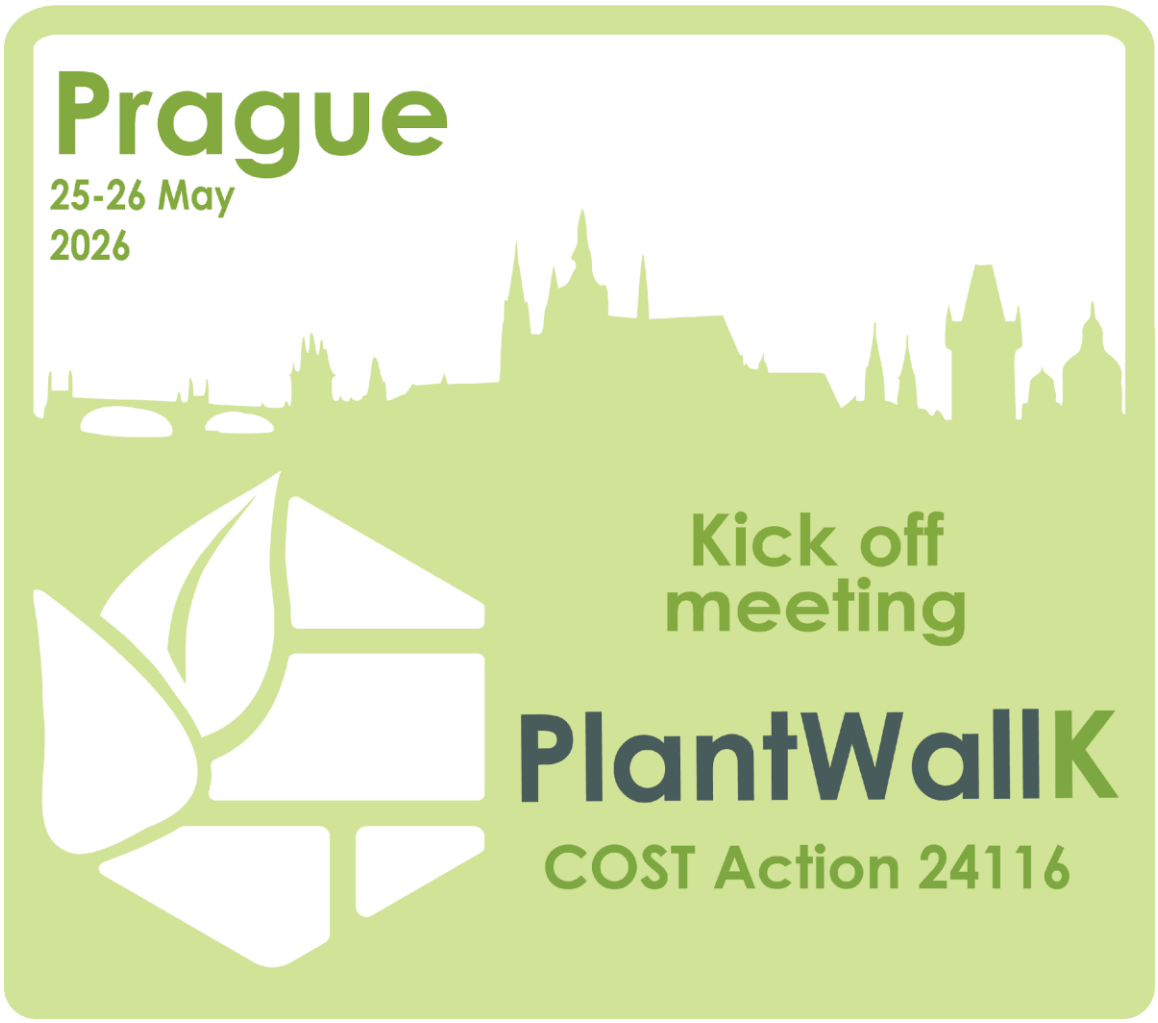
Book of Abstracts



Talks

Ordered according to the program schedule

From touch to strength: cell wall remodelling during wheat thigmomorphogenesis

Annalene Hansen, John Doonan and **Maurice Bosch**

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Mechanical stimulation from environmental forces such as wind, rain or touch induces thigmomorphogenesis, a suite of adaptive responses that can enhance plant resilience. Japanese farmers have utilised mechanical stimulation for centuries - treading on wheat seedlings to improve crop performance and stress tolerance. Despite its long-standing recognition, the structural basis of thigmomorphogenesis in monocot crops remains poorly understood, particularly the contribution of cell wall remodelling to mechanically induced stem strengthening. To address this, spring and winter wheat varieties were subjected to daily mechanical stimulation for 2-4 weeks. Treatment caused marked phenotypic and structural changes, including reduced plant height (up to 35%), increased stem stiffness (up to 80%), and elevated chlorophyll content. To identify drivers of increased stiffness, we analyzed key cell wall and anatomical parameters, including lignin content and composition, cellulose crystallinity, pectin modification, and stem tissue structure. While total lignin levels and cellulose crystallinity were not consistently affected, mechanical stimulation significantly increased sclerenchyma thickness and pectin methyl esterase (PME) activity and shifted lignin composition towards a higher syringyl:guaiacyl (S:G) ratio. These findings suggest that cell wall matrix remodelling and tissue-level reinforcement, rather than bulk lignin accumulation, are major contributors to mechanically induced stem stiffening in wheat. Mechanical stimulation also altered grain phenolic accumulation and testa pigmentation, suggesting broader effects on cell wall-associated phenylpropanoid metabolism. Together, these results identify the plant cell wall as a central regulator of mechanoadaptive growth in wheat and demonstrate that controlled mechanical stimulation promotes targeted wall remodel

Can force generation in plant cells be predicted with Lockhart's Law?

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Through turgor pressure, plant cells generate forces that far exceed those attainable by muscle contraction in animals. Maintaining turgor while growing involves a constant production of osmoticum to drive water into the cells, making it possible to pierce the soil and remain upright against the pull of gravity. Thus, water movements are key to understanding the different strains and stresses to which plant cells subject themselves. In the case of Characean internodal cells, the turgor pressure positively regulates the growth rate phenomenologically abiding to a threshold fluid like constitutive law, coined Lockhart's law. Ignoring other regulations, this framework provides one of the simplest descriptions of the interaction between a growing cell and its environment. Combining water potential equilibrium to Lockhart's law, we quantitatively predicted how a growing cylindrical cell which exhibits elongation and twisting will interact with either a spring or a torsion spring. To test our predictions and evaluate our hypothesis robustness, we mounted an experimental set-up to monitor every quantity involved in Lockhart's growth law and their evolution during the interaction with an obstacle by combining a pressure probe to monitor turgor in real time and home-made glass obstacles of known rigidity to measure the force exerted at the cell's apex

Mechanical and Growth Anisotropy in *Chara corallina*: Challenging Green's Hypothesis

Etienne Couturier

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Paul Green hypothesized that growth anisotropy of plant cylindrical organs could be controlled by cell-wall elastic strain. The present study aimed to challenge this hypothesis through a robust experimental and analytical framework. By combining live cell imaging of *C. corallina* internodal cells with controlled turgor pressure manipulation, we simultaneously measured, for the first time, both the growth strain rate tensor and the elastic compliance tensor derived from multiaxial mechanical testing in the same cell. Under Green's hypothesis, a significant correlation should be observed between the two tensors in all directions. Our results revealed a moderate yet significant correlation between multiaxial elastic compliances and growth strain rates most pronounced in the axial direction. The ratio of axial-to-radial elastic compliance was significantly correlated with the ratio of radial-to-axial growth strain rates. In contrast, other quantities, such as the radial compliance components or the orientations of the two tensors relative to the cell axis showed no significant correlation. Furthermore the growth strain rate tensor was strongly age-dependent in both magnitude and orientation, unlike the elastic compliance. Finally, analysis of intra tensor variability revealed that axial and radial components were strongly correlated for both tensors, with a lowered correlation in the principal axis decomposition

Multiscale modelling of cell adhesion and separation in plant

Ben Malek Rawen and **Grégory Mouille**

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Cell adhesion plays an essential role in the development and growth of plants. The essential nature of this process makes difficult its study and the underlying mechanisms remain obscure. We propose to use a non-essential plant organ as a model system to investigate these processes. In tomato, type VI glandular trichomes store defence metabolites in an intercellular cavity localized between four secretory cells. The genesis and maintenance of this cavity occur through the localised control of cell adhesion via conserved genetic/biochemical regulation and mechanical processes. The dynamic changes in the material properties of the cell wall are a key element to understand its deformation and the consequent mechanical stresses that lead to the fracture of the middle lamella leading to cell separation and the formation of the cavity. In this project we aim to understand how these mechanical aspects relate to biochemical changes in the cell wall during the formation of the cavity. We took advantage of the genetic variability in the formation of this cavity that we observed in tomato and the use of various transformants, to dissect these mechanisms. We first investigated the nature and properties of the trichome cell wall during the course of cavity formation then we made use of the homogenization theory and of the recent developments in continuum damage mechanics to reconstitute a multiscale model of the development of the cavity in type VI trichomes. A modular architecture was used to implement the model and tune the level of its complexity. It leads to a useful generic tool for the scientific community to explore the various morphogenesis processes in plants. Finally, this work provides fundamental insights into the formation of storage cavities through schizogeny in plants, but more generally in the maintenance and regulation of cell adhesion in plants.

Reconstituting plant cell wall architecture: A biomimetic platform to study cell wall-pathogen mechanobiology

Maya Kleiman

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While the plant cell wall is a complex, dynamic network of polysaccharides, its inherent chemical density often masks the individual roles of surface physics and mechanics in plant-environment interactions. This research presents a biomimetic approach to decouple these cues by fabricating synthetic root mimics that replicate the structural topography, mechanical stiffness, and enzymatic reactivity of the natural cell wall. We employ a multicomponent matrix integrated with key cell wall glycans including carboxymethyl cellulose (CMC), cellulose nanofibers (CNF), xyloglucan, and pectin. By modulating the ratios of these biopolymers, we can precisely tune the matrix porosity and surface stiffness. This allows for the high-resolution study of how specific cell wall mechanics influence the penetration mechanisms of parasitic nematodes. Furthermore, the inclusion of specific cell wall polysaccharides enables these mimics to serve as functional substrates for Cell Wall Degrading Enzymes (CWDE). This provides a reactive, biomimetic interface that simulates the biochemical and mechanical breakdown of the wall during pathogen invasion. Beyond fundamental mechanobiology, this platform serves as the blueprint for sustainable, polysaccharide-based materials. We are currently leveraging these glycan architectures to develop "pesticide-free" physical traps—biodegradable, non-toxic systems that lure and capture pests by mimicking the physical and enzymatic cues of the host cell wall

Multiscale modelling of cellulose microfibril formation: role of hemicelluloses in bundling and crystallisation

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The formation of cellulose microfibrils in plant cell walls is a complex multiscale process governed by intermolecular interactions, hydration, and the presence of hemicelluloses. While experimental studies have revealed structural features of mature cell walls, the molecular mechanisms underlying microfibril bundling and crystallisation during early-stage assembly remain poorly understood. In particular, the role of chemically diverse hemicelluloses in regulating these processes is still unclear. In this work, we combine atomistic and coarse-grained molecular dynamics simulations with experimental characterization to investigate the formation and evolution of cellulose microfibril assemblies. From a bottom-up perspective, we model cellulose chains emerging from biomimetic arrangements inspired by cellulose synthase complexes and follow their bundling and crystallisation pathways under varying conditions, including the presence of xylans and mannans with different chemical substitutions. These simulations are complemented by experimental studies on model cellulose-hemicellulose composites using electron microscopy and X-ray scattering techniques to quantify nanostructural features such as fibril spacing, registry, and crystallinity. To bridge scales and enable data-driven insights, we analyse simulation and experimental datasets jointly, identifying correlations between chemical structure, hydrogen-bonding networks, and emergent microfibril organization. This integrated approach provides new understanding of how hemicelluloses modulate cellulose assembly pathways, either promoting ordered crystallisation or stabilising disordered, hydrated interfaces. The presented work contributes to the development of multiscale modelling frameworks for plant cell walls and highlights the importance of combining simulations with experimental data, in line with the objectives of interdisciplinary initiatives such as COST Action PlantWallK.

From tissue reconstructions to mechanical models of plant cell walls.

Elsa Gascon

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Mechanical stresses have been recognized to play a major role during morphogenesis. In plants, these forces are borne by cell walls and emerge from both tissue geometry and cell expansion. The tissue topology, specified during cell division, is determinant for the repartition of stresses: differences in mechanical properties among adjacent cells and tissues generate mechanical interactions whose resolution gives rise to complex organ shape. In developing inner tissues, stresses are difficult to quantify experimentally, making mechanical simulation a powerful complementary approach whose relevance depends on the accuracy and robustness of the underlying digital structure. We developed experimental protocols and numerical methods to generate high-quality 3D tissue reconstructions compatible with finite-element methods (FEM), while preserving realistic cell geometry and tissue topology. Our interdisciplinary approach relies on recent advances in tissue clearing, confocal imaging, and developments in image processing, mesh generation and mechanical simulations. It applies to two complementary systems in Arabidopsis thaliana: the root cortex at cellular and subcellular scales, and the seed coat at tissue and organ scales. In particular, we introduced a 3D numerical reconstruction of seed coat layers at cellular resolution, using simplicial complexes to abstract the topology of multilayered organs, thereby reflecting the natural discretisation observed in certain plant tissues. This framework reveals geometric distinctions between cellular layers and provides a solid basis for biomechanical modelling of plant cell walls.

AI for Comparative Experiments: A Vision for Adaptive Discovery in Biology

Filip Šroubek

UTIA, Czech Academy of Sciences

Experimental science is fundamentally comparative: systems are perturbed and their responses are observed. However, in complex biological systems, many effects remain hidden because they are not captured by predefined analytical descriptors. This limitation contributes to fragmented understanding, especially when integrating complex data across different experimental modalities and scales. In this talk, I will present a forward-looking vision of how artificial intelligence can reshape comparative experiments into adaptive discovery systems. The key idea is to move beyond fixed analysis and instead learn representations that reveal the true effects of perturbations directly from data. In the longer term, such representations could guide experimental design, enabling more efficient and informative exploration of complex systems. This is an emerging and highly interdisciplinary direction that we aim to develop in the coming years. Using biological problems as motivation, I will outline how this approach could integrate with large-scale experimental pipelines, such as those investigating cytoskeletal dynamics and membrane trafficking processes. We invite researchers across biology and related fields to engage with this vision and help shape a new paradigm of AI-driven experimental science.

Flexible Pectin Nanopatterning Drives Cell Wall Organization in Plants

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Plant cell walls are abundant sources of materials and energy. Nevertheless, the nanostructure of cell walls, specifically how pectins interact with cellulose and hemicelluloses to construct a robust and flexible biomaterial, is poorly understood. X-ray scattering measurements are minimally invasive and can reveal ultrastructural, compositional, and physical properties of materials. Resonant X-ray scattering takes advantage of compositional differences by tuning the energy of the incident X-ray to absorption edges of specific elements in a material. Using Tender Resonant X-ray Scattering at the calcium K-edge to study hypocotyls of the model plant Arabidopsis thaliana, we detected Ca order that suggests flexible pectin nanopatterning in plant cell walls, capable of switching states depending on pectin content or cross-linking ion identity. We found that this patterning is tightly interdependent with cellulose microfibril arrangement. Particularly, our results indicate that increased Ca concentration leads to a specific cross-linking modality where nodes (Ca junctions in pectin polymers) move significantly closer to each other. This suggests that there is a fraction of uncross-linked, demethylesterified pectin that provides sites for potential cross-linking and might be tunable to influence wall stiffness. Moreover, applying Zn or La instead of Ca modifies pectin nanopatterning in ways that might affect wall mechanics and/or modify local wall properties. Both discoveries might show mechanisms related to plant control over cellular growth and shaping during development. Finally, we identified Ca-HG assemblies as potential rescuing factors for wall integrity in xyloglucan-deficient plants. Our results indicate direct structural interlinks between components of the plant cell wall at the nanoscale and reveal mechanisms that underpin both the structural integrity of these components and the molecular architecture of the plant cell wall

Brillouin Microscopy for Quantitative Analysis of Plant Cell Wall Mechanics Across Systems

Laura Bacete

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Mechanical properties of plant cell walls regulate growth, morphogenesis, and stress responses, yet remain difficult to measure in living tissues. Brillouin microscopy is an optical technique based on light scattering by thermally driven acoustic waves, which provides access to local viscoelastic properties without physical contact or labels, and can be applied to intact, hydrated samples. I will present how my group uses Brillouin microscopy to study cell wall mechanics across diverse plant systems. Our work includes Arabidopsis roots, root hairs, and leaf pavement cells, as well as cotton fibres and aspen wood samples. We combine Brillouin imaging with genetic and chemical perturbations of wall composition, supported by correlative confocal microscopy and quantitative analysis pipelines adapted to heterogeneous wall regions. Together, these approaches enable cross-system comparisons that link wall composition, mechanical state, and function, and define practical strategies for quantitative plant cell wall mechanobiology

Developing synthetic fluorescent cell wall and membrane probes for plants: navigating a maze

Maarten Besten

Wageningen University and Research

From a physical perspective plants are highly interesting organisms, they deal with immense pressure from both the outside and inside but still manage to grow, divide and adapt to changes in their environment. To gain further insight in how plants propagate, grow and defend themselves against biotic and abiotic threats, microscopy has become an indispensable technique to study these processes. In the recent decades major improvements have been made regarding microscopy, fluorescent protein design and synthetic probe synthesis which allow researchers to continuously push the boundaries of what we can visualize and measure in cells. However, most of these improvements in the field of synthetic fluorescent probes are focused mammalian cell research, creating a disparity between plant cell and mammalian cell research. Most of these probes developed for mammalian cells do not work in plants which we believe is caused by the presence of a cell wall. This structure, which is lacking in mammalian cells, provides plants with mechanical support and acts as a barrier against environmental stresses. It consists of a complex network of polysaccharides that is highly dynamic and changes its local composition depending on its surroundings. This complex network acts as a barrier through which many fluorescent probes cannot penetrate. In our lab we developed 2 modular systems, CarboTag and LipoTag, that can be used to develop synthetic probes for the plant cell wall and membrane respectively. With our set of probes we were able to image cell morphology as well as perform functional imaging on plant cell walls and membranes (including porosity, pH and lipid order). We aim to develop a toolbox of synthetic fluorescent probes specifically tailored towards plants that will provide researchers with more options to study mechanotransduction in plants using fluorescence microscopy, hopefully reducing the disparity between plant and mammalian cell imaging.

Keynote lecture 1:

Carbohydrate microarrays, tools and challenges

Peter Ulvskov

University of Copenhagen

Comprehensive microarray Polymer Profiling, CoMPP, is an analytical technique developed twenty years ago that combines sequential extraction with printing of extracts on microarrays and probing with specific carbohydrate epitope specific proteins, most notably monoclonal antibodies. The technique has been very successful if the metric is publications often resulting from collaborative efforts but unsuccessful if the metric is the number of laboratories in which CoMPP has been implemented. A microarrayer robot is not a cheap instrument but there are other barriers. A suite of software tools that establishes an effective CoMPP pipeline will be presented and an experimental example will be given for illustration. While the suite of tools hopefully will lower the entry barrier, there is another challenge: New cell wall polysaccharides are discovered in land plants and in algae but there are no laboratories dedicated to developing new antibodies to complex polysaccharides. Add to this that certain polysaccharides appear to be very lowly immunogenic. Can a joint PlantWallk effort ensure the continued development of new probes so that CoMPP techniques remain relevant?

Plant cell walls - analysis of their involvement in metal metabolism by mapping distribution and speciation via µXRF, µXANES and HPLC-ICPMS

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Trace metals (TMs, e.g. Cu, Fe, Mn, Mo, Ni, Zn) are essential for organisms as active centres of enzymes, as about one third of all proteins are metalloproteins. Therefore, TM homeostasis in plants is at the core of many challenges that agriculture and human society are currently facing. Cell walls play an important role in this in terms of both, apoplastic transport and formation of barriers against unintended uptake. In our group, we are contributing to this field by several powerful and recently improved methods. These are in particular: - µXRF tomography in shock-frozen hydrated state at synchrotrons and 2D mapping with a benchtop system living samples, both for measuring element distribution from macroscopic to sub-cellular resolution - µXANES tomography in shock-frozen hydrated state at synchrotrons for revealing speciation (redox state, ligands) with cellular resolution - Sector-field ICP-MS with desolvating injection coupled to different LC methods (AEC, CF, HIC, SEC) for revealing binding to proteins after extraction from plant tissues. These analyses improve the mechanistic knowledge of metal deficiency/toxicity stress, the role of trace elements in plant defence response to pathogens, and metal metabolism in general. Our model systems are diverse photosynthetic organisms with emphasis on crops and metal hyperaccumulator plants, mostly using biochemical and biophysical methods. Two important recent application examples of this work are Morina et al. (2025) and Andresen et al. (2023), as well as Küpper et al. (2025) for the latest HPLC-ICPMS methods development.

Küpper H*, Bokhari SNH, Konik P, Dycka F, Flores-Sanchez IJ, Andresen E, Jaime-Pérez N, Colussi A, Morina F (2025) Metalloproteome factory: from crude extract to identification of metalloproteins via 3-dimensional HPLC-ICP-sfMS. *Analytical Chemistry* 97, 17641–17649, https://doi.org/10.1021/acs.analchem.5c02775

Morina F*, Kuvelja A, Brückner D, Mojović M, Nakarada Đ, Bokhari SNH, Vujić B, Falkenberg G, Küpper H* (2025) How eriophyid mites shape metal metabolism in leaf galls on *Tilia cordata*. *New Phytologist* 246, 2222–2242. https://doi.org/10.1111/nph.70103

Andresen E, Flores-Sanchez IJ, Brückner D, Bokhari SNH, Falkenberg G, Küpper H* (2023) Sublethal and lethal Cd toxicity in soybean roots specifically affects the metabolome, Cd binding to proteins and cellular

distribution of Cd. *Journal of Hazardous Materials* 442, 130062. https://doi.org/10.1016/j.jhazmat.2022.130062

Surface chemical characterization of plant cell walls by XPS and chemical imaging

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Plant cell wall research has traditionally relied on microscopy, biochemical fractionation, and bulk spectroscopic techniques however, quantitative information on the outermost surface chemistry remains limited despite its importance for hydration behaviour, interfacial interactions, adhesion properties, and biochemical accessibility. X-ray Photoelectron Spectroscopy (XPS), probing the uppermost nanometers (~5 nm) of a material surface, offers a highly surface-sensitive analytical tool capable of resolving elemental composition and chemical states in complex biological matrices. Recent studies have demonstrated that XPS can successfully distinguish lipid, protein, and polysaccharide fractions in biological cell walls and detect compositional changes associated with growth conditions and environmental stress. These findings indicate strong methodological potential for plant cell wall systems, where surface chemical heterogeneity remains insufficiently explored. In particular, C1s and O1s spectral deconvolution enables discrimination of major functional groups associated with cellulose-, hemicellulose-, lignin-, and pectin-derived surface components through C-C/C-H, C-O, C=O, and O-C=O contributions. Beyond conventional spectral analysis, XPS chemical imaging provides spatially resolved information on the distribution of chemically distinct regions across heterogeneous plant-derived surfaces, supporting visualization of local compositional variability. The integration of XPS-based surface imaging provides a transferable methodological framework from material science to plant biology, helping to address current analytical gaps in surface-sensitive plant cell wall characterization.

Calcium deposition and cell wall integrity in persimmon abscission zones: evidence of cellular adaptation

Julia Morales

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In persimmon (*Diospyros kaki* cv. 'Rojo Brillante'), fruit abscission represents a major agronomic challenge, yet the mechanisms linking nutrient dynamics with tissue structural integrity remain poorly understood. In particular, the role of calcium as a regulator of cell wall stability and its impact on the structural organisation of abscission zones requires further mechanistic insight. This study investigates the relationship between calcium distribution and cell wall architecture within the fruit abscission zone, integrating structural and compositional analyses at the tissue level. Fruits were subjected to different calcium fertilisation strategies, including foliar and root applications, alongside untreated controls. Two anatomically distinct abscission zones were examined using Cryo-scanning electron microscopy (Cryo-SEM) to characterise cellular organisation and identify structural features associated with abscission processes. In parallel, energy-dispersive X-ray spectroscopy (EDX) was employed to determine the elemental composition of observed crystalline structures. The results revealed the formation of calcium-containing crystals specifically in the abscission zone II of treated fruits, whereas no such structures were observed in untreated samples. Notably, despite the presence of these crystalline deposits, no evidence of cellular disruption or lysis was detected in the surrounding tissues. This suggests that cells within the abscission zone are able to accommodate high local calcium concentrations through adaptive mechanisms, likely involving compartmentalisation and/or regulated integration within the cell wall matrix, thereby preserving tissue integrity. These findings provide new insights into how calcium contributes to the regulation of cell wall properties and tissue cohesion in critical developmental zones. By combining advanced imaging with elemental analysis, this work contributes to the development of integrative methodologies for studying cell wall structure-function relationships and supports a multiscale perspective linking nutrient management, cellular adaptation, and tissue biomechanics. This approach opens new avenues for interdisciplinary research aimed at improving crop resilience and reducing yield losses.

Dynamic Remodeling of the Cell Wall Pectin Matrix Underpins Desiccation Tolerance in the Resurrection Plant *Ramonda serbica*

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Desiccation tolerance in the ancient resurrection plant *Ramonda serbica* requires extraordinary mechanical flexibility of the cell wall to accommodate drastic changes in cell volume. This study investigates the molecular mechanisms governing cell wall plasticity during dehydration and rehydration. A fundamental aspect of this adaptation is the plant's ability to follow significant cell shrinkage through invagination and wrinkling of the cell wall, without loss of its structural continuity. Integrated transcriptomic and biochemical analyses, coupled with immunohistochemical localization using monoclonal antibodies (JIM5, JIM7), revealed a coordinated regulation of the pectin matrix. Our results highlight a strategic modulation of the degree of pectin methylesterification through the balanced activity of pectin methylesterases (PMEs) and their inhibitors (PMEIs). In desiccated tissues, the accumulation of de-methylesterified homogalacturonan and arabinogalactan proteins is responsible for maintaining the structural integrity of the wall. Simultaneously, the preservation of highly esterified pectin regions provides the necessary elasticity for wall folding. These modifications are closely associated with the accumulation of cell wall-bound protective proteins, H2O2 regulating enzymes, and polyphenols. Our findings suggest that the dynamic shift in pectin chemistry is a fundamental adaptation that allows *R. serbica* to endure multiple desiccation-rehydration cycles, establishing cell wall plasticity as a cornerstone of its survival strategy.

Keynote lecture 2:

Cortical microtubules as cell wall architects: a modelling perspective

Eva Deinum

Wageningen University & Research, The Netherlands

The anisotropic properties of plant cell walls largely derive from the cellulose microfibrils are deposited, and with which orientations. This process is by and large guided by cortical microtubules. These cortical microtubules are part of a dynamic collective called the cortical microtubule array, that appears during interphase and integrates many cues – mechanical, developmental, geometrical, etc. Collectively, they determine cell wall aspects like major expansion axes and patterned local reinforcements.

In this lecture, I will address how microtubule-based microtubule nucleation and microtubule flexibility affect the way different cues are weighted, as well as the ability of the array to adopt specific patterns.

Dynamic Control of the Cellulose Synthase Complex: BIK1 Phosphorylates TTL3 to Stabilize Growth in High Salinity

Miguel Botella

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Cellulose biosynthesis must be tightly regulated to maintain plant growth and cell wall integrity under environmental stress. The cellulose synthase complex (CSC) dynamically responds to stress conditions, yet the mechanisms controlling its regulation remain incompletely understood. We recently reported that TETRATRICOPEPTIDE THIOREDOXIN-LIKE (TTL) proteins are dynamic CSC-associated components that relocalize from the cytosol to the plasma membrane contributing to CSC stabilization during stress. Using different approaches we found that phosphorylation of Ser93 at the IDR regulates TTL3 recruitment to the CSC. A phosphonull version of TTL3 constitutively associates with the CSC and is functional, whereas a phosphomimic form shows aberrant localization and fails to complement the salt-sensitive phenotype of ttl mutants. We further identify the receptor-like cytoplasmic kinase BOTRYTIS-INDUCED KINASE1 (BIK1) as the kinase that phosphorylates TTL3 at Ser93. In vitro kinase assays demonstrate direct phosphorylation of TTL3 by BIK1, and protein interaction analyses show that both proteins associate in vivo. Consistently, loss of BIK1 alters TTL3 localization, supporting a regulatory role for BIK1 in TTL3 dynamics. Despite the biochemical and genetic interaction with BIK1, TTL proteins do not contribute to canonical immune responses, indicating that the BIK1-TTL3 module specifically regulates cellulose biosynthesis during abiotic stress. Together, these results uncover a phosphorylation-dependent mechanism controlling TTL3 recruitment to the CSC and reveal a role for BIK1 in maintaining cellulose biosynthesis during salt stress.

Reactive oxygen species and the cell wall fortification

Ivan Kulich

Biology Centre CAS, České Budějovice, Czech Republic

Reactive oxygen species (ROS) generated by plant NADPH oxidases play roles in many signaling pathways, but their exact molecular functions remain elusive due to the difficulty of measuring their dynamics in living tissues. In this talk, I will present a direct method for measuring rapid bursts of apoplastic hydrogen peroxide on the root surface, allowing high-resolution monitoring of ROS production in real time. Using this approach, we found that the NADPH oxidases RBOHC and RBOHF are jointly required for ROS bursts triggered by auxin, extracellular ATP, the RALF1 peptide, and mechanical bending. I will further show that these diverse stimuli converge on calcium signaling, which is essential for NADPH oxidase activation in planta. Interestingly, while auxin-induced ROS bursts are dispensable for rapid root growth inhibition and gravitropism, mechanical bending activates RBOHC/F via the stretch-activated calcium channel MCA1. This mechanically triggered ROS production is crucial for adaptive cell wall fortification and efficient root–soil penetration

Correlative TIRFM/A-ESEM Approach for Nanoscale Analysis of Plant Plasma Membrane Protein Organization

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Correlative light and electron microscopy (CLEM) is increasingly important in plant cell biology for resolving the nanoscale organization of the plasma membrane (PM), where key regulatory processes occur within submicrometric domains. Among these, auxin efflux carriers of the PIN-FORMED (PIN) family exhibit lateral heterogeneity closely linked to their function in auxin transport and plant development. Here, we introduce Advanced Environmental Scanning Electron Microscopy (A-ESEM) as a new method for high-resolution imaging of wet plant samples in their native state. Our research presents a CLEM workflow that combines total internal reflection fluorescence microscopy (TIRFM) with A-ESEM to investigate protein organisation at the cell periphery. This approach enables direct correlation of immunofluorescence signals with electron-dense Au nanoparticles representing individual PIN molecules, without additional sample processing following TIRFM imaging. Taking advantage of the non-invasive nature of A-ESEM, we quantitatively assessed the number of PIN molecules within PM nanodomains with nanometer-scale spatial precision.

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What atomic force microscopy can tell us about plants

Anna Fučíková

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In this contribution, we examine the applications of Atomic Force Microscopy (AFM) in plant biology. Beyond high-resolution imaging, AFM enables measurement of mechanical and topographical properties of plant cells and tissues in near-native states. We highlight key parameters obtained by AFM-such as surface roughness, cell wall elasticity (Young's modulus) etc. -and discuss the technical challenges and limitations of using this method in complex plant systems.

Cell wall microdomain remodeling: integrated microtechnique approaches

Vincent Burlat

Université de Toulouse, CNRS, INP, Laboratoire de Recherche en Sciences Végétales

Over the years, we contributed demonstrating the involvement of molecular modules controlling cell wall microdomain remodelling using Arabidopsis seed mucilage secretory cells as a model. These modules involve homogalacturonans methylation and acetylation patterns, homogalacturonan modifying enzymes, peroxidases and extensins. We developed integrated approaches and technologies including microtranscriptomics data mining, in situ hybridization, reverse genetics, immunofluorescence phenotyping on tissue array serial sections coupled to immuno FRET-FLIM analysis, TEM microphenotyping, protein molecular modeling, molecular docking and functional in vivo validation through confocal microscopy observation of complementation translational fusions, or MS/MS oligosaccharide profiling of seed surface abrasion fractions. An overview of this journey will be presented and future foreseen development will be discussed.

Shape2Fate: a morphology-aware deep learning framework for tracking endocytic and exocytic carriers at nanoscale

Adam Harmanec

Institute of Information Theory and Automation, Czech Academy of Sciences

The plasma membrane is continuously reshaped by two opposing processes - exocytosis, which delivers material to the cell surface, and endocytosis, which retrieves it. Maintaining this balance is essential for cell function, yet how individual exocytic and endocytic events are coordinated in space and time remains poorly understood in both animal and plant cells. This question is especially compelling in plants, where endocytosis must work against the high turgor pressure of the cell - and does so using plant-specific protein machinery that has no counterpart in animal or yeast systems. We present Shape2Fate, a morphology-aware deep-learning pipeline that detects, tracks, and classifies individual exocytic and endocytic carriers in live-cell total internal reflection fluorescence structured illumination microscopy (TIRF-SIM) movies at ~100 nm resolution. Trained on synthetic data and exploiting carrier shape evolution rather than fluorescence intensity, Shape2Fate achieves expert-level tracking and outcome classification across diverse cell types, imaging conditions, and microscope platforms. Applying Shape2Fate to constitutive secretion and insulin-stimulated GLUT4 exocytosis in mammalian cells, we uncover two opposing exo-endocytic coupling architectures, establishing that the spatial rules linking exocytosis to endocytosis are pathway-specific rather than universal. Because Shape2Fate is trained on physics-based synthetic data rather than manually annotated images, it is readily adaptable to new biological systems by updating the synthetic generator to match the morphological and imaging regime of interest. TIRF-SIM has already proven instrumental for resolving clathrin-coated pit maturation and plant-specific endocytic machinery in Arabidopsis, and adapting Shape2Fate to plant cells would offer the prospect of quantifying, for the first time at the single-event level, how individual endocytic pits mature and how their morphological progression relates to the activity of plant-specific machinery. Shape2Fate is openly available, enabling

Posters

Listed in alphabetical order

Deciphering Spatiotemporal Variations in Plant Cell Wall Mechanics via AFM Nanoindentation

Prashant Anand

Karlsruhe Institute of Technology, Germany

This study explores the nanomechanical properties of plant cell walls and their dynamic modulation during growth, with a particular focus on maize (*Zea mays*) root tissues. Plant cell wall mechanics are fundamental to regulating cell expansion, yet quantitative characterization of internal wall properties under near-native conditions remains technically challenging. To address this, the authors employ an advanced atomic force microscopy (AFM)-based nanoindentation approach, enabling direct measurement of mechanical parameters in internal tissues without chemical fixation, dehydration, or extensive sample perturbation. The analysis reveals a pronounced spatiotemporal variation in cell wall stiffness and elasticity along the root developmental gradient. Specifically, cell walls in the elongation zone exhibit significantly reduced apparent elastic modulus and increased compliance compared to those in mature regions, indicating localized wall loosening associated with growth. These findings support the concept that cell wall extensibility is tightly regulated through biomechanical remodeling, likely mediated by alterations in polysaccharide architecture and cross-linking interactions. Furthermore, the study demonstrates the robustness of AFM as a high-resolution tool for probing in situ biomechanical heterogeneity, providing quantitative insights into the interplay between wall structure and function. Overall, this work advances the understanding of plant cell wall biomechanics and establishes a methodological framework for investigating growth-related mechanical properties at the cellular and subcellular levels.

The plasma membrane as a mechanotransduction element in plants

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Animal cells dynamic behaviors inevitably bring attention to membranes and membranes dynamics as key parts of mechanosensing and mechanotransduction (Le Roux et al. 2019). On the other side, the dominant view in plant biology that plant cells are highly pressurized elements enclosed in a stiff and load-bearing wall has often skewed the attention away from plant cell fine and fast mechanosensing processes and from the role of plant membranes in these. Recent reports uncovered that plant cells can sense and respond to very gentle mechanical stimulation, thus questioning the view of plant cells as highly pressurized and stiff structures. Gentle touch has been shown to elicit calcium and glutamate responses within milliseconds (Howell et al. 2023 Bellandi et al. 2022), as well as rearrangement of ER and actin within seconds (Hardham et al. 2008). Interestingly, how these processes are mediated or what is their impact on downstream signalling remains largely unknown. We propose that membranes could be the mechanotransduction elements that allow perception and response to fine mechanical stimuli in plant cells, mediating fast cellular responses to touch. We hypothesize that increase in membrane tension upon mechanical stimuli could affect membrane lipid composition at the touch site, which in turn would affect intracellular signalling. While it has been shown that changes in membrane lipid composition instruct developmental processes involving changes in mechanical stress pattern (Boutté and Jaillais 2020 Doumane et al. 2021 Dubrovsky et al. 2008) and are associated to interaction with microbes (Guyon et al. 2025 Qin et al. 2020 2021), the direct connection between exogenous mechanical stimulation, membrane tension and changes in lipid composition in plants has not yet been made. We propose to map membrane tension, membrane lipid composition and membrane microdomains dynamics upon mechanical stimulation, and to investigate the link between these and intracellular responses triggered by mechanical stimulation.

Fantastic CNGCs and how they are regulated by ARO

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Cyclic nucleotide-gated Ca^{2+} channels (CNGCs) are major players in plant signaling orchestrating plant response to multiple environmental stimuli. Despite many available studies the regulation of CNGC activity remains insufficiently understood. Here, we show that Armadillo Repeat Only (ARO) proteins act as key regulators of CNGCs, and are indispensable for CNGC activity. However, AROs alone are insufficient for enabling CNGC functioning. We, therefore, introduce a concept of a “door with two locks” where CNGC activity is regulated in parallel by ARO binding and other stimuli, such as phosphorylation. Finally, we show that AROs compete with calmodulin for CNGC binding and exhibit opposite effects on the channel activity.

Cellulose microfibril deposition and cell wall mechanics in moss cell morphogenesis.

Laiju Chandran Kalathodi

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Morphogenesis intricately relies on the coordinated interplay between the cytoskeleton and the cell wall, where cellulose microfibril organisation and wall mechanics are important determinants of cellular shape. FASS knockout lines, a component of the plant microtubules (MT)-regulating TTP complex, were first described as preprophase band-less mutants, and later also as exhibiting strong defects in interphase microtubule nucleation and reorientation dynamics. Although the mutant still undergoes overall normal histogenesis, loss of FASS function contributes to the defects in post-cytokinetic complexity of cell morphogenesis. Since the coordination of microtubules with cell wall (CW) cellulose microfibril orientation plays a pivotal role in determining the direction of cell elongation, we analysed the anisotropy and orientation changes of cortical MTs arrays together with selected mechanical and compositional features of the CW in moss *Physcomitrium dwarf* fass mutants and wild type. Imaging using fluorescent cellulose stains, high-resolution scanning electron microscopy, Raman spectroscopy, and Brillouin microscopy revealed considerable differences in the organisation of cell surface structures, cellulose microfibril patterns, and wall mechanical properties in mutants compared to wild type resulting in a significantly stiffer CW in fass mutant. Their correlation with microtubular cytoskeleton orientation and dynamics provides further insights into moss morphogenesis. This also provides a methodological framework for the quantitative analysis of cellulose microfibril arrays and cell wall mechanics in moss.

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Exploring *Arabidopsis genomic* variation to identify new players in cell wall biogenesis

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We recently reported a genome-wide association screen (GWAS) of 310 Arabidopsis accessions, finding single nucleotide polymorphisms (SNPs) associated with phenotypic variation in multiple leaf epidermis traits. Several of them reflected the cell wall composition, such as callose content, various autofluorescence parameters, or ease of mechanical breakage of trichoms. Three of the cell wall-related traits, namely callose content, trichome autofluorescence and overall epidermis autofluorescence intensity, exhibited significant correlation with temperature, precipitation or radiation-related climatic parameters of the site of origin of the screened accessions (Bezvoda et al 2025 doi: 10.1111/pce.15357). Mining of the publicly available GenoClimV2 database for environmentally correlated sequence variation in 32 candidates from our screen that exhibited SNPs associating with the above-listed three cell wall-related phenotypic traits revealed two genes with identical SNPs associating with the same climatic parameters that correlated with wall-related traits in our screen, suggesting a possible cell wall-related role for these two hitherto uncharacterized genes, AT4G00440 and AT4G01140.

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Cell wall remodeling drives leaf cell shape and packing in *Arabidopsis thaliana* in response to temperature

Prabal Das

Max Planck Institute of Molecular Plant Physiology

Plants undergo morphological and developmental changes as adaptive responses to temperature fluctuations, enabling them to maintain growth and function under varying temperature conditions. While progress has been made on several fronts, a complete understanding of cellular and genetic mechanisms underlying these responses is lacking. In particular, temperature-dependent changes in the morphology of leaf epidermal pavement cells and mesophyll cells, as well as the packing of mesophyll cells that maintains structural integrity during cell expansion, remain largely unexplored. Here, we investigate the cellular architecture and molecular mechanisms that govern the plasticity of these different leaf cell shapes in *Arabidopsis thaliana* under varying temperature conditions. Using a combination of natural variation among Arabidopsis accessions, confocal imaging, molecular perturbations, cell wall biochemical assays, immunolabeling, and quantitative morphometric analysis, we characterize temperature-dependent changes in cell shape, cell packing, and cell wall organization. To bridge the gap between morphology and molecular composition, we performed biochemical assays of the cell wall to quantify changes in matrix polysaccharides. Our results reveal that temperature-induced shifts in cell shape and size are coupled with remodeling of the cell wall polysaccharides. By linking cell level morphometrics to distinct biochemical signatures of the cell wall matrix, we aim to advance our understanding of how changes in the wall matrix facilitate tissue-scale adaptation under a changing climate.

The pectic part of the cell wall as holder of the plant regeneration potential.

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Compared to animals, plants have evolved remarkable capacities to repair tissues and even regenerate new organs or whole plants upon injury. The ETHYLENE RESPONSE FACTOR 114 (ERF114) and ERF115 play a paramount role in this process enabling for example callus formation, de novo root regeneration and root meristem regeneration through the upregulation of described downstream targets. However, the upstream signals linking wounding to ERFs induction and regeneration are still unknown. Through the combination of metabolomic analysis, pharmacological and genetic approaches, we found that expression of the ERFs is linked with pectin modifications in *Arabidopsis thaliana* but also poplar. Pectin fragments called oligogalacturonides (OGs) can activate the immune response as damage associated molecular patterns but were never linked to a root developmental process such as regeneration. Here, we report for the first time that OGs carry a root regeneration promoting potential. OG treatment could induce expression of the ERFs and promote a faster root organogenesis in an ERF115-dependent manner. This positive effect was dependent on the size but not the esterification status of OGs. Lastly, we found that OGs act additively with auxin treatment in promoting root regeneration, revealing a promising tool to achieve regeneration in recalcitrant plant species.

Fluorescent markers useful for studying plant cell wall protein secretion

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The research activity of our working group at the University of Salento (Italy) focuses on understanding cell wall remodelling, with particular emphasis on the in vivo biosynthesis and secretion of its polymeric components, including polysaccharides, proteins, and glycoproteins. Special attention has been devoted to investigating cell wall protein secretion through the development and application of fluorescent markers. In this context, the group has extensively characterized the secretion pathways of enzymatic proteins targeted to the cell wall. These include polygalacturonase-inhibiting proteins of *Phaseolus vulgaris* (PvPGIP2), pectin methylesterase inhibitors of Arabidopsis (AtPMEI1), and xyloglucan endotransglucosylase/hydrolases of Arabidopsis (AtXTH11, AtXTH29, and AtXTH33). Notably, for these xyloglucan endotransglucosylases/hydrolases, distinct secretion routes have been identified, involving both conventional and unconventional protein secretion pathways, with the latter associated with EXPOs (Exocyst-Positive Organelles)-mediated trafficking. The group is currently investigating the secretion of three different pectin methylesterases (AtPME12, AtPME18, and AtPME34) into cell wall. In this context, the role of actin nucleation has been examined by crossing stably transformed fluorescent PME seedlings of *Arabidopsis with* arpc5 mutants, previously reported to exhibit alterations in cell wall composition, including changes in pectin deposition. These ongoing studies are providing insights into the differential involvement of the actin cytoskeleton, supporting its role in conventional secretion (AtPME12) and suggesting its slight or absent involvement in unconventional secretion pathway (AtPME18 and AtPME34).

Impact of Low and Medium-Intensity Pulsed Electric Fields (PEF) on Tomato Pectin Nanostructure: A Comparison of Green and Red Maturity Stages

Marianna Giancaterino

BOKU University

Pulsed Electric Field (PEF) is a non-thermal technology increasingly used for processing fruits and vegetables, yet its effects on plant biomolecules remain poorly understood. This study investigates the impact of low- and medium-intensity PEF on the physicochemical properties of pectin fractions from tomatoes at two maturity stages (green and red). Monopolar exponential decay pulses (1.0 and 10.0 kV/cm 1.5 and 151 kJ/kg) were applied to alcohol-insoluble residue (AIR). Three pectin fractions—water-soluble (WSP), chelator-soluble (CSP), and diluted alkali-soluble (DASP)—were analyzed using atomic force microscopy (AFM) and HPLC-UV/VIS. PEF induced significant structural changes in all fractions, with effects strongly dependent on ripeness. In red tomatoes, WSP fibre length decreased (up to 50%) with increasing field strength, whereas in green tomatoes it increased. Monosaccharide analysis indicated reduced pectin linearity regardless of maturity stage. Overall, PEF modifies pectin nanostructure in a manner that can mimic natural ripening, with practical implications such as improved peelability in tomatoes.

Regulatory control of stem architecture and vascular development by the LEAFY-COTYLEDON1-LIKE4 (L1L4) master transcription factor in tomato

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Although plant stems are essential for structural integrity and nutrient translocation, the genetic mechanisms governing their composition remain a key area of study. This study utilized genome-edited mutants to investigate the regulatory function of the master transcription factor LEAFY-COTYLEDON1-LIKE4 (L1L4), an NF-YB subunit, within tomato (*Solanum lycopersicum*) stems. Disruption of L1L4 coding sequence resulted in lodging stems characterized by modified mechanical properties and reorganized cell wall components, specifically lignin, cellulose, and pectin. Beyond structural changes, compositional analysis identified a diverse array of high-value organic molecules within the stem extractives. Our research identifies L1L4 as a master switch for stem structure, governing the assembly of essential cell wall materials. By providing a precise genetic tool to remodel lignin and internal architecture, L1L4 offers a solution to the dual challenge of enhancing crop hardiness while streamlining industrial processing of plant biomass.

Cell-to-cell adhesion and plasmodesmata - what do these two have in common?

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Maintaining the cell wall during plant development is no easy task. Although cell walls might seem rigid, they must be constantly modified by the living and growing cells encased within to meet those cells' needs. One such tool is the actin cytoskeleton, which is known to be involved in the keeping of the cell wall integrity. However, the actin cytoskeleton is managed by an abundance of actin-binding proteins, among them the ARP2/3-WAVE/SCAR module. The ARP2/3 complex is a nucleator of branched actin filaments, and the WAVE/SCAR complex is its activator. K.O. mutants show cell wall-related phenotypes, which are thought to result from impaired actin cytoskeleton, hindering the proper delivery of cell wall-building and modifying materials. It is becoming clear that ARP2/3 utilisation is rather specific, tied to a particular situation, place or time. In our work, we show developmentally dependent localisation of the WAVE/SCAR complex. Cell growth is a major driving force of the cell-to-cell adhesion defects observed in mutants, and we found that the timing and place of the WAVE/SCAR subunit appearance within a cell correlate with the cell's elongation status. Further, active cell-to-cell communication appears to be required for maintaining the WAVE/SCAR domain.

Using Proteomics in Cell Wall Research

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Cell wall proteins account for only 5–10% of the extracellular matrix however, despite their low abundance, they play a crucial role in cell wall dynamics. Proteomics and other ”omics” approaches, such as transcriptomics and metabolomics, provide high-throughput, comprehensive insights into the proteins, genes, and metabolites that comprise this dynamic structure. These approaches allow the identification of qualitative and quantitative differences across treatments and developmental stages. Importantly, changes in protein abundance do not necessarily reflect changes in activity, as protein function is often regulated by post-translational modifications. Therefore, integrating high-throughput data with complementary methodologies, such as microscopy and other analytical techniques, can significantly enhance our understanding of cell wall dynamics and provide valuable large-scale data for predictive modeling.

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Lectins as Molecular Tools for Studying Plant Cell Wall Glycans

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Protein-carbohydrate interactions represent one of the most prevalent signaling pathways within cells, as well as between cells and their surrounding environment [1]. Lectins are a specific class of proteins of non-immune origin that specifically and reversibly bind carbohydrates and can precipitate glycoconjugates without altering their structure [2]. They are widely distributed across almost all forms of life, with plant lectins being among the most abundant and extensively studied. In plants, lectins play diverse roles, including involvement in growth, development, immune responses, and defense against pathogens [3]. The plant cell wall is a complex and dynamic structure primarily composed of polysaccharides – a heterogeneous mixture of cellulose, hemicelluloses and pectins, and in some extent proteins and phenolic compounds [4,5]. It plays a crucial role not only in cell division, growth, and differentiation but also in plant responses to environmental constraints [5]. Given that plant polysaccharides represent natural ligands for lectins, these proteins offer significant potential as tools for investigating the structure and distribution of complex glycan architectures within cell wall. Lectins can be integrated into simple analytical platforms or labeled (e.g., with fluorescent probes), enabling rapid and practical analysis of cellular glycosylation patterns. Such approaches allow monitoring of dynamic glycan changes and comparative analysis of different biological samples using histochemical and imaging techniques [6]. Our research is based on the development of a simple and accessible protocol for the isolation and partial purification of lectin from green lentil (Lens culinaris) seeds. The purity of the isolated lectin was estimated by comparison with a commercial lectin standard, and it was further characterized in terms of sugar specificity. Additionally, the protein was fluorescently labeled and evaluated for its binding affinity toward different monosaccharides and complex glycan structures on eukaryotic cell surfaces.

Cell wall remodelling in plant defence strategy to toxic trace elements – immunocytochemical analysis

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Plant defence strategy against toxic trace metals and metalloid ions (e.g. Pb, Cd, Cu, Zn, Hg, Al and As - TMs) includes, not only the deposition of these toxic elements ions, in the regular cell wall (CW), but also CW remodelling. This process leads to an increase of CW thickness and the formation of local CW thickenings, which enhances CW's capacity to bind and sequester TMs. The results of our long-term research on the response of various plant species to various TMs have shown that CW remodeling and CW thickenings formation is a common defense strategy against TMs - CW thickenings occurred in plant species belonging to different phyla, both in tip growing and diffuse growing cells and in response to different TMs The nature of the CW thickenings was analysed using a battery of monoclonal antibodies (mAb) identifying the CW compounds, in particular pectin epitopes: LM7 - low-methylesterified (up to 20% LM7-P) LM19 - low-methylesterified (up to 40% LM19-P) LM20 - highly methylesterified (esterification not lower than 80% LM20-P) PAM1 - with ca. 30 non-methylesterified GalA residues (blockwise PAM1-P) 2F4 - fraction of pectins cross-linking with metal ions (2F4-P). Moreover, mA anti-cellulose - detects cellulose in crystal form (CEL Plant Probes), anti-callose antibody - detects callose (CAL Biosupplies) in confocal (CLSM) and in transmission electron microscope (TEM). The results showed that all tested CW thickenings were particularly abundant in two main components, known as the key components of TMs sequestration within plant CWs: low-methylesterified pectins, up to 40% (pectin epitope LM19) capable to bind toxic TMs ions and callose – which prevents metal ions migration into the protoplast. Moreover, CW thickenings contained also other CW components e.g. 2F4- fraction of pectins and PAM 1 – fraction of pectins which could also be involved in binding TMs It is worth emphasizing that using EDS X-ray microanalysis, connected with TEM, we demonstrated the CW thickenings were the apoplast domains which usually exhibited high concentrations of Pb,

Cd, Cu, Zn, Hg, Al and As and these TMs colocalized with gold particles indicated presence of pectin fractions (in particular LM19-pectin epitope) able to bind TMs ions - identified by immunogold technique. Thus, CW remodelling and formation of CW thickenings abundant in pectin fractions able to bind TMs and callose may be considered as the important mechanism in plant defence strategy which leads to the increase of the apoplast capacity for TMs sequestration and lets the plant cope with TMs.
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Root Cell Wall Class III Peroxidase Responses to Chitosan in Pea

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Chitosan is a plant elicitor that modulates structural and biochemical processes in the plant cell wall. Here, we investigated the effects of chitosan on class III peroxidases (POX) involved in root cell wall remodeling in pea (*Pisum sativum* L.) seedlings. Cell walls were isolated from roots of hydroponically grown plants treated with chitosan (150, 300, and 500 mg Lâ»ą), and the activity of ionically and covalently cell wall-bound POX was analyzed at different developmental stages. Chitosan strongly influenced cell wall-associated POX in a stage-dependent manner. Ionically bound POX activity was significantly inhibited in younger plants, while older plants showed a dose-dependent response. In contrast, covalently bound POX activity increased with higher chitosan concentrations, with a more noticeable effect in older plants. Isoelectric focusing revealed new cationic POX isoforms (pI 9.3–9.6) induced by higher chitosan levels, suggesting enhanced oxidative cross-linking of cell wall polymers. These findings indicate that chitosan causes developmental stage-specific changes in root cell wall-associated class III peroxidases, emphasizing their key role in cell wall remodeling and adaptation to elicitor-induced stress.

Quantification of growth and mechanical stress of primary cell walls

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We consider plants as physical objects and focus on the mechanical perspective. From this perspective turgid (living) plant cells are pressurised vessels, which means that the cell walls are under tensile in plane stress while protoplast is under compression. This picture becomes more complicated when we focus on layers of individual cell walls: the layers facing protoplast (i.e. the recently formed layers) buckle when the wall is released from the tensile stress. This is a manifestation of differences in elastic strain and presumably also stress between the wall layers, which we confirmed by experiments and model. On the other hand, in organs comprising tissues made of turgid cells that differ in wall thickness, stiffness or cell lumen, tissue stresses are generated that are superimposed on the stresses at the cell scale. The tissue stresses are manifested by deformation of tissues that are isolated from the organ. The stresses are generated because walls of adjacent cells that build the tissue and organ are strongly adherent. The adhesion originates during cytokinesis when a common double wall is formed separating sister cells. Another fundamental implication of this adherence is that plant tissues exhibit symplastic growth that is an irreversible deformation of the cell wall system in which the continuum condition is observed. Noteworthy, adjacent cells in a tissue often grow with different anisotropy and growth rate. This heterogeneity is a prerequisite for morphogenesis but at the same time may lead to shear stresses at the common double cell walls. We developed a protocol to measure growth of the organ surface at the subcellular scale. The obtained results facilitated recognition of growth heterogeneity within individual wall surfaces but also of a supracellular growth pattern that is a manifestation of coordination of growth of cells at the tissue scale.

THE ROLE OF GLYCAN-PROTEIN INTERACTIONS IN PLANT CELL WALLS AND POSSIBLE APPLICATIONS

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The plant cell wall is a dynamic, flexible, and complex structure that supports plant growth, while serving as a barrier and a platform for interactions with other cells and organisms. Although it is primarily composed of glycans - cellulose, hemicellulose and pectin, its structure is highly diverse and intricate. Proteins and lipids can be covalently attached to glycans through a process known as glycosylation. Moreover, glycans and their glycoconjugates – glycoproteins, glycolipids and proteoglycans, play an important role in cell communication and pathogen defense, as well as in adhesion, recognition, information transfer and the formation of physical barriers. Glycan-protein interactions represent key mechanisms for maintaining vital functions, as they regulate growth, preserve structural integrity, and take part in recognition and signaling processes. Glycoproteins, such as extensins and arabinogalactan proteins, interact and crosslink with cellulose, hemicellulose and pectin to organize the cell wall matrix, thereby promoting cell wall strengthening, defense and growth. Furthermore, receptor proteins, including lectin receptor kinases and wall-associated kinases specifically bind glycans to detect environmental changes caused by pathogen invasion. Consequently, this type of glycan binding activates intracellular signaling pathways that induce defense mechanisms against invading microbes, contributing to plant survival. In contrast, some bacterial proteins can recognize and bind glycans on plant cell walls to initiate a symbiosis. Even though these interactions are recognized as important, there is a limited number of studies in this area. Glycan mircoarrays are being used for rapid profiling of glycan-binding proteins to investigate their binding properties and specificities. Nevertheless, deeper research into glycan-protein interactions in plant cell walls could contribute to advances in plant glycobiology, biotechnology, medicine, and agriculture.

The Role of the Cell Wall in Zinc Redistribution in a Heterogeneous Tobacco Root System

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Zinc (Zn) is a biologically essential element—it is a structural component of numerous proteins, including enzymes and transcription factors. It is an indispensable element in the diet of humans and animals, and plants constitute its primary source in the food chain. In my research, I investigate the mechanisms of Zn transport in the roots of tobacco (*Nicotiana tabacum* L.) grown under conditions of heterogeneous Zn distribution in the growth substrate medium. A heterogeneous system means that one layer of the substrate (either upper or lower) contains Zn, while the other is deprived of it. This experimental setup allows the study of the plant's capacity to redistribute the element within the root system. In my research I employ micro–X-ray fluorescence (μXRF), a technique that enables direct analysis of the spatial localization of Zn in plant tissues without the need for chemical digestion. μXRF analysis revealed that in roots of plants grown under heterogeneous conditions, Zn distribution is relatively uniform throughout the entire root system, even when only part of the system had direct contact with the element. This finding suggests active redistribution of Zn between different parts of the root system. In addition, we observed localized Zn accumulation at the junctions of lateral roots with the primary root. This may suggest that these regions function as specific "transport nodes," where potential local redistribution of elements between the xylem and phloem occurs, which could facilitate Zn delivery to developing lateral roots. A possible hypothesis is that the cell wall structure in these junctions — including the degree of lignification, the presence of pit formation in xylem walls, and the reorganization of pericycle and endodermal cells during lateral root formation — may create conditions that favor local Zn accumulation. Such anatomical adaptations could partially explain why higher concentrations of the element are observed in these regions. To better understand the structural basis of this phenomenon,

Esca-linked symptomatology leads to pectin de-esterification and xyloglucan remodelling in grape berries

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Esca is a major grapevine (*Vitis vinifera*) trunk disease, yet grape cell wall (CW) responses in this context remain poorly characterised. We compared CWs from symptomatic vs asymptomatic berries. Alcohol-insoluble residue (AIR) was characterised by trifluoroacetic acid (TFA) hydrolysis followed by gas chromatography-mass spectrometry (GC-MS), and fractionation with 1,2-diaminocyclohexanetetraacetic acid (CDTA) and 4M NaOH for Comprehensive Microarray Polymer Profiling (CoMPP) using 31 CW glycan-directed probes (monoclonal antibodies and carbohydrate-binding modules). Symptomatic berries showed pectin with lower methylesterification and increased Ca^{2+}-pectate "egg-box" motifs, alongside reduced arabinogalactan-protein (AGP) epitope accessibility in both CDTA and NaOH extracts. Symptomatic berries also indicated higher glucose and a tendency to higher xylose, with lower fucose and galactose, and decreased probe binding to specific xyloglucan epitopes in NaOH fractions. These patterns suggest reduced xyloglucan side-chain substitution and tighter glycan-glycan association that consolidate the CW matrix. We propose that Esca-linked hydraulic stress accompanies wall remodelling towards a less porous matrix network enhancing barrier and turgor functions. This work adds a CW perspective to Esca and proposes a wall-remodelling mechanism, laying the groundwork for studies on impacts to grape and wine yield and quality

How metals shape the structure and function of the cell wall in response to biotic stress

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The cell wall is a primary barrier and the first signalling trigger of plant response to pathogens and pests. Metals such as Mn, Ca, Cu and Fe have been shown to regulate cell wall cross-linking and structure, as well as redox processes, which are crucial for plant defence response and pathogen growth. Additional metals, such as Zn, may also play important roles. Our previous work demonstrated that Zn priming enhances the immunity of pepper plants against the necrotrophic pathogen Botrytis cinerea (Kuvelja et al., 2024). In another system, the expression of Mn-binding apoplastic germin-like proteins increased in response to gall formation, accompanied by changes in the cell wall redox state and differential expression of metal-related genes (Morina et al., 2025). Nevertheless, many aspects of metal metabolism within the cell wall remain poorly understood. Our work focuses on elucidating the mechanisms underlying metal-induced modifications of the cell wall under biotic stress, with particular emphasis on proteomic profiling and metal metabolism. We apply ultra-trace metal speciation analyses using HPLC–ICP-MS to identify and quantify cell wall-bound metalloproteins associated with priming and defence responses. In parallel, synchrotron-based techniques, including micro-XRF tomography and XANES, are used to characterize changes in metal distribution and ligand coordination within the cell wall. Our model systems include economically important species and Arabidopsis as a model with well-established genetic resources, challenged with Botrytis cinerea. Our work contributes to a deeper understanding of metal dynamics in the cell wall, including binding, sequestration, and redistribution. It improves understanding how metals interfere with pathogen and pest modifications of the cell wall on one side, and host remodelling in response to biotic stress on the other. This knowledge supports the development of disease-resistant crops with improved nutrient efficiency and reduced fertilizer input.

Morina, F., Kuvelja, A., Brückner, D., Mojović, M., Nakarada, Đ., Bokhari, S.N.H., Vujić, B., Falkenberg, G. and Küpper, H., 2025. How eriophyid mites shape metal metabolism in leaf galls on Tilia cordata.

New Phytologist, 246(5), pp.2222-2242. Kuvelja, A., Morina, F., Mijovilovich, A., Bokhari, S.N.H., Konik, P., Koloniuk, I. and Küpper, H., 2024. Zinc priming enhances Capsicum annuum immunity against infection by Botrytis cinerea–From the whole plant to the molecular level. Plant Science, 343, p.112060.

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Impact of cell wall metabolism on the epigenetically controlled cell identity in moss

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Vegetative development of the model moss *Physcomitrium patens* involves two major growth stages – the two-dimensional protonema and the three-dimensional leafy gametophore. The filamentous protonema comprises three cell types – the meristematic apical cell, the photosynthetically active chloronema and the less photosynthetically active or inactive caulonema cells, responsible for the spreading of the plant. Cell identities and developmental transitions are governed be the expression of specific sets of genes, determined by the organization of chromatin in the nucleus. The histone methyltransferase Polycomb repressive complex 2 (PRC2) establishes epigenetic repression of developmental genes, which specifies cell identities in plants. In moss, PRC2 controls the transition from two-dimensional to three-dimensional growth and prevents apogamy. We found that PRC2 is required to maintain the photosynthetically active chloronema and to prevent precautious terminal differentiation into non-photosynthetic caulonema. To identify the underlying molecular pathways, we profiled the transcriptome of primary chloronema in wild-type and PRC2-depleted plants, and compared this to the sets of genes directly targeted by PRC2 for repression. We identified genes involved in cell-wall metabolism, in particular the cellulose synthase genes (CESAs), as the major category of PRC2 targets that are upregulated in the PRC2 mutant chloronema. Using the herbicide dichlobenil, we could show that inhibition of cellulose synthesis in the PRC2-depleted caulonema rescues the developmental phenotype and promotes the growth of photosynthetically active chloronema. Based on these results, we aim to understand how the metabolism of plant cell wall influences the developmental and metabolic cell identity and how epigenetic mechanisms finetune central metabolic balance and plant development.

Live-cell tracking of homogalacturonan dynamics using an oligocationic peptide probe

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Homogalacturonan (HG) is a chemically and spatially dynamic pectic cell wall component whose methyl-esterification patterns significantly influence wall biomechanics and plant development. Despite the availability of specific mAbs, tools for tracking HG in its native context within live tissues remain limited. We developed a novel peptide-based probe (Lys10) that recognizes negatively charged, demethylated HG through strong anionic interactions. Specificity analysis with a dot blot assay confirmed its binding to HG, with signal intensity inversely proportional to the degree of methyl esterification. Molecular docking and molecular dynamics simulations further supported a stable, high-affinity interaction between Lys10 and HG. The calculated Kd was comparable to that of antibodies. Externally applied Lys10 quickly penetrates live tissues, enabling live-cell imaging of cell wall HG in roots. Using Lys10 for time-lapse imaging of root hair elongation, we observed rapid redistribution of the signal between the tip and shaft, indicating localized HG remodeling during tip growth. To explore the possibility of developing a genetically encoded marker, we generated stable *Arabidopsis lines* expressing HG-binding oligopeptides fused to mCherry and targeted to the secretory pathway. Overall, our results demonstrate that Lys10 is a versatile HG-recognition tool with potential for long-term, real-time imaging, facilitating studies on HG dynamics.

Plant Proteomics: Exploring Function, Diversity, and Future Foods

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Plant proteomes represent a complex and largely underexplored layer of biological information with direct implications for nutrition, crop performance, and sustainable food systems. Advances in mass spectrometry-based proteomics, combined with bioinformatic tools, now enable comprehensive characterization of protein composition, structure, and function across diverse plant species and tissues. These approaches support the evaluation of protein quality, functional properties, and variability driven by genetic background and environmental conditions. In parallel, proteomics provides a powerful framework to investigate genetically modified and selectively bred crops, offering molecular insight into traits associated with stress tolerance and adaptation to changing climates. Integrating analytical and computational strategies expands our understanding of plant protein diversity and supports the rational development of alternative protein sources and future crops with improved resilience and functionality.

PECTIN REMODELING AT THE CROSSROADS OF PLANT-PATHOGEN INTERACTIONS: INTEGRATIVE APPROACHES AND CHALLENGES

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The plant cell wall is a dynamic interface that integrates mechanical, biochemical, and immune signals to regulate plant responses to abiotic stress and pathogen interactions (Lionetti and Metraux, 2014 Vicré and Lionetti, 2023 Del Corpo et al., 2024). Pectin-derived oligosaccharides generated during plant-pathogen interaction act as elicitors of plant defense responses, and emerging research lines are increasingly focused on the identification and extraction of bioactive oligosaccharides from agro-industrial waste, highlighting their potential for sustainable crop protection applications (Greco et al., 2024). Within this framework, pectin methylesterification is a central regulatory process, controlled by pectin methylesterases and their regulatory network, which modulate cell wall mechanics, integrity, and signaling in a highly context-dependent manner (Lionetti et al., 2012). Deciphering the spatiotemporal regulation of pectin methylesterification and its integration with cell wall mechanics, signaling, and plant-pathogen interactions demands the integration of complementary experimental approaches. Genetic, biochemical, and glycan analytical methods provide quantitative insights into pectin structure, its modulation, and the functional validation of enzyme activity, enabling the dissection of isoform-specific functions (Lionetti et al., 2007 Lionetti, 2015). In parallel, additional layers of information are provided by imaging-based methods (Lionetti et al., 2017 Coculo et al., 2023). Together, these approaches link pectin remodeling with key processes such as damage signaling, receptor activation, and hormonal regulation. Despite these advances, major bottlenecks remain. The functional specialization of pectin methylesterase isoforms is still poorly understood, molecular probes often lack specificity, and current methodologies struggle to integrate enzyme dynamics with cell wall composition, mechanics, and signaling across spatial and temporal scales. These limitations are particularly critical under stress conditions, where plant and pathogen activities dynamically compete to shape cell wall properties. Addressing these challenges requires the development of standardized and validated tools and multidisciplinary integration of biochemical, imaging, and computational approaches, including state-of-the-art data analysis. In line with the PlantWallK COST Action (CA24116), strengthening collaboration and shared infrastructures will be key to advancing a systems-level understanding of cell wall remodeling and improving plant resilience to environmental stress.

Coculo, D. et al (2023). Plant Physiology and Biochemistry 201:107865. Del Corpo, D. et al (2024). Plant Communications 5:100931. Greco, M.et al (2024). Plant Physiology and Biochemistry 217:109213. Lionetti, V. (2015). Front.Plant Sci. 6:1–8. Lionetti, V., and Metraux, J. P. (2014). Front.Plant Sci. 5:612. Lionetti, V. et al (2007). Plant Physiol 143:1871–1880. Lionetti, V. et al (2012). J.Plant Physiol. 169:1623–1630. Lionetti, V. et al (2017). Plant Physiol. Jan 12.:pii: pp.01185.2016. Vicré, M., and Lionetti, V. (2023). Front Plant Sci 14:1230438.

PR1 differentially occupies cell wall spaces in cotyledons and leaves upon induction

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PATHOGENESIS-RELATED 1 (PR1) transcript abundance has long been used as a marker of salicylic acid (SA)–dependent activation of plant defense however, its precise role in plant immunity remains unclear. As part of efforts to elucidate its function, we investigated PR1 localization and secretion in *Arabidopsis thaliana*. The mCherry-tagged PR1 protein, upon induction by SA, exhibits differential localization patterns in seedling leaves. On the adaxial side of wild-type plants, PR1 localizes predominantly to epidermal cell walls, whereas on the abaxial side, the signal is primarily detected in the vacuole. Only after prolonged induction does PR1 also accumulate in the cell wall compartment on the abaxial side. Mutants affecting the secretory pathway, including exocyst-deficient lines, display altered PR1 localization patterns. Similarly, mutants impaired in pathogen perception show a shift in localization preference toward mesophyll cell walls rather than epidermal cell walls. Modifications of the PR1 sequence—consistent with observations from transient expression studies in *Nicotiana benthamiana* (Pečenková et al., 2022)—also disrupt its localization. Given the extracellular localization of PR1, we further investigated whether its presence in the cell wall is associated with epidermal cell morphology. Detailed analyses revealed several significantly altered cell shape parameters in both the loss-of-function pr1 mutant and PR1-overexpressing lines. The biological significance of these observations remains to be elucidated.

How do neighboring plant cells coordinate division orientation in tissues? An actin point of view

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Orientation of cell division is essential to maintain tissue organization in land plants, where cells are immobile and surrounded by cell walls. It has long been established that microtubule structures, such as the preprophase band, determine the division plane. However, recent observations suggest that additional mechanisms contribute to the orientation of cell division in multicellular contexts. Perturbation of the actin cytoskeleton in specific root epidermal cells leads to division defects in neighboring cells, raising questions about potential non-cell-autonomous mechanisms that regulate orientation. In this project, I use cell type specific actin perturbation system (DeAct) to investigate how actin regulated processes in one cell influence division orientation in neighboring cells. The current focus is on establishing transgenic lines to enable reproducible analysis of division orientation and mitotic progression. In parallel, preliminary experiments are exploring potential mechanisms underlying this effect, including the role of actin-mediated trafficking and cell wall modifications at the cell interface. Together, this work aims to unravel how positional cues are transmitted between neighboring cells to coordinate division orientation, providing new insight into how plant tissues maintain their architecture during their growth and development.

Role of cell wall in Zn-tropism

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As sessile life forms, plants must constantly respond to environmental factors. One example of such adaptations to environmental factors is tropism. In our experiments, we found that 60% of plants grown in a Zn-deficient environment shifted their root growth direction toward an increasing Zn concentration gradient. We found that the Zn concentration between the two sides of the root growing near the Zn source was uneven. A particularly high amount of Zn was bound to the cell wall directly exposed to the Zn gradient. Furthermore, pectin methylesterase activity was lower in tropic roots, while expression of a pectin methylesterase inhibitor was elevated in this type of root. In short, we expect that Zn binding alters epidermal pectin elasticity, reducing cell elongation on the Zn-proximal side and promoting root bending toward the source, as opposite cells elongate more. Understanding how plants find Zn in the environment may contribute to increasing the concentration of this element in plants and consequently reducing the need for pharmacological Zn supplementation.

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Extraction and characterization of cellulose from *Arabidopsis thaliana* wild type and CSC-related mutants

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Plant cell walls are complex structures whose composition and organization are tightly regulated by genetic factors. *Arabidopsis thaliana* mutants provide a valuable model to investigate how alterations in cell wall biosynthesis affect cellulose structure and extractability. In this work, cellulose was isolated from *Arabidopsis thaliana* wild type (Col-0) and two cell wall mutants (*prc1-1* and *ttl134*). An exhaustive extraction protocol was optimized to efficiently remove non-cellulosic components (extractives, hemicelluloses, and lignin) while preserving the native cellulose fraction. Extraction yields were determined for each genotype, enabling comparison of cellulose recovery as a function of genetic background. The different isolated cellulose fractions were then characterized by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and X-ray diffraction (XRD). ATR-FTIR provided information on the chemical composition of the samples, confirming the effective removal of lignin and hemicelluloses, while XRD provided insight into cellulose crystallinity. Differences between Col-0 and mutant lines suggest that genetic modifications influence both cellulose organization and its extractability. These results contribute to a better understanding of plant cell wall architecture and highlight the relevance of integrating plant genetics with cellulose processing and characterization approaches. Keywords: Plant cell wall Cellulose *Arabidopsis thaliana* Cell wall mutants.

Unraveling the Functional Basis of Stigma Position Regulation in Tomato

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Stigma position (SP) represents a key reproductive trait in tomato (*Solanum lycopersicum* L.), directly affecting pollination efficiency, fertility, and fruit set. In many traditional and vintage cultivars, the stigma is located at or protrudes beyond the staminal cone, promoting cross-pollination while reducing self-fertility. Moreover, high temperatures often induce stigma exertion even in self-pollinating cultivars, leading to reduced fertilization efficiency and low fruit set. Consequently, deciphering the genetic and molecular mechanisms governing SP is essential for breeding productive and climate-resilient varieties. This study aims to improve tomato resistance to high-temperature stress by functionally validating potential genes and providing novel insights into SP regulation. We integrated SNP-based genotypic and SP-related phenotypic data from our work and public repositories and analyzed them using Meta-GWAS framework. Several QTLs significantly associated with SP were identified across genetically diverse landraces and cultivars, with polymorphic regions detected on several chromosomes. For candidate gene validation, we selected two agro-morphologically similar but contrasting landraces in terms of stigma position: Scatolone di Bolsena (SCA) and Marmande ancienne (MAR). Top candidate genes have been selected, and CRISPR/Cas9-based gene editing was undertaken to generate targeted mutations via Agrobacterium-mediated transformation. The putative transformant explants are currently in the elongation stage, producing new shoots from the callus tissue under selection. We will acclimatize the gene-edited plantlets to the greenhouse and confirm target gene modifications in subsequent stages. Following confirmation, modified lines will undergo phenotypic assessments under heat-stressed conditions, and cell wall-related pathways will be investigated using gene expression analyses (qPCR/RNA-Seq).

Suberin deposition to cell walls of barley mpk3 mutants leads to resistance against Fusarium graminearum.

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After F. graminearum inoculation, HvMPK3 KO lines showed differential regulation of proteins involved in the synthesis of suberin, including lipid transfer proteins (LTPs), GDSL esterase/lipase (containing "GDSL" aminosequence motif) and peroxidases. In accordance with proteomic analysis, microscopic observations validated increased suberin accumulation in the roots of HvMPK3 KO lines, which most likely contributed to the arrest of F. graminearum infection. According to obtained data, TALEN-based knockout of HvMPK3 leads to barley resistance against Fusarium root rot due to the suberin deposition to the surface of the roots.

Deciphering the dual role of fungal xylanases and plant xylanase inhibitors in plant cell wall integrity and immunity

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The plant cell wall represents both a structural barrier and a dynamic signaling interface during plant–microbe interactions. Among the cell wall–degrading enzymes produced by phytopathogens to overcome the plant cell wall, fungal endo-β-1,4-xylanases play a central role in the hydrolysis of xylan, the major component of hemicellulose. Gene disruption studies have demonstrated the contribution of several xylanases to pathogen virulence. Xylanases produced by Fusarium graminearum and Botrytis cinerea exhibit dual functionality: in addition to enzymatic degradation of hemicellulose, they display necrotizing activity and trigger plant immune responses independently of their catalytic function. Conversely, plant xylanase inhibitors modulate pathogen virulence by blocking both the hydrolytic and necrotizing activities of fungal enzymes, thereby influencing disease progression and host resistance. To dissect these interactions, an integrated methodological approach combining protein biochemistry, molecular biology, microscopy, and in planta functional approaches was employed. This study provides a methodological framework for investigating the interactions between fungal xylanases and plant cell wall–associated xylanase inhibitors as critical determinants of plant resistance. Future research will focus on identifying the plant molecular components involved in the perception of fungal xylanases and in the downstream signaling pathways that mediate immune activation.

Cell wall modifications of root apoplastic barriers in response to nutrient stress in crop species

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Root apoplastic barriers (endodermis and exodermis), strengthened by cell wall modifications, play a key role in regulating water and nutrient movement. We applied histochemical methods for detection of lignification and suberization to analyze how the exodermis responds to nutrient stress in various crop species. The exodermis, an important protective layer in root outer cortex, responded to nutrient limitations. Nitrogen deficiency in particular strongly promoted the lignification of exodermal Casparian bands and deposition of suberin lamellae, with roots adjusting locally to their immediate environment. This response was conserved across different plant groups, highlighting its role in plant adaptation. Overall, the formation of the exodermis represents an important mechanism by which plants optimize resource uptake and cope with nutrient stress and soil heterogeneity, with broad relevance for plant performance and crop efficiency. This work was supported by the Charles University (project GAUK No. 336122) and from the project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

Multiplex Silencing of SlPG, SlPL, SlTBG4, and SlExp1: A Combined Approach to Extending Tomato Shelf Life

Selman Uluisik

Burdur Mehmet Akif Ersoy University

Fruit softening is a key factor affecting the shelf-life of tomato, post-harvest losses, and consumer acceptance. In climacteric fruits like tomato, softening is primarily driven by the disassembly of the cell wall matrix, especially pectin remodelling. This process involves several enzymes including polygalacturonase (PG), pectate lyase (PL), β-galactosidase (TBG4), and expansin (Exp1) whose actions are highly coordinated. In this study, we designed and assembled a multiplex CRISPR/Cas9 construct using GoldenBraid cloning technology, targeting simultaneously four key tomato fruit softening genes (SlPG, SlPL, SlTBG4, and SlExp1). Following successful transformation of this construct into the tomato, the presence of the Cas9 transgene in the regenerated R0 lines was confirmed. In the context of the project, T1 plants were produced, molecular screenings were performed to identify Cas9-free stable edited lines were selected. We are now going to phenotype the mutant T2 fruits based on their postharvest performance during April and May.

Habitat-Related Biochemical Differentiation in Two Balkan Endemic Scilla Species

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Scilla lakusicii Šilić and *Scilla litardierei* Breistr. (Asparagaceae) are closely related Balkan endemic species inhabiting contrasting karst habitats of the Dinarides. *S. lakusicii* usually occurs in open rocky limestone habitats, rocky grasslands, and rock crevices of the southeastern Dinarides, whereas *S. litardierei* mainly inhabits wet meadows on the flat bottoms of karst poljes. These ecologically distinct habitats suggest possible differences in physiological and biochemical adaptations between the two species. In this study, cell walls were isolated from leaf tissues of both Scilla species. The levels of ionically bound cell wall proteins and the activity of ionically bound class III peroxidases (POX) were measured to assess interspecific differences. Class III peroxidases are essential in regulating cell wall structure, including lignification and cross-linking of wall components, and changes in their activity may indicate adaptive responses to environmental stresses. Additionally, differences in the chemical composition of cell wall acetone extracts were analyzed using gas chromatography–mass spectrometry (GC–MS). The results are discussed in the context of biochemical and metabolic differences that may serve as adaptive responses to contrasting habitat conditions within the Dinaric karst ecosystems.

Keywords: *Scilla lakusicii*, *Scilla litardierei*, Class III peroxidases, karst, GC-MS.

Insight into the role of hemicellulose in mechanics of *Pinus sylvestris* hypocotyls

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Hemicelluloses are one of the three main structural components of primary cell walls, where they influence cell expansion and maintain structural integrity. Conifers represent an economically significant group of trees. However, the relations between chemical composition and mechanical properties of their primary cell walls have not been fully elucidated yet. We investigate the role of three key hemicelluloses (xyloglucans, xylans, and mannans) in regulation of mechanical properties of primary cell walls of conifers. To achieve this, etiolated pine hypocotyls were subjected to enzymatic treatments followed by mechanical tests. Additionally, using immunolabeling, we analysed the spatial distribution of different hemicelluloses within the hypocotyl tissues. We show that enzymatic treatment with xyloglucanase reduced both the Young's modulus and the strength of hypocotyls. Trials with different modes of enzyme application show that they are critical for the mechanical test results.

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Impact of allopolyploidy on the tubulin divergence in Brassicaceae

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Tubulins are essential cytoskeletal proteins required for microtubule assembly, cell division and plant development. Although tubulins are among the most conserved proteins in eukaryotes, plant genomes encode multiple α-, β- and γ-tubulin isotypes whose evolutionary relationships and functional differentiation remain incompletely understood. This study examines how tubulin gene families evolve and diversify in flowering plants despite strong functional constraints.

Using a comparative genomics framework, we have unravel evolutionary trajectories of key lineages of tubulin gene isotypes in the genomes of allopolyploid Brassicaceae and their parental species, in particular: allohexaploid Camelina sativa and its diploid progenitors; and in complex polyploid Brassica representatives; which were compared to model species Arabidopsis thaliana and A. lyrata.

The results demonstrate that while tubulin gene sequences are characterised by high evolutionary stability, the panel of evolutionary retained genes significantly varies in species. Despite main tubulin classes originating from ancient whole-genome duplication events, current well-known tubulin isotype diversity in model species, like A. thaliana, may be a specific feature of this particular species or Brassicaceae. For instance, our analysis uncovered the fact that

presence of two distinct γ-tubulins in A. thaliana, which has been long-term disputable in terms of functional necessity, appears to be a unique feature of this species and not observed in distant relatives. Continuous gain, loss and re-emergence of different α- and β-tubulins was also observed among the analysed species.

Taken together, these findings provide a refined evolutionary framework for plant tubulin and demonstrate how essential cytoskeletal components can undergo controlled diversification while preserving fundamental cellular functions. The results also raise question to which degree cytoskeletal research performed on model species may be extrapolated to other plants, acknowledging uncovered tangled evolution of tubulins. The presented findings establish tubulins as informative gene models for studying evolutionary constraint and different gene family organisation in plant genomes.

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