identified and purified using DNA-based flow-sorting because the chromosomes usually cluster together within a flow karyotype, forming composite peaks. However, two options exist towards true single chromosome sequencing: i) flow-sorting into individual wells of a PCR plate (or into tubes); and ii) chromosome enrichment using flow-sorting with subsequent microfluidic processing. The later approach enables high-throughput analysis, statistically improves sequence coverage, and reduces price and bias. Here, we describe a method for single-chromosome sequencing that uses a microfluidic platform to capture, amplify, and barcode the individual chromosomes. Chromosome encapsulation in “gel microbeads” allows lysis of chromosomes and tagmentation of DNA, while a “microfluidic double merger" efficiently pairs each chromosome with a unique oligonucleotide barcode, allowing together single-chromosome sequencing. Prior to down-stream analyses, the sequencing data are de-multiplexed by barcode, resulting in subsets of reads originating from a single chromosome. This high-throughput and low-bias method will enable a wide range of (cyto)genomic studies, such as allocating of genes or scaffolds to individual chromosomes, precisely delimiting translocation events, and studying recombination. In the future, the method is foreseen to be utilized in organisms with large genomes (e.g., Triticeae crops) as well as organisms with numerous small chromosomes (e.g., sugarcane).

THE RELATIONSHIP BETWEEN CALLOSE SYNTHASE EVOLUTION AND STRUCTURE

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The biosynthesis of callose, a cell wall polysaccharide essential for various plant developmental processes, is facilitated by large integral membrane enzymes called callose synthases (CalSs). CalSs are encoded by a multimember family of *GLUCAN SYNTHASE-LIKE* (*GSL*) genes and are predicted to contain 16 transmembrane helices and three functional subdomains – Vta1 domain (unknown function), FKS1 domain (homologous to yeast glucan synthase-like FKS1) and glycosyltransferase domain (the presumed catalytic pocket). However, this organization is not conserved throughout the plant lineage, with specific CalS homologs lacking the Vta1 domain and others evolving novel functional regions. Such non-random history of independent domain acquisitions and losses is consistent with their peripheral localization in the structural models and could have pronounced functional implications. In our work, we also provide the first insight into the possible roles of the Vta1 domain within the CalS structure.

This work was supported by GA UK project nr. 270823 and Czech Science Foundation project nr. 22-35680M.

DIY HYDROPONIC SETUP FOR EASY PLANT ROOT TRANSIENT TRANSFORMATION

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Agrobacterium-mediated transient transformation plays a crucial role in plant research, offering fast an effective method to study various aspects of plant biology. Traditional methods for seedlings cultivation like on agar plates or filter paper are inadequate for prolonged cultivation, necessitating multiple transplantations during the process.

To address these challenges, we propose a hydroponics-based system for efficient root transformation of seedlings. The modified deep water culture method utilizes a diluted nutrient solution and readily available laboratory equipment and pet store products. Multiple gene constructs can be introduced into multiple plants simultaneously, eliminating the need for cumbersome plant movements.

Furthermore, we explore the potential of this hydroponic system for recombinant protein production. The secretion of proteins into the media can increase protein yields and simplify purification. We utilise a composite plant system with transgenic roots for

enhanced protein production and an untransformed shoot for nourishing the "root factory,". This obviates the need for complex extraction procedures like leaf homogenization and protein precipitation, streamlining purification processes.

Our investigations primarily focus on two model plant species, *A.thaliana* and *N. benthamiana*. Notably, our approach allows root transformation since early developmental stages without compromising root integrity. Moreover, it facilitates simultaneous transformation of multiple genotypes or even a mixture of different plant species using a single construct or multiple constructs concurrently. We anticipate that our hydroponic system will find wide applicability across numerous areas of plant research.

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DEVELOPMENT OF HIGHLY SENSITIVE AND SPECIFIC HPLC ANALYSIS OF GLUTATHIONE REDOX STATUS IN PLANTS

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Redox regulations and antioxidative defence play a central role in the acclimation of plants to their environment. Glutathione represents an essential component of the cellular antioxidative defence system, which keeps levels of reactive oxygen species (ROS) under control. Glutathione is also related to the sequestration of xenobiotics and heavy metals[1]. Under physiological conditions, the glutathione pool of plant cells can be found mainly in its reduced form (GSH), whereas modulation of the level of its oxidised form (GSSG) corresponds to the extent of oxidative stress[2], which makes glutathione a suitable candidate as a stress marker[1]. We aimed to develop a sensitive method to analyze reduced and oxidised glutathione levels in small samples of plant tissues or plant cell culture. We tested method of glutathione derivatisation followed by high performance liquid chromatography (HPLC) separation and fluorescence detection. We used monochlorobimane (MClB), which binds to the thiol group of GSH to form a fluorescent adduct. GSSG needs to be converted to GSH by the action of a suitable reducing agent[3]. Tobacco cell culture and pea seeds were used as plant material and commercial glutathione was used as a calibration standard. The method was optimised to increase the signal intensity, reduce the content or eliminate interfering components, and protect the thiol groups of GSH from oxidation during sample preparation and analysis. The applicability of the developed method was also verified by analysis of tobacco cells treated with hydrogen peroxide as well as with the oomycete elicitor cryptogein, which leads to a decrease in the GSH/total glutathione ratio.

REFERENCES
1. Tausz M., Šircelj H., Grill D.: Journal of Experimental Botany 55, 1955 – 1962 (2004).
2. Hajdinák P., Czobor Á., Lorincz T., Szarka A.: Periodica Polytechnica Chemical Engineering 63, 1 – 10 (2019).
3. Cossetti C., Giovamberardino G. D., Rota R., Pastore A.: Nature Communications (2018).

PREDICTIONS OF PIN-LIKES PROTEIN STRUCTURES

Jing Xu [a], Katja Rapp [a], Chengzhi Ren [a], Nibedita Priyadarshini [a], Jürgen Kleine-Vehn [a,b]

The abstract is available in the abstract book of Student conference.

Errata

PERFUSION ADD-ON FOR COMMERCIAL MICROSCOPIC CHAMBERS

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Monitoring plants in microscopic chambers is one of the best approaches for live imaging. However, applying treatments often requires long pretreatments or removing the chamber from the microscope and manipulating the sample, which can increase the risk of creating artefacts or damage. For example, observing living root hairs and studying their responses to treatments can be challenging. Root hairs are delicate and susceptible to mechanical stress and other factors. To overcome these problems, we have designed a 3D-printed perfusion add-on that fits into a standard microscope chamber and allows the real-time study of the structures of interest in response to different treatments, without the need for complicated manipulation or potential loss of focus during microscopy.

PROFILING HISTONE MODIFICATIONS BY CHIP-SEQ

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Chromatin immunoprecipitation (ChIP) has become a standard method of choice for detecting genomic regions associated with proteins of interest. Is it routinely used to identify the location of chromatin-associated proteins, transcription factors, histones or histone post-translational modifications. ChIP can be coupled to quantitative PCR (ChIP-qPCR) to analyse single loci, or to high-throughput sequencing (ChIP-seq) to analyse genome-wide distribution of the associated bait proteins.

Here I will present our strategies for profiling the distribution of histone post-translational modifications on single locus and genome-wide levels using native ChIP (nChIP) or ChIP using crosslinked chromatin (X-ChIP). We have optimized protocols for ChIP-seq starting with low amounts of plant tissues, including root tips or dissected embryos of Arabidopsis thaliana. Recently, we also implemented spike-in X-ChIP and developed protocols for X-ChIP using green algae. I will present the experimental considerations and workflow for ChIP, DNA extraction, Illumina sequencing library preparation and bioinformatic pipeline for Illumina data analyses.

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FISH SIGNAL IMAGE ANALYSIS AND BIOINFORMATIC ANNOTATION AS TOOLS FOR RETROSPECTIVE INVESTIGATION OF RETROTRANSPOSON TRANSPOSITION ACTIVITY

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Retrotransposons are a dominant component of most eukaryotic genomes. These are essentially genetic elements capable of making their own copies and incorporating them into new locations in the genome. Although the mechanistic aspects of retrotransposon reproduction have been extensively studied, it remains unclear when and under what circumstances does the highest increase in new copies occur. Thanks to the presence of sex-specific chromatin (sex chromosomes), dioecious plants make it possible to study maternal and paternal activity of retrotransposons in plants and how stable this activity is. Based on observations of the distribution of retrotransposons on sex chromosomes, we propose a model where retrotransposons preferentially reproduce

in either the paternal or maternal lineage. The model can be validated by quantifying retrotransposons on individual chromosomes using FISH signal analysis in ImageJ and by bioinformatic analyses of short reads and genome assembly. Furthermore, additional retrospective study of retrotransposon activity in other plant species and across distinct populations will allow us to identify the most important factors responsible for retrotransposon activation under natural conditions (position of active copies, epigenetic regulation, role of transcription factors or other activators) and thus contribute to a deeper understanding of the biology of these important players in eukaryotic evolution.

Acknowledgement: This research was supported by the Czech Science Foundation (19-15609S and 22-00364S).

FUNCTIONS OF THE EXO84A EXOCYST SUBUNIT IN ARABIDOPSIS POLLEN

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Pollen grain germination and pollen tube elongation are crucial biological processes in seed plants that involve polarized secretion. Precise regulation of this process requires the exocyst tethering complex that targets secretory vesicles specifically to the plasma membrane. The exocyst consists of eight subunits, assembled into two modules. We focus on the EXO84 exocyst subunit in pollen here. Out of three EXO84 isoforms present in Arabidopsis, EXO84a represents the main isoform functioning in the male gametophyte. EXO84a interacts with EXO70 and SEC15 subunits in the Y2H system similar to EXO84b in the sporophyte. Mutants in EXO84a generate short pollen tubes that often show an aberrant morphology. EXO84a-GFP is localized in growing tips of pollen tubes similar to other exocyst subunits. We conclude that EXO84a is a crucial component of the exocyst complex in Arabidopsis pollen required for efficient polarized secretion.

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