INTERNATIONAL PLANT PROTEOMICS ORGANIZATION

6th InPPO Conference

May 15 – 18 2025
BANFF SPRINGS HOTEL
Banff, AB, Canada

PROGRAM

InPPO Conference

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Welcome

Situated on the eastern slopes of the Rocky Mountain range in Banff National Park, Canada, the town of Banff represents a United Nations Educational, Scientific, and Cultural Organization (UNESCO) World Heritage Site. Importantly, it is also situated on Treaty 7 Territory, which is the ancestral lands of the Niitsitapi from the Blackfoot Confederacy, along with the Siksika, Kainai, and Piikani First Nations; the Îyârhe Nakoda of the Chiniki, Bearspaw, and Goodstoney First Nations; the Tsuut'ina First Nation and the Métis Nation of Alberta, representing a historic location for the intersection of peoples, languages and cultures.

By meeting on this historic site at the beautiful Banff Springs Hotel, the 6th International Plant Proteomics Organization Conference similarly aims to intersect diverse topics and peoples from around the world to "Cultivate Innovation through the Integration of Plant Science and Proteomics".

The scientific program features keynote and invited speakers from around the world covering innovation within proteomics themes of single-cell/cell-type, interactions and modifications, technological advances, climatic resiliency, crops and agriculture, unconventional plants, and artificial intelligence in plant discovery.

It is our pleasure to welcome you to this exciting event and be inspired by intersections between plants and proteomics from across the globe.

Jennifer Geddes-McAlister
University of Guelph

R. Glen Uhrig
University of Alberta

InPPO 2025 Co-Chairs

6th International Plant Proteomics Organization Conference
May 15 to 18, 2025
Banff Springs Hotel, Banff, AB, Canada

AGENDA

Thursday, May 15, 2025	
13:00	REGISTRATION OPENS - Riverview Lounge All Posters are to be mounted – New Brunswick Room All Sessions will take place in Alberta Room
16:00	WELCOME Conference Chairs: Jennifer Geddes-McAlister, R. Glen Uhrig
16:15 – 18:30	**Session 1 – Proteomics for Climate Resiliency** Chair: Devang Mehta, KU Leuven Co-Chair: Boyan Liu, University of Guelph
16:15	KEYNOTE **Harvey Millar**, The University of Western Australia, AUS *Proteomics for Climate Resiliency: Developing and adapting proteomic data for wider decision making in a changing world*
16:45	**Christoph Rampitsch**, Agriculture and Agrifood Canada, CAN *Peptidomics in Rusted Wheat. Endogenous rust peptides identified from short open reading frames by de novo sequencing*
17:05	**Devang Mehta**, KU Leuven, BEL *Closing gaps in plant chronobiology: from genome to proteome and from molecules to future geographies.*
17:25	**Natalia Bykova**, Agriculture and Agrifood Canada, CAN *Crosstalk between the cellular redox state, NO, ROS, and phytohormones integrates major physiological processes upon abiotic stress impact on seed dormancy control in wheat*
17:40	**Lauren Grubb**, University of Alberta, CAN *Defining a role for REVEILLE genes in the plant response to drought and salt stress using quantitative proteomics*
17:55	**Myah Crosby**, University of Guelph, CAN *Starch Phosphorylases Regulated by Glutathionylation*
18:15 – 20:00	WELCOME RECEPTION *New Brunswick Room with Exhibitors & Posters*

Friday, May 16, 2025	
07:30	LATE REGISTRATION – Riverview Lounge BREAKFAST - New Brunswick Room with Exhibits & Posters
08:30 – 10:00	**Session 2 - Artificial Intelligence in Plant Proteomics** Chair: Jennifer Geddes-McAlister, University of Guelph Co-Chair: Lauren Grubb, University of Alberta
08:30	KEYNOTE **Sachin Rustgi,** Clemson University, USA *Screening Wheat and Peanut Genotypes with Reduced Immunogenicity Using Protein Profiling*
09:00	**Marie Brunet**, University of Sherbrooke, CAN *Learning translation: deep-learning guided proteomics in plants*
09:20	**Mathieu Lavallée-Adam**, University of Ottawa, CAN *TBA*
09:40	**Zhiyong Wang**, Carnegie Institution for Science, USA *Crosstalk between phosphorylation and O-glycosylation regulates metabolism, growth, and immunity*
10:00-10:30	COFFEE BREAK New Brunswick Room with Exhibits & Posters Sponsored By
10:30 – 12:30	**Session 3 – Proteomics in Unconventional Plants** Chair: Ting-Ying Wu, Academia Sinica Co-Chair: Qiaomu Li, University of Alberta
10:30	KEYNOTE **Michelle Colgrave**, Edith Cowan University, AUS *Novel foods, the science behind evaluating health attributes, allergenicity and food safety*
11:00	**Ting-Ying Wu**, Academia Sinica, TW *The Landscape of Protein Phosphorylation Dynamics under Heat Stress in Marchantia*
11:20	**Ramesh Katam**, Florida A & M University, USA *Root proteomics and protein interaction studies showed shift in biosynthetic pathways to different salt stress levels in Pistachio*
11:35	**Niels Maywald**, University of Guelph, CAN *Next-Generation Intercropping: Synergistic Nitrogen Fixation for Sustainable Agriculture*
11:50	**Haleema Tariq**, McGill University, CAN *Paenibacillus polymyxa alters the metabolic and proteomic profile of Cannabis sativa*
12:05	**Jacqueline Thomson**, University of Guelph *Mechanistic analysis of tall fescue (Shedonorus arundinaceus) and its fungal endophyte, Epichloë coenophiala*

Friday, May 16, 2025, cont.	
12:30– 13:45	LUNCH BREAK 12:30 TO 13:30 SEMINAR – THERMO FISHER PRESENTATION Alberta Room
13:45 – 15:30	**<u>Session 4 – Advancing Proteomics Technologies in Plants</u>** Chair: R. Glen Uhrig, University of Alberta Co-Chair: Mohana Talasila, University of Alberta
13:45	KEYNOTE **Aleksandra Skirycz,** Michigan State University, USA *From interaction to function, what can we learn about metabolites from knowing their protein partners?*
14:15	**Sixue Chen**, The University of Mississippi, USA *Systems biology of C3 to CAM Transition, toward Improving Crop WUE*
14:35	**Bryan Hau,** The University of Western Australia, AUS *Identification and characterisation of the molecular drivers of malting performance in Hordeum vulgare*
14:50	**Vibha Srivastava,** University of Arkansas, Division of Agriculture, USA *Phosphoproteomics for unraveling novel targets of SnRK1 kinase in rice*
15:05	**Monique van Schie**, Wageningen University, NL *Location, location, location: exploration of the subcellular proteome of plants*
15:30 – 16:00	COFFEE BREAK New Brunswick Room with Exhibits & Posters Sponsored By
16:00	LIGHTNING TALKS
	- Poster 111 - Sujani Rathnayake, University of Guelph
	- Poster 104 – Utpal Bose, Edith Cowan University
	- Poster 105 – Mohana Talasila, University of Alberta
	- Poster 100 – Qiaomu Li, University of Alberta
	- Poster 109 – Boyan Liu, University of Guelph
	- Poster 121 – Genc Haljiti, Bavarian Centre for Biomolecular Mass Spectrometry
	- Poster 122 – Francis Bourassa, University of Sherbrooke
	- Poster 123 – Ankita Sehrawat, University of Delhi
16:30	POSTER SESSION Presenters of even-numbered posters should be at their posters between 16:30 – 17:30 and presenters of odd-numbered posters should be at their posters between 17:30 – 18:30 pm
18:30	FREE TIME

Saturday, May 17, 2025	
07:30 – 09:00	BREAKFAST & InPPO General Meeting (All Welcome) Host: Chrisoph Rampitsch Alberta Room
09:00 – 10:30	**Session 5 – Agricultural Proteomics (Session I)** Chair: Justin Walley, Iowa State University Co-Chair Jiaxi Lu, University of Guelph
09:00	KEYNOTE **Georgia Tanou**, Hellenic Agricultural Organization – DIMRA, GRC *Deciphering the Molecular Basis of Cold Stress Response in Kiwifruit: A Multi-Omics Perspective*
09:30	**Olivia Wilkins**, University of Manitoba, CAN *A single nucleus atlas of development altering stress*
09:50	**Nasim Alijanimamaghani,** University of Guelph, CAN *Impact of 15ADON and 15ADON/3NX Fusarium graminearum Chemotypes on Wheat and Corn Proteomes in Ontario, Canada*
10:05	**Guido Giordano**, Technical University of Munich, DE *Progress on the Crop Proteome Atlas (Year 3): a focus on barley germination and malting*
10:30– 11:00	COFFEE BREAK New Brunswick Room, with Exhibits & Posters
11:00	**Session 5 – Agricultural Proteomics (Session II)** Chair: Justin Walley, Iowa State University Co-Chair Jiaxi Lu, University of Guelph
11:00	**Justin Walley**, Iowa State University, USA *Regulatory architecture of disease resistance in maize revealed by multi-omic systems genetics*
11:20	**Industry Sponsored** **Jennifer Geddes-McAlister**, University of Guelph, CAN *Temporal wheat proteome remodeling by deoxynivalenol reveals novel detoxification signatures and strategies across cultivar*
11:40	**Hui Cao**, The University of Western Australia, AUS *Dissecting 70 years of wheat breeding through proteome-wide association study*
11:55	**Nora Foroud**, Agriculture and Agri-Food Canada, CAN Characterization of the Brachypodium mitogen-activated protein kinase MKK4/5-MPK3/6 pathway

Saturday, May 17, 2025, cont.	
12:15 – 13:30	LUNCH BREAK – Early Career Researcher Session (All Welcome & Lunch Provided) Chair: Guido Giordano, Technical University Munich Alberta Room
13:30	FREE TIME
18:30	RECEPTION Mt. Stephen Hall
19:00	DINNER & DANCING, Award Presentation Mt. Stephen Hall

Sunday, May 18, 2025	
07:30 – 08:30	BREAKFAST New Brunswick Room with Exhibits & Posters
08:30 – 10:15	**Session 6 - The Modified Plant Proteome** Chair: Christoph Rampitsch, Agriculture and Agrifood Canada Co-Chair: Sujani Rathnayake, University of Guelph
08:30	KEYNOTE **Ive de Smet**, VIB-UGENT, Centre for Plant Systems Biology, BE *Leveraging temperature-mediated phosphoproteomes to identify conserved signaling modules in plants*
09:00	**Pitter Huesgen**, University of Freiburg, DE *Tracing protease activities in plant immunity*
09:20	**Juergen Eirich**, University of Muenster, DE *Posttranslational Modifications and Their Role in Plant Acclimation to Light*
09:40	**Tagnon Missihoun**, University of Québec Trois-Rivières, CAN *Abscisic acid and Aldehyde dehydrogenases influence protein carbonylation in Arabidopsis thaliana*
09:55	**Mark Roosjen,** Wageningen University, NL *Pushing the limits - fast signaling in plants*
10:10	**Chris White-Gloria**, University of Calgary *Phospho-proteomics uncovers vast range of substrates for chloroplastic protein phosphatase SLP1*
10:25 – 10:50	COFFEE BREAK New Brunswick Room, with Exhibitors & Posters
10:50 – 12:30	**Session 7 – Plant Interactomes** Chair: Shouling Xu, Carnegie Institution at Stanford Co-Chair: Niels Maywald, University of Guelph
10:50	KEYNOTE **Pengcheng Wang**, Institute of Advanced Biotechnology, CN *Chloroplast phosphonetworks in Arabidopsis*
11:20	**Shouling Xu**, Carnegie Institution at Stanford, USA *Mapping Architecture of Protein complexes in Arabidopsis using XL-MS*
11:40	**Kishor Ingole**, University of Cambridge, UK *Unravelling the Cell-type specific SUMOylome of Arabidopsis roots by mass spectrometry (MS)*
12:00	**Kyle Bender**, University of Zurich, CH *Quantitative proteomics of plant receptor kinase signaling*
12:15	**Linfei Guo**, Wageningen University, NL *Proteomic analysis of growth-stress interaction*
12:30	WRAP-UP & CLOSING Conference Chairs: Jennifer Geddes-McAlister, R. Glen Uhrig

PRESENTATIONS:

Keynote

Colgrave, Michelle
Edith Cowan University

Novel foods, the science behind evaluating health attributes, allergenicity and food safety

A major challenge facing the world is providing protein security in the face of a growing global population. To this end, we have started exploring different crops and food sources from pulses, insects, algae, fungi and more. As we pivot towards these under-utilised resources, potential exists for increasing prevalence of allergy. Emerging protein sources often possess high protein content and health-promoting benefits, but they can also contain proteins that can trigger life-threatening anaphylaxis. In this presentation, the role of proteomics as a powerful tool to characterise both nutritional and antinutritional proteins in emerging protein sources will be discussed.

PRESENTATIONS:

Keynote

De Smet, Ive
VIB-UGent Center for Plant Systems Biology

Leveraging temperature-mediated phosphoproteomes to identify conserved signaling modules in plants

Plants respond to mild warm temperature conditions by increased elongation growth of organs to enhance cooling capacity, in a process called thermomorphogenesis. In addition, to regulate gas exchange with the environment and to control abiotic stress relief, plants regulate stomatal opening. To identify regulators of these processes that are conserved in flowering plants, we mapped changes in protein phosphorylation in both dicots and monocots exposed to warm temperature. Here, I will present some of the conserved signaling modules that we identified and the role of some individual phosphosites.

PRESENTATIONS:

Keynote

Millar, Harvey
The University of Western Australia

Proteomics for Climate Resiliency: Developing and adapting proteomic data for wider decision making in a changing world

Proteomics, at its core, is a powerful tool for capturing snapshots of protein profiles from biological material, providing insights into the organization of protein machinery within cells. By inference, comparing proteomes can reveal potential changes or differences in cellular function. Proteomics is most valuable when integrated with transcriptomic or metabolomic datasets and correlated with physiological measurements, enhancing our understanding of cellular processes. However, typical proteomic data outputs often fall short of being directly applicable to other plant and crop science disciplines or industrial pipelines—particularly those focused on evaluating complex ecosystem-level phenomena such as environmental change and climate adaptation, or developing new genotypes better suited to these conditions through breeding or genetic modification. This represents a missed opportunity for three key reasons which all relate to what proteins really are. First, proteins drive plant function, providing a more direct explanation of altered phenotypes than gene expression studies, while still being gene-linked. Second, proteins play a central role in nitrogen storage and demand within cells, a crucial factor in plant growth and both agricultural and ecosystem productivity. Third, protein abundance and post-harvest properties largely determine digestibility and nutritional value for humans and animals, as well as the suitability of plant products for various food applications. In this talk, I will share our journey from fundamental plant proteomics in model species to wheat proteomics research focused on salinity, hypoxia, and grain filling. I will discuss the challenges of integrating this work with other disciplines. Then I will explore our ongoing efforts to enhance the scale, speed, and decision-making tools necessary to embed wheat proteomics into actionable strategies for climate resilience, using temperature tolerance and nitrogen-use efficiency as examples.

PRESENTATIONS:

Keynote

Rustgi, Sachin
Clemson University

Screening Wheat and Peanut Genotypes with Reduced Immunogenicity Using Protein Profiling

The U.S. Food and Drug Administration recognizes nine major food allergens, five of which are plant-based. Among these, wheat and peanuts are dietary staples and key protein sources, but their seed storage proteins can trigger immune responses in genetically predisposed individuals. In wheat, gliadins and low-molecular-weight glutenins are linked to allergies and autoimmune conditions such as celiac disease. In peanuts, Ara h1, h2, h3, and h6 are the major allergenic proteins. Some Ara h proteins resemble alpha-amylase trypsin inhibitors, suggesting defensive roles against pests and pathogens. Peanut allergies affect ~3% of the U.S. population, while wheat-related disorders impact ~7%. Both crops are polyploid, complicating conventional breeding for reduced allergenicity. Two complementary strategies were pursued: (1) screening diverse germplasm, including mutants, landraces, and introgression lines, for reduced allergenic proteins and protein biomarker-assisted pyramiding of missing protein phenotypes, and (2) genome editing of key immunogen genes. Analytical methods included PAGE with densitometry, mass spectrometry, ELISA, and Western blotting, enabling identification of promising genotypes. CRISPR, particularly Cas12a, supports multigene editing, though delivery remains a challenge. In this study, constructs targeting allergen genes were delivered using biolistics in both crops, wheat pollen magnetofection, and viral vectors in peanut. Biolistics proved broadly effective, while magnetofection and viral delivery enabled in planta expression without tissue culture. Selected peanut lines are being screened by Western blotting using Ara h2-specific antibodies and IgE from allergic individuals. Top candidates will advance to basophil activation tests. Wheat genotypes will be assessed using T-cell lines from celiac patients sensitive to alpha-gliadins.

PRESENTATIONS:

Keynote

Skirycz, Aleksandra
Michigan State University

From interaction to function, what can we learn about metabolites from knowing their protein partners?

Functional diversity reflects the immense chemical diversity of living organisms that produce hundreds of thousands of small molecule compounds, most of which remain to be chemically and functionally characterized. Because small molecules rarely work on their own but rather via interactions with proteins, following the proverbial "tell me who your friends are, and I will tell you who you are," identification of protein interactors can be used to unravel the function of a metabolite. The complex and dynamic protein-metabolite interactions (PMIs) network underlies all biological processes but remains under-characterized. In my group, we adapted co-fractionation mass-spectrometry (CF-MS), a well-established approach to map protein assemblies, for proteome and metabolome-wide identification of the protein-metabolite complexes. CF-MS experiments combine the separation of native complexes with MS analysis of the obtained fractions and use the similarity of elution profiles, referred to as co-elution or co-fractionation, to delineate interactors. CF-MS enables the untargeted identification of complexes without needing a protein or a metabolite bait. The PMI networks generated in the group comprise tens of annotated metabolites and hundreds of unknown metabolic features. During my seminar, I will discuss how we use the obtained interaction data to uncover novel regulatory functions of compounds, focusing on dipeptides and cyclic dipeptides and their role in regulating central carbon metabolism and organismal health in plants and animals.

PRESENTATIONS:

Keynote

Tanou, Georgia
ELGO DIMITRA

Deciphering the Molecular Basis of Cold Stress Response in Kiwifruit: A Multi-Omics Perspective

Kiwifruit is highly sensitive to ethylene and exhibits a rapid respiration rate, which accelerates fruit softening and limits postharvest life. Cold storage is widely employed to maintain fruit quality and extend shelf life; however, due to its subtropical origin, prolonged exposure to low temperatures can induce physiological disorders. Cold stress triggers extensive molecular and biochemical modifications, leading to transcriptional reprogramming and metabolic shifts. In this study, we employed a multi-omics approach to elucidate the molecular basis of kiwifruit cold acclimation during storage. Fruits were harvested and stored at 0°C with 95% RH for 15 and 90 days, and tissue-specific responses in the pericarp and placenta were assessed. A comprehensive analysis integrating metabolomics, proteomics, transcriptomics, and whole-genome bisulfite sequencing was performed at different postharvest time points. This systems-level investigation revealed key molecular signatures associated with cold stress, including transcriptional, epigenomic, proteomic, and metabolic adaptations. Integrative bioinformatic analysis of RNA-seq and proteomic datasets identified several transcription factors (TFs) implicated in cold stress response. Functional characterization using biotechnological approaches highlighted the roles of C2H2, GARP-G2-like, HMG, WRKY, NAC, GRAS, and MYB-related TF families in cold acclimation. These findings provide new insights into the regulatory networks governing cold stress tolerance in kiwifruit and propose potential molecular biomarkers for breeding strategies aimed at improving postharvest resilience.

Contributing Authors
M. G. Kollaros, M. Michailidis, M. Samiotaki, C. Polychroniadou, D. Valasiadis, A. Karamanoli, C. Bazakos, A. Molassiotis, G. Tanou

PRESENTATIONS:

Keynote

Wang, Pengcheng
Institute of Advanced Biotechnology

Chloroplast phosphonetworks in Arabidopsis

Chloroplasts play central roles in photosynthesis and diverse metabolic processes, including the biosynthesis of amino acids, vitamins, and lipids, as well as the regulation of signal transduction pathways. However, the phosphorylation-based regulation of chloroplast functions remains poorly understood, with few kinases and substrates identified to date. In this study, we combined proteomics and phosphoproteomics approaches to systematically identify protein kinases potentially localized in chloroplasts in *Arabidopsis thaliana*. We identified 484 kinase candidates, of which 38 were confirmed to localize to chloroplasts via transient expression assays. Seventeen of these had been previously reported, while 21 represent newly identified chloroplast-localized kinases. Using the KALIP2 (Kinase Assay Linked Phosphoproteomics 2) method (Wang, *PNAS*, 2020), we characterized the in vitro kinase activity of 19 chloroplast kinases and identified 1,296 putative phosphorylation substrates. This enabled the construction of the first chloroplast phosphorylation network, linking 19 kinases with their substrates. Functional validation showed that CPK16 and CPK29 phosphorylate the calcium-sensing receptor (CAS) at multiple sites, modulating chloroplast-associated immune responses. Additionally, PKL proteins localized in chloroplasts were found to regulate targets potentially involved in chloroplast function. Our study establishes a comprehensive phosphorylation network in *Arabidopsis* chloroplasts and offers new insights into kinase-mediated regulatory mechanisms, providing a valuable resource for understanding how environmental signals influence chloroplast functions.

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PRESENTATIONS:

Oral

Alijanimamaghani, Nasim
University of Guelph

Impact of 15ADON and 15ADON/3NX Fusarium graminearum Chemotypes on Wheat and Corn Proteomes in Ontario, Canada

Fusarium Head Blight (FHB) in small grains and Gibberella Ear Rot in corn, caused by *Fusarium graminearum*, are significant diseases in small grains and corn due to their production of harmful mycotoxins. In Ontario, in addition to the predominant 15ADON chemotype, a newly emerging chemotype (15ADON/3NX) has been identified, which produces novel mycotoxins (NX and 3ANX) in highly infected corn. In this study, wheat and corn inoculated with 15ADON and 15ADON/3NX strains and analyzed for mycotoxin production and proteomic changes. Results showed both chemotypes produced more deoxynivalenol (DON) in wheat than corn, with 15ADON producing 7.3 times more DON in wheat and 15ADON/3NX producing 5 times more. Although DON concentrations were similar between chemotypes, 15ADON/3NX caused more severe disease symptoms in corn. There was a strong correlation between DON and disease severity in both wheat ($R^2 = 0.936$) and corn ($R^2 = 0.888$). Among the 15ADON strains, 180379 was the most toxigenic in both corn (57.3 µg/g) and wheat (380.6 µg/g). However, among the 15ADON/3NX strains, 252441 in wheat (245.6 µg/g) and 2525444 in corn (57.8 µg/g). To better understand the impact of the chemotypes on wheat, we performed proteomics profiling on the samples. We defined a core modified proteome influenced by mycotoxin presence, along with 3NX-exclusive proteome remodeling that suggests activation of specific defenses depending upon chemotype. This experiment was repeated in 2024 and will also be repeated in 2025 to allow for multi-year data comparison and provide unprecedented insight into the relationship of plant defense responses to mycotoxin contamination across multiple agricultural grains.

Contributing Authors

Seyedehsanaz Ramezanpour2, Jason A. McAlister2, Jennifer Geddes-McAlister2, Gursahib Sign1, David Hooker1

PRESENTATIONS:

Oral

Bender, Kyle
University of Zurich

Quantitative proteomics of plant receptor kinase signaling

Cellular responses to external stimuli are governed at the molecular level by signal transduction networks. In this context, endogenous and exogenous signals are perceived by receptors that activate signaling to regulate the activity of downstream effectors to reprogram cellular states. In plants, plasma membrane-localized receptor kinases (RKs) serve as the primary receivers of extracellular molecular signals and activate signal transduction through cytosolic protein kinase domains. While considerable effort has been made to understand how RK-mediated signaling is regulated, a quantitative understanding of RK complex composition and regulation in living plant cells is lacking. To address this knowledge gap, we developed quantitative proteomics workflows to characterize RK complex composition and dynamics. Analysis of several immune-related RKs by affinity-enrichment mass spectrometry reveals novel facets of RK signaling: 1) Ligand perception results in recruitment of co-receptors but has little effect on RK complex composition; 2) Activated RK complexes are likely present at relatively low stoichiometries; and 3) RKs may associate in functionally related receptor clusters. In addition to the analysis of RK complexes, we have established a simple, quantitative phosphoproteomics workflow allowing for the identification and quantification of more than 30,000 and 27,000 phosphorylation sites, respectively, in a single experiment, which opens avenues for the identification of receptors for orphan ligands and novel signaling components. Collectively, our results provide insights into RK signaling in an in vivo context and provide a framework to address critical questions in RK biology, including a path forward to understand mechanisms governing signaling specificity in RK-activated pathways.d

Contributing Authors
Kyle W. Bender, Henning Mühlenbeck, Laura Herold, Jana Ordon, Alvaro D. Fernandez Fernandez, Keran Zhai, Jack Rhodes, Antje Dittmann, and Cyril Zipfel

PRESENTATIONS:

Oral

Brunet, Marie A.
University of Sherbrooke

Learning translation: deep-learning guided proteomics in plants

Thousands of functional coding sequences have eluded annotations. These overlooked coding sequences are often small and at unsuspected genomic loci. Several approaches have been developed throughout the last decade, trying to identify these non-canonical coding sequences. However, due to experimental limitations and inherent biases, our interpretation of the proteomic landscape is most likely underestimated, and our understanding is limited to the sensitivity and specificity of our methodologies. Accurate annotation of functional elements holds crucial implications, there is a dire need for tools enabling an exhaustive evaluation of proteomes. The astounding success of deep learning on sequence modeling tasks, combined with high-quality omics data, provides hope in the search for an approximation of the universal features that underlie translation. Here, we present FOMOnet, a deep learning model that performs end-to-end segmentation of a transcript with single-base resolution. On human sequences, FOMOnet displays a ROC AUC of 99.8% and a PR AUC of 99.7% with no overfitting and an estimated false positive rate of 0.52%. Furthermore, despite being trained on human sequences, FOMOnet shows impressive performance in Arabidopsis thaliana. On transcripts with known coding sequences (39,346 in TAIR10), FOMOnet predicts the annotated sequence for 55% (21,653), and for an additional 15.5% it predicts an alternative initiation codon. FOMOnet failed to predict 28.6% of annotated sequences (11,259), with these mostly present on transcripts with short UTRs. FOMOnet also predicted 163 transcripts as bi-coding (2 coding sequences) and predicted a different coding sequence from the one annotated for 172 transcripts.

Contributing Authors
Francis Bourassa, Ihor Arefiev, Marie A. Brunet

PRESENTATIONS:

Oral

Bykova, Natalia
AAFC

Crosstalk between the cellular redox state, NO, ROS, and phytohormones integrates major physiological processes upon abiotic stress impact on seed dormancy control in wheat

Climate changes result in greater annual variation in susceptibility of wheat to preharvest sprouting and seed quality damage due to high sensitivity of wheat to abiotic stresses. Adaptation of seed dormancy trait in wheat cultivars to climate changes requires comprehensive understanding of abiotic stresses impact on the interaction between genotype and environment. In this study, hexaploid wheat (Triticum aestivum L.) populations grown in the field or under controlled environment, integration of global quantitative proteomic profiling with genome-wide RNA-seq analysis of transcriptome, quantitative redox proteomics, targeted metabolomics, and analysis of gene functions using CRISPR/Cas9-mediated gene editing were employed. Differentially abundant proteins and expressed genes governing complex seed dormancy trait, and redox responding proteins regulating levels of reactive oxygen species (ROS) in cell wall, extracellular compartment, and mitochondria were revealed. Changes in the intracellular redox state modulated by accelerated production of reactive oxygen species (ROS), nitric oxide (NO) and its derivatives, antioxidative systems, phytohormonal signaling and their reciprocal regulation of cellular oxidative metabolism play important roles in seed dormancy control. The increase in redox state under the hypoxic conditions during early imbibition of seeds facilitates excessively high levels of ROS and NO, which support energy metabolism prior to radicle protrusion and are critically important for redox signaling via post-translational modification of proteins and mediation of phytohormonal responses, for breaking endodormancy. In germinating seeds, their energy status and redox regulation of major signal transduction events control endodormancy, promote germination, and establish phytohormone profiles essential for triggering major developmental processes.

Contributing Authors
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PRESENTATIONS:

Oral

Cao, Hui
The University of Western Australia

Dissecting 70 years of wheat breeding through proteome-wide association study

Wheat (*Triticum aestivum* L.) is the staple food for 30% of the human population and a trusted source of both calories and nutrition. Over the past century, wheat breeding programs have heavily prioritized on delivering high-yielding varieties to address food security, however these intensive selection processes have caused up to 69% losses in genetic diversity in modern bread wheat. Furthermore, the success in steady yield growth has significantly lowered grain protein content over the past few decades, despite the need to maintain protein content as a quality standard for wheat. To explore the genetic basis of these trends, a Multiparent Advanced Generation Inter-Cross (MAGIC) population comprising over 500 recombinant inbred lines that captures the genetic variations across 70 years of UK wheat breeding history (1935 - 2004) was used in this study. Genome-wide association studies (GWAS) of this population have mapped 136 QTLs across 73 phenotype/year combinations. Through high-throughput quantitative proteomics using an optimized rapid 15 min gradient method, we quantified ~1000 proteins across the entire population. Further Proteome-Wide Association Studies (PWAS) by statistically associate protein profiles to QTLs enables us to identify the individual causal gene (the marker) within the complex QTL region. This study provides new insights into how wheat grain protein compositions have evolved and its impacts on grain quality over 70 years of wheat breeding history in UK, as well as demonstrates the potential of using advanced proteomics to leverage wheat grain quality study and accelerate modern high quality wheat breeding for future needs.

Contributing Authors
Hui Cao, James Cockram, Richard Mott, A. Harvey Millar

PRESENTATIONS:

Oral

Chen, Sixue
The University of Mississippi

Systems biology of C3 to CAM Transition toward Improving Crop WUE

Human population is expected to reach 9 billion by 2050, and crop productivity needs to increase to feed the growing population. Unfortunately, freshwater shortage and adverse environmental conditions have posed grand challenges to global food security. How to increase crop water use efficiency (WUE) is an urgent question. Here systems biology is applied to learn from a unique photosynthesis mechanism - crassulacean acid metabolism (CAM). Mesembryanthemum crystallinum (common ice plant) a facultative CAM plant, which can shift from C3 to CAM. Through multi-omics, many transcripts, proteins and metabolites were identified to be altered. For example, eighteen transcription factors were identified during the C3 to CAM transition. One of the transcription factors is homeobox 7(McHB7), and it was validated to play a role in ice plant stress tolerance.

Contributing Authors
Bowen Tan, Qijie Guan, Noe Perron, Craig Dufresne

PRESENTATIONS:

Oral

Crosby, Myah
University of Guelph

Starch Phosphorylases Regulated by Glutathionylation

Eukaryotic cells store carbohydrates as α-glucans when the cell is nutritionally rich and mobilize this stored energy to meet various energy requirements, as well as buffer against internal and environmental stressors. Algae and land plants contain α-1,4-glucan phosphorylases (α-GPs), called starch phosphorylases (SPs), which play a key role in mediating the storage and mobilization of starch. Plastidial SP (Pho1) of land plants is thought to be a multi-role enzyme in which its roles contribute to plant health during abiotic stress. Abiotic stressors affect the cellular redox balance, which may result in increased reactive oxygen species (ROS), oxidative stress, and oxidative damage to proteins and tissues. Oxidative stress also disrupts the redox state of glutathione, favouring the oxidative form and leading to disulfide bond formation between a glutathione and protein's cysteine, called glutathionylation. Typically, most enzymes are inhibited by oxidation however, Pho1 increases in catalytic activity under increased oxidation in in vitro experiments using the agent diamide. Quantitative analysis has determined that Pho1 activity increases with diamide and glutathione titrations, suggesting protein modification via glutathionylation. Additionally, labelling with a biotinylated form of glutathione (BioGEE) and chemiluminescence detection determined that plastidial and cytosolic SPs are likely glutathionylated. Sequence alignment and computational analyses have identified potential cysteines of SPs as sites of glutathionylation. These results demonstrate a potentially universal modification within land plants in response to cellular oxidation. These results also support the contribution of SPs to plant abiotic tolerance and cellular resilience through the regulation of starch metabolism.

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PRESENTATIONS:

Oral

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Posttranslational Modifications and Their Role in Plant Acclimation to Light

Plants exhibit significant physiological adaptations in response to various light conditions. Regulation of protein activity through post-translational modifications (PTMs), rather than alterations in RNA expression or protein abundance, plays a crucial role in mediating these adjustments. While individual players have been extensively studies, a holistic view of PTMome dynamics remains elusive, rendering numerous cellular responses uncharacterized. To address this gap, we performed a (time-resolved) investigation of the modified proteome in Arabidopsis thaliana, to specifically examine phosphorylation, lysine and N-terminal acetylation, and cysteine-based redox switches. Using advanced liquid chromatography tandem mass spectrometry (LC MS/MS)-based proteomics, combined with tailored enrichment and sample preparation protocols, we identified and quantified thousands of proteins along with their respective PTM sites. Our analyses revealed intricate links between primary metabolic pathways and PTM-mediated regulatory networks, shedding new light on the extent to which photosynthetic and stress-related responses remodel global cellular functioning. Furthermore, our comprehensive multi-omic profiling approach offers unprecedented insights into the complex interplay of genetic and environmental factors governing plant metabolism. Our findings contribute significantly to the comprehension of post-translational modifications (PTMs) and their vital part in shaping plant responses to variable light conditions. Unravelling the manifold impact of PTMs on plant metabolism not only expands our foundational knowledge but also sets the stage for subsequent studies targeting broader biological concepts in plant sciences.

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Characterization of the Brachypodium mitogen-activated protein kinase MKK4/5-MPK3/6 pathway

The mitogen-activated protein kinase (MAPK) family in plants are key signaling enzymes required for a wide variety of cellular processes from development to stress response and plant defense. The MAPK pathways are reasonably well characterized in the model dicot species *Arabidopsis thaliana* and to a lesser extent in rice. While rice was the first model for monocots, it has not proven to be as representative of the Poaceae (grass) family of monocots, which include the cereal crop species such as wheat and barley. The MAPK family of genes have been identified in the Poaceae model crop, *Brachypodium distachyon*, but characterization of the MAPK pathways has received little attention. With the long-term goal of characterizing the YODA MAPK pathway in cereals, involved in both plant development and plant defense, we have investigated the downstream MAPKs, MKK4/5-MPK3/6 in B. distachyon. In *A. thaliana*, AtMKK4 and AtMKK5 are activated by YODA through phosphorylation at threonine and serine residues of the AtMKK4/5 activation loop: TMDPCNS. Meanwhile, these enzymes are deactivated through phosphorylation immediately upstream of the activation loop, specifically at S-X3-T of TMDPCNSSVGT, where the serine residue is part of the activation loop. It is hypothesized that steric hindrance from phosphorylation at S-X3-T prevents phosphorylation of threonine in the activation loop. To test this, we used in vitro kinase assays with recombinantly expressed BdMKK4 and BdMKK5, introducing phosphosmimetic mutations in the activation loop: T-X5-S-X3-T. Specific combinations of threonine and/or serine residues were substituted with acidic residues (glutamic acid and aspartic acid) to mimic phosphorylation. Activity assays targeting the downstream MAPKs, BdMPK3 and BdMPK6, were performed. Phosphorylation was confirmed by western blot analyses and further verified by mass spectrometry. Our results show that the E-X5-D-X3-T, E-X5-S-X3-T, and to a lesser extent T-X5-D-X3-T are sufficient for phosphorylation of BdMPK3/6, and the T/E-X5-S/D-X3-E was inactive. As a next step, we aim to perform in vitro kinase assays to screen downstream targets of BdMKK4/5E-X5-D-X3-T-BdMPK3/6 for phosphorylation of candidate downstream transcription factors involved in plant development and/or defense.

Contributing Authors

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PRESENTATIONS:

Oral

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Temporal wheat proteome remodeling by deoxynivalenol reveals novel detoxification signatures and strategies across cultivars

Fusarium head blight (FHB) is a globally devastating fungal disease resulting in reduced grain yield and quality, along with contamination of grains with dangerous mycotoxins. Consumption of such mycotoxins by humans through processed food or livestock through feed has downstream implications for human and animal health. This interconnectivity across the environment, animal, and human health defines the One Health problem of threatened food safety and security. In this study, we explore remodeling of the wheat proteome upon exposure to a common mycotoxin, deoxynivalenol (DON). We investigate cultivar-specific responses to DON exposure in FHB-susceptible (Norwell) and -resistant (Sumai#3) cultivars across a continuum of exposure (i.e., 24 and 120 hours post inoculation), and upon low (i.e., 0.1 mg/mL) and high (1.0 mg/mL) levels of the mycotoxin. This complex experimental design enables us to tease apart the dynamic relationship between each cultivar and DON tolerance. Specifically, we define precise proteins and broad categories of remodeling that are common (i.e., reduction in photosynthesis) and unique (i.e., glycosyltransferase) to the cultivars and align with anticipated protective mechanisms. Moreover, we adapted an in vitro DON tolerance expression system and determined that induction of ubiquinol oxidase provides heightened protection for yeast growth relative to the negative control, as well as increased protection compared to a well-defined DON detoxifying protein. Our study suggests a new avenue for identification and characterization of novel DON detoxifying proteins as putative biomarkers for selected breeding strategies. Such strategies support the production of wheat varieties with increased tolerance to DON for improved global food safety and security.

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Progress on the Crop Proteome Atlas (Year 3): a focus on barley germination and malting

Plants are crucial for human nutrition. Despite challenges posed by their complexity, proteomics is more and more employed to study these organisms, providing insights that surpass what can be learnt with transcriptomics and genomics. The next frontier for plant proteomics is large-scale analysis, across diverse species, varieties, and organs, providing a more comprehensive understanding of plant systems and their molecular mechanisms. At ThessInPPO2022, I presented the project 'The Proteomes that Feed the World', which aims at mapping the proteomes of 100 crops most important for human nutrition. After nearly two years, we have completed sourcing and biobanking, finalized protein extraction for more than half of the crops and completed mass-spec measurements for around one third of the species. Preliminary results reveal high-quality data, with around 10,000 proteins identified per sample, which enables comparative analyses across species and organs. This atlas will represent a key resource for the plant scientific community. As part of this initiative, my research focuses on barley, a critical crop for the food and beverage industries. Through spatially and temporally resolved proteomics, I developed the most comprehensive barley seed proteome to date, uncovering unexpected tissue-specific patterns for gibberellin metabolism during germination. Furthermore, with an extensive time-course germination analysis across a panel of 20 barley ancestors, landraces, and cultivars, representing milestones in barley breeding for beer production, I found significant quantitative changes in the enzymatic machinery critical for barley germination and malting. These findings advance our understanding of barley domestication and open opportunities for targeted breeding.

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Defining a role for REVEILLE genes in the plant response to drought and salt stress using quantitative proteomics

The plant circadian clock allows for precise regulation of responses to developmental and environmental cues at the appropriate time within a 24 h photoperiod. Consisting of a series of interconnected transcription factor feedback loops, each expressed at specific times of day within the photoperiod, more than 30% of *Arabidopsis* genes show circadian regulation. REVEILLE (RVE) genes exhibit highest expression in the afternoon, and act as activators of evening-expressed clock genes, including TOC1 and PRR5. Specifically, RVE4, RVE6 and RVE8 have been identified as regulators of both plant growth and development, as well as of plant abiotic stress responses, such as hot and cold. Additionally, our previous work, as well as work in soybean, has implicated RVEs in the plant response to drought and salt stress. However, there is a lack of understanding of how RVEs control osmoregulation, particularly relating to changes at the protein level. Here, we describe a quantitative proteomic approach to defining the diel osmotic- and salt-induced proteome of the triple mutant rve4 6 8. Key findings and targeted validation reveal an osmoregulatory role for RVE proteins.

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PRESENTATIONS:

Oral

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Proteomic analysis of growth-stress interaction

Plants, as sessile organisms, integrate various internal and environmental signals, such as hormones, light, and temperature, through intracellular networks that regulate growth and defense. This project aims to elucidate the role of key protein complexes, specifically BAP-D and COP1/SPA, in modulating these processes. By focusing on the composition and post-translational modifications of these complexes in response to growth cues and stress, we aim to understand how they mediate signal integration. Using protein proximity labeling, we will map interactions and modifications to uncover the regulatory mechanisms that govern plant growth under stress conditions.

PRESENTATIONS:

Oral

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Identification and characterisation of the molecular drivers of malting performance in Hordeum vulgare

The end goal of barley malting is the degradation of storage proteins and carbohydrates, which provides yeast its essential nutrients and sugars for beer brewing. The process relies on synthesis of various enzymes during the germination process in different parts of the grain. Although malting has been around since Ancient Egypt, there are still improvements to the process and the molecular regulation of what occurs that still contains key unknown processes. Investigations into the barley proteome throughout the malting process has yet to be thoroughly investigated thoroughly. Here, protein-level differences between malting and feed barley cultivars and identify molecular drivers for the malting process. A comparison of barley grains for 17 barley cultivars showed differentially abundant proteins in significant functional groups. To assess the functionality of these, protease activity was measure via Activity-Based Protein Profiling, which identified a major storage protein degrading enzyme. Tissue-specific proteomics was performed to determine the localisation of proteins, which suggested that proteins are differentially synthesised/transported between tissues inside the grain. To follow up, protein turnover experiments determining the ageof proteins, as well as which proteins are most rapidly synthesised can be performed. Additionally, these turnover experiments will investigate changes in the degradation rate for proteins in the starchy endosperm during germination and show protein activity dictated by polypeptide age and post-translation modifications. These proteomic investigations into the major differences between malting and non-malting cultivars aims to advance barley research, breeding, and the malting process leading to improved process sustainability.

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Tracing protease activities in plant immunity

Proteolytic processing is a common, (mostly) irreversible and site-specific post-translational protein modification that alters protein activity, interactions and location. Protein termini provide an unambiguous readout of processing, but are often low abundant and therefore rarely detected in standard proteome analyses. Over the last two decades, multiple enrichment methods have been developed to overcome this problem, including our Hypersensitive Undecanal-mediated N-TERmini enrichment (HUNTER). We have applied these methods to identify proteolytic processes in plant immune responses, including hypersensitive cell death. Despite successes, results have often been hampered by a lack of reproducibility and still insufficient sensitivity. In recent years, data independent acquisition (DIA) methods, combined with advances in mass spectrometry instrumentation and new computational tools, have massively improved the reproducibility and sensitivity of mass spectrometry-based proteomics. We have now established analysis of N-terminal peptides enriched by HUNTER in library-free DIA mode using FragPipe. We evaluated the performance of DIA-HUNTER by rigorous benchmarking to traditional DDA-analysis across multiple instruments. We consistently observe substantial increases in reproducibly quantified N-terminal peptides in DIA-mode compared to DDA-mode, independent of the mass spectrometry system used.

PRESENTATIONS:

Oral

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Unravelling the Cell-type specific SUMOylome of Arabidopsis roots by mass spectrometry (MS)

Post-translational modifications (PTMs) are integral to biological systems, yet the mechanisms governing their specificity remain poorly understood. SUMOylation, a PTM mediated by the Small Ubiquitin-like Modifier (SUMO), plays a pivotal role in diverse cellular pathways across eukaryotes. Unlike ubiquitination or phosphorylation, SUMOylation in plants is regulated by a set of 32 genes, presenting a simplified model to investigate PTM-regulation. Our research focuses on unraveling how SUMO mediates environmental signals into physiological responses, contributing to fundamental "rules of life" underlying development and stress adaptation. We have characterized the spatial dynamics of the entire SUMO system in Arabidopsis root cells, providing an unprecedented resource that uncovers cell-specific SUMO component localization and regulation. To date, cell-type-specific PTM characterization has not been attempted in any plant species. Upon sensing a stimulus, Arabidopsis roots likely employ distinct PTM-mediated signaling pathways to transduce signals from outer to inner cells. To investigate this, we generated transgenic lines expressing His-tagged-SUMO1KGG under cell-type-specific promoters. SUMO1KGG-modified proteins were enriched using Ni-NTA under denaturing conditions. A second enrichment using K-ε-GG antibodies that recognizes the remnant of modified SUMO1KGG on target proteins upon tryptic digestion was performed followed by LC-MS/MS analysis. This approach identified ~1600 SUMO1-conjugated proteins, revealing cell-type-specificities in SUMOylation. These findings suggest unique roles for different cell-types in signal transduction upon stimulus recognition. Our study paves the way for cell-type-specific characterization of a PTM, offering insights into the SUMOcode in plants. This work provides a foundation for editing and optimizing SUMOylation pathways, enabling researchers to develop crops resilient to climate change and environmental challenges.

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Root proteomics and protein interaction studies showed shift in biosynthetic pathways to different salt stress levels in Pistachio

Pistachio (Pistacia vera L.) is an economically important tree nut that thrives in semi-arid and arid environments. This study aimed to investigate the physiological and molecular mechanisms that contribute to stress tolerance in the UCB-1 cultivar. Over a period of 100 days, five one-year-old pistachio rootstocks were subjected to four different saline water regimes. The rootstocks employed a sodium exclusion strategy to cope with salinity stress. Total proteins were extracted from the pistachio roots exposed to varying concentrations of NaCl. These proteins were then analyzed using high-throughput LC-MS/MS spectrometry, with the results compared to the Citrus database. In total, over 1,600 protein IDs were identified. Comparative analysis revealed that 245 proteins were more abundant, while 190 proteins were less abundant across three different stress levels. Proteins involved in carbohydrate metabolism, glycolytic processes, stress response, defense mechanisms consistently showed overexpression under all stress conditions. In contrast, proteins associated with biosynthesis, transport, and trafficking exhibited increased expression at low to moderate stress levels, but their response decreased at higher salinity concentrations. Additionally, proteins related to amino acid metabolism, lipid metabolism, protein modification and folding, signal transduction, and translation showed increased expression in response to higher salinity stress. The protein interaction network, mapped to orthologs in Arabidopsis thaliana, revealed clusters associated with these proteins. Under control conditions, most proteins were involved in chloroplast and cytosolic pathways. As salinity levels increased, the focus of these pathways shifted, highlighting carbohydrate metabolism under moderate salinity stress and amino acid metabolism under severe salinity stress.

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Next-Generation Intercropping: Synergistic Nitrogen Fixation for Sustainable Agriculture

The overuse of synthetic nitrogen fertilizers in agriculture has led to severe environmental consequences, including soil degradation, water contamination, and greenhouse gas emissions. To address these challenges, this research project focuses on genetically modifying common bean plants (Phaseolus vulgaris) to enhance the release of biologically fixed nitrogen from their root nodules. By making this nitrogen available to neighboring non-nitrogen-fixing crops, this approach aims to reduce reliance on synthetic fertilizers and promote more sustainable agricultural practices. To achieve this goal, we employ advanced molecular techniques, including proteomics, to identify key genes involved in nitrogen release from root nodules. Once these genes are identified, targeted genetic modifications will be introduced to optimize nitrogen transfer. The effects of these modifications will then be systematically evaluated under controlled conditions, assessing their impact on plant growth and nitrogen uptake by associated crops. This research holds significant potential for improving agricultural sustainability. By enhancing the natural nitrogen cycle, it could reduce fertilizer dependency, lower environmental pollution, and support ecosystem health. Additionally, increased nitrogen availability may improve soil fertility and boost crop yields while mitigating the ecological damage associated with conventional fertilization methods. Ultimately, our findings will provide valuable insights into plant-microbe interactions and the genetic regulation of nitrogen release, paving the way for innovative strategies in environmentally friendly agriculture.

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PRESENTATIONS:

Oral

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Closing gaps in plant chronobiology: from genome to proteome and from molecules to future geographies.

Climate-change is causing a gradual northward shift in the growing regions of many crop plants. Previous research by us and others has found that plants use their circadian clock to sense changes in their geographical environment, such as differences in light intensity and quality to shape their growth and development. As climate change forces agriculture to expand into more Northern latitudes, we need to understand how the clock functions in different environments and how we can tweak it to engineer future-proof crops. My newly established research group is attempting to decipher how plant circadian clock processes external light signals that change with latitude, building upon our recent discovery that the circadian clock is key to how plants respond to changes in twilight length. We are also researching how the circadian clock subsequently controls gene regulation to impact a variety of biological processes. We know from decades of fundamental research that the circadian clock in plants consists of highly interconnected transcriptional-translational feedback loops that control the expression of approximately 40% of all genes. However, little is yet known about how these rhythms in transcription translate to rhythmic protein expression. By developing a new mass-spectrometry technique for quantitative proteomics, we are producing a circadian protein atlas in plants, providing unprecedented global insight into the timing of gene expression by the clock. We are now seeking to use such new proteomics approaches to characterize how plant chronobiology responds to environmental change with the aim of engineering latitudinal adaptability in crop plants.

PRESENTATIONS:

Oral

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Abscisic acid and Aldehyde dehydrogenases influence protein carbonylation in Arabidopsis thaliana

Improving crop tolerance to stress conditions requires a deep understanding of plant responses to environmental stress. Protein carbonylation is a key oxidative modification involved in plant stress responses. To decipher how protein carbonylation impairs plant tolerance to the oxidative stress, we profiled the carbonylated proteome of Arabidopsis thaliana leaves treated with the phytohormone abscisic acid (ABA), which is produced in response to a variety of stresses and controls the expression of stress-responsive genes in plants. The carbonylated proteomes were obtained by affinity capture and subjected to liquid chromatography coupled with tandem mass spectrometry. We identified 180 carbonylated proteins. Of these, 26 proteins became carbonylated upon ABA treatment whereas 163 proteins initially carbonylated in untreated samples were no longer detected in the ABA-treated samples. This indicated a dynamic control of protein carbonylation within the plant cells. To obtain further insight into how protein carbonylation could be controlled in vivo, we compared the carbonylated proteome between the wild-type plant and mutants of stress-responsive aldehyde dehydrogenase enzymes. This comparison revealed a surprising susceptibility of the plant photosystems to carbonylation and the protective role of the aldehyde dehydrogenases in the cytosol and chloroplasts.

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Oral

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Peptidomics in Rusted Wheat. Endogenous rust peptides identified from short open reading frames by de novo sequencing

Puccinia triticina (*Pt*) is an obligate fungal parasite that causes leaf rust on wheat. This disease occurs annually and potentially results in large yield losses. Host-pathogen communication at the protein level has been well-studied in this and similar pathosystems, but the potential roles of peptides (smaller than 10 kDa) has not been examined. Small peptides, transcribed from short open reading frames, have been reported from model fungal species. These peptides are generally not represented in genomic databases because they often do not resemble hypothetical protein ORFs. This research investigates the role(s) of such peptides in the wheat-rust interaction, using top-down LC-MS analyses to detect novel peptides. For the LC-MS approach we evaluated several approaches, including N-terminal enrichment and C_4 RP-HPLC, before settling on SEC-HPLC. Enriched peptides were left intact or digested with trypsin and then analyzed by LC-MS in a high-resolution Orbitrap mass spectrometer. Automated *de novo* sequencing, which does not require any databases, was used to obtain candidate peptide sequences and these were queried against wheat and rust genomic sequences to eliminate fragments resulting from protein turnover or breakdown. To find potential short open reading frames, peptides were mapped back on to the genomic sequence of *Pt*, while accounting for both codon redundancy, all reading frames and potential *de novo* sequencing errors (e.g. AB to BA reversals). We have found several candidate peptides with no significant homology to the *Pt* nor to the *Triticum aestivum* genomes, but which match short open reading frames on the *Pt* genome. The most recent research progress will be presented.

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PRESENTATIONS:

Oral

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Pushing the limits - fast signaling in plants

Plants are masters of perception, reacting to a myriad of biochemical and physical cues in a constant changing environment. Due to their sessile life, plants must rely on local cell-based signal processing to perceive and react sufficiently fast to a multitude of stimuli. The ability to respond quickly is crucial for sustaining growth, defense and metabolism and thereby be able to survive challenges associated with pathogens, resource limitations and mechanical perturbations. Sophisticated protein phosphorylation networks are underlying these fast responses shaping the cellular response. In the past decade plant sciences have moved our understanding of these networks further, however this understanding is on the operation of minutes and hour long timescales. Through phosphoproteomic analysis we have been able to show that plants respond on the seconds time scale to plant hormones, plant peptides and to mechanical perturbation. Our findings provide novel insights in phosphorylation dependent signaling networks and moreover should lead to a paradigm shift of how we study phosphorylation dependent plant signaling networks.

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Phosphoproteomics for unraveling novel targets of SnRK1 kinase in rice

SnRK1 is the plant ortholog of the evolutionarily conserved SNF1/AMPK protein kinase family that plays a crucial role in regulating cellular metabolism and energy balance in the cell. However, the SnRK1 pathway in these processes is not well-understood. This study aims to understand the SnRK1 pathway through phoshoproteomics and transcriptomics approaches. Rice contains three functional paralogs of the kinase subunit of SnRK1 complex: OsSnRK1A, OsSnRK1B, and OsSnRK1C. Of these, OsSnRK1B and OsSnRK1C share high sequence homology and functional redundancy. We subjected ossnrk1b+c seedlings exposed to extended darkness, mimicking starvation, to phoshoproteomics, proteomics, and RNA-seq. With the hypothesis that differential phosphorylation of the direct SnRK1 targets in this mutant will illuminate SnRK1 pathway, we identified over 100 hypo-phosphorylated peptides consisting of the known and the novel targets. Searching these peptides in the proteome data of the ossnrk1b+c mutant showed that both stabilization and degradation of the targets occurred through SnRK1 signaling. In addition to the well-known processes, the network analysis revealed that membrane trafficking including vesicle-mediated transport and cytokinesis are regulated by SnRK1. Phosphoproteomics revealed functional activators of membrane transport pathway as the novel targets of SnRK1. Together with transcriptomic analysis that showed anabolic and stress-related pathways were misregulated in the ossnrk1b+c mutant, our phosphoproteomics data unravels SnRK1targets during metabolic adjustment in response to starvation in rice seedlings.

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PRESENTATIONS:

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Paenibacillus polymyxa alters the metabolic and proteomic profile of Cannabis sativa

Cannabis is a medicinal plant whose metabolites can be used for treating pain, neurological disorders, relaxation of muscles, and many other ailments. Growing healthy cannabis plants for medicinal use is important for high flower yields and their associated therapeutic metabolites. Plant-beneficial microbes can be suitable tools to enhance the cannabinoid and terpene content in cannabis while augmenting overall plant growth and productivity. In the present study, two strains of Paenibacillus polymyxa were tested for their effects on root development, biomass accumulation, photosynthesis rate, chlorophyll content, cannabinoids and terpene accumulation as well as differential expression of proteins. The strains had been selected based on their potential for in vitro biocontrol activity against the Botrytis cinerea (fungal pathogen of cannabis) and their capacity to produce lytic enzymes. Shotgun proteomics of cannabis flower buds revealed modulation of several proteins associated with plant growth and stress tolerance, through multiple signalling pathways. Several proteins involved in major metabolic pathways, such as carbon metabolism, photosynthesis, respiration, and photorespiration, were found to be upregulated. Among them are glucan endo-1,3-beta-D-glucosidase succinate, dehydrogenase, glucose-6-phosphate isomerase, and sucrose synthase. Based on the proteomic profiles of mature cannabis flowers, we conclude that P. polymyxa inoculants alter a multiprotein regulatory mechanism that enhances cannabis growth and development. The results of this study provide insight into the molecular mechanisms underlying the positive effects of the beneficial P. polymyxa strains on cannabis growth at the molecular level.

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Mechanistic analysis of tall fescue (Shedonorus arundinaceus) and its fungal endophyte, Epichloë coenophiala

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PRESENTATIONS:

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Location, location, location: exploration of the subcellular proteome of plants

The development of organelles is an important hallmark of the evolution of eukaryotic cells. Organelles offer unique cellular micro-environments tuned to protein functions and allowing regulation by separating or concentrating proteins. The systematic assessment of the localization of proteins is therefore an important goal in describing the organization of cells and in protein functional analysis. Fractionation and mass spectrometry-based methods allow mapping protein localization at proteome-wide scale. However, the presence of a rigid cell wall and organelles such as chloroplasts and large vacuoles render such approaches challenging in plants, and no exhaustive subcellular proteome maps have yet been reported. We adapted existing methods from the broader spatial proteomics field for use in plants, and optimized existing data analysis strategies, which allowed us to generate high-quality spatial proteomes of Arabidopsis thaliana. We have localized 6230 proteins in whole seedlings, and 8595 in roots. Furthermore, we have localized 4658 proteins in the liverwort Marchantia polymorpha, allowing comparative analysis of proteome organization. Finally, we used this approach to detect 908 proteins whose location changes in response to the protein trafficking drug Brefeldin A. We also experimentally validated predictions on protein localization made by our maps using confocal microscopy. Our data represents the first high-resolution proteome-wide resource of protein subcellular localization in plants, and offers a strategy to study a range of processes at the subcellular level.

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Crosstalk between phosphorylation and O-glycosylation regulates metabolism, growth, and immunity

Plant growth is regulated by hormonal, nutritional, and environmental signals. Cellular responses to these signals are mediated by dynamic posttranslational modifications, including phosphorylation, O-GlcNAcylation (O-linked-N-acetylglucosamine, O-GlcNAc), and O-fucosylation (O-fucose). In Arabidopsis, hundreds of kinases and phosphatases control protein phosphorylation in diverse signaling pathways, whereas two homologous proteins, named SPINDLY (SPY) and SECRET AGENT (SEC), catalyze protein O-fucosylation and O-GlcNAcylation, respectively, of serine and threonine residues. Genetic evidence indicates the essential roles of SPY and SEC in broad developmental and physiological processes, as the single spy and sec mutants display various developmental defects while the spy sec double mutant is embryo lethal. We have identified hundreds of O-fucosylated proteins and O-GlcNAcylated proteins as well as hundreds of substrates of the BIN2 kinase in Arabidopsis using mass spectrometry. The O-glycosylated proteins include mostly nuclear and cytosolic proteins that can also be phosphorylated, revealing potential crosstalk between O-glycosylation and phosphorylation signaling pathways. I will present examples of crosstalk between O-glycosylation and phosphorylation in regulating carbon metabolism and immunity.

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PRESENTATIONS:

Oral

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Regulatory architecture of disease resistance in maize revealed by multi-omic systems genetics

Complex traits such as disease resistance have been traditionally studied using quantitative genetics. Here, we use systems genetics to integrate disease severity and multi-omic quantitate trait loci (QTL) to uncover biological networks underlying interaction with northern leaf blight (NLB), a yield-limiting disease of maize. Specifically, we integrated transcriptome, (phospho)proteome, and metabolome measurements to map molecular QTL and build predictive regulatory networks following NLB infection. These inferred networks identified a critical signaling module that was genetically validated comprised of a kinase termed NLB SUSCEPTIBLE KINASE 1, a bHLH transcription factor, and the lignin biosynthesis enzyme BROWN MIDRIB 2. Our results demonstrate the feasibility of high-throughput mapping of genetic determinants of gene-product levels and demonstrates the power of systems genetics to identify upstream regulatory genes that confer resistance to NLB that can inform future strategies for crop protection.

PRESENTATIONS:

Oral

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A single nucleus atlas of development altering stress

TBD

Contributing Authors
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PRESENTATIONS:

Oral

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Mapping Architecture of Protein complexes in Arabidopsis using XL-MS

Functional annotation of genes remains a challenge in the post-genomic era, even for model species such as Arabidopsis thaliana. Protein-protein interaction (PPI) networks are essential for understanding protein function, and cross-linking mass spectrometry (XL-MS) has become a powerful tool for mapping these interactions. Here, we present an XL-MS platform that combines the PhoX cross-linker and extensive fractionation to achieve proteome-wide interaction mapping in Arabidopsis. Using cell lysates, chloroplasts, and nuclei, we identified a total of 47,119 unique cross-links, including 43,592 intra-protein and 3,527 inter-protein cross-links, allowing us to construct a PPI network with 1,230 proteins and 1,446 PPIs. STRING analysis supported 62.4% of the inter-protein cross-links, while 37.6% revealed novel interactions. This study provides insights into protein structures, complex topologies, and conserved and unique plant interactions, improving functional annotation and advancing our understanding of cellular processes while generating new biological hypotheses.

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PRESENTATIONS:

Oral

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Phospho-proteomics uncovers vast range of substrates for chloroplastic protein phosphatase SLP1

In plants, like all other eukaryotes, reversible protein phosphorylation, controlled by protein kinases and phosphatases, is an important regulator of various aspects of cell biology. Fundamental to plant metabolism is the local chloroplast metabolism, a collection of processes tightly controlled by post translational modifications such as protein phosphorylation. Following our group's bioinformatic discovery of chloroplast-localized protein phosphatase SLP1, our phosphoproteomics study revealed that SLP1 regulates the phosphorylation of over 120 proteins in the chloroplast. Upon alignment of phospho-sites, an acidic phosphorylation motif appears which is indicative of casein kinase (CK) phosphorylation. Interestingly, this implies that SLP1 is the protein phosphatase that opposes the action of major chloroplastic protein kinase cpCK2. This gives SLP1 a supposedly widespread role and places it at the center of chloroplast metabolism, making it an attractive phosphatase to inspect.

Contributing Authors
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PRESENTATIONS:

Poster

Poster 100

Li, Qiaomu
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Using Integrated Proteomics Analysis to Characterize Roles of Splicing Kinase1 (SK1) in Arabidopsis thaliana

Transcriptional regulation (e.g. Alternative Splicing, AS) and post-translational modifications (e.g. phosphorylation) are commonly employed by eukaryotic cells to coordinate growth and stress reprogramming. Alternative splicing (AS), including intron retention and exon skipping, is mediated by the spliceosome, a multi-protein complex composed of splicing factors, such as serine/arginine-rich proteins. Protein phosphorylation is involved in regulating a wide range of biological processes by modulating protein features (e.g. protein-protein interactions). In plants, phosphorylation has been associated with AS regulation, yet the molecular mechanisms remain unclear. Here, we aim to use multi-omics approaches to investigate regulatory roles of SK1 in AS. First, we employed proximity labelling TurboID methods, resolving a total of 106 SK1 candidate interactors. Among these, several AS-related GO terms were over-represented, such as Prp19 complex (GO:0000974) and U2-type pre-spliceosome (GO:0071004). In addition, multiple GO terms involved in transcription were also enriched, including histone deacetylation (GO:0016575) and transcription corepressor activity (GO:0003714), suggesting that SK1 might directly fine-tune transcription, along with AS. Subsequent phosphoproteomic analysis of sk1 mutants found 2526 differentially abundant phosphopeptides, attributed to 1247 unique phosphoproteins. Combined with interactome results, multiple overlapping proteins were uncovered, including arginine/serine-rich splicing factors. We then confirmed their interaction with SK1 using a combination of firefly luciferase complementation and bimolecular fluorescence complementation assays. Moreover, we found that co-expression of SK1 with target splicing factors increased their accumulation compared to their expression alone, suggesting that phosphorylation induced by SK1 impacts protein stability.

Contributing Authors
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PRESENTATIONS:

Poster

Poster 101

McAlister, Jason
University of Guelph

Mycotoxin-driven proteome remodeling of Triticum aestivum defines host responses and pathogen adaptation to emerging threats.

Fusarium head blight (FHB) is a devastating fungal disease impacting the global cereal crop industry. The causative agent, Fusarium graminearum, produces dangerous mycotoxins, which reduce grain quality and yield, as well as impact the health of livestock and humans upon consumption. Deoxynivalenol is the most prominent mycotoxin produced by the fungus with the type B trichothecene, 15-acetyl DON (15ADON) chemotype, prevailing in many regions; however, recent evidence supports the rise of prevalence and detection of new mycotoxins, such as the type A trichothecenes, NX and 3ANX. Within this study, we applied mass spectrometry-based proteomics for dual perspective (host and pathogen) proteome profiling of FHB in wheat under diverse mycotoxin pressured. Our findings supported the assessment of proteome remodeling of Triticum aestivum (wheat) inoculated in the field with isolates of 15ADON+3ANX or 15ADON compared to an uninfected control for identification of 5,386 wheat proteins. We observed differential responses of the host dependent upon mycotoxin class: 15ADON+3ANX and 15ADON, and further precision towards isolate-specific remodeling of protein abundance profiles. Specifically, we observed significant increases in production of proteins associated with fungal defense response and catabolic processes (e.g., arabinan, xylan, polysaccharide) across both mycotoxin classes based on Gene Ontology by Molecular Function, with only the combinatory class (15ADON+3ANX) driving changes in hydrogen peroxide catabolic processes, lipid metabolism, and lipid transport. From the fungal perspective, we detected 1,766 fungal proteins with virulence- and mycotoxin-associated proteins with differential abundance across the isolates, supporting a diverse range of host response to infection. Overall, this study provides an in-depth assessment of protein-level changes by both the plant and pathogen driven by mycotoxin contamination, supporting detection of defense response mechanism specific to emerging mycotoxins.

Poster 101 Continued

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PRESENTATIONS:

Poster

Poster 102

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Advancements in Click Chemistry Techniques for Nascent Proteome Analysis

Click chemistry is a set of novel technologies that can be used for labelling of cellular components in vivo via efficient and relatively simple chemistry, facilitating enrichment of molecules of interest. One application of this chemistry is to incorporate tags into newly synthesized proteins, where the “click” reaction facilitates the enrichment of exclusively the nascent proteome, washing away the older, high-abundance proteins to intensify signals of interest during mass spectrometry. Pioneered in mammalian cell culture models, these techniques are not equally advanced in plants, in part because of difficulty navigating the unique plant responses to the supplied compounds. We have optimized a protocol for nascent proteome enrichment via click chemistry in Arabidopsis that allows for simple and fast tagging of newly synthesized proteins with drug concentrations up to 20-fold lower than previously observed in the literature, using fewer steps, and detecting thousands of unique proteins enriched from small amounts of crude plant matter extract, while simultaneously avoiding previously observed stress phenotypes associated with increased labeling compound concentrations. These improvements grant a more efficient, cheaper, and more accessible workflow for this form of proteomics in plants, allowing for broader and more effective use of these technologies.

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PRESENTATIONS:

Poster

Poster 103

Dufresne, Craig
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Optimizing Plant Phosphoproteomic Data Independent Acquisition: micro-Pillar Array Columns and Traditional Packed Bed Technology

Plant research has come to the forefront of modern science as crop output, environmental impacts, and land resources have been required to be balanced with economics. Arabidopsis thaliana, a model plant system, provides the ideal set of characteristics for study of plant proteomics: short lifecycle, known genome, ease of cultivation, and proven methods of genetic modification. Just as mammals undergo natural cycles of life, plants experience cycles such as seasonal weather changes, day and night photoperiods and changes in temperature or growth media conditions. Many of these changes are mediated by phosphorylation, often challenging due to low phosphoprotein levels which can be enhanced with phosphopeptide enrichment coupled with data independent acquisition using modern chromatography systems such as micro-Pillar Array Columns. Methods Soil grown Arabidopsis thaliana Col-0 plants were grown under a 12h light: 12h dark photoperiod and extracted using an SDS-based SP3 bead method (Leutert et al 2019; Mehta et al 2022), with phosphoproteins enriched using Zi4+IMAC HP (ReSyn) as previously described (Leutert et al 2019), using a King Fisher APEX. Data Independent Acquisition (DIA) on a hybrid Orbitrap-Astral detector was optimized with specific focus on optimal injection times when compared to loaded amounts. Four chromatography systems are evaluated: seventy-five and one-hundred fifty micron ID particle packed bed traditional columns, semiporous nano micro-Pillar Array Colum, and high throughput non porous capillary column. This will allow comparisons between packed bed and pillar arrays with both nano and capillary flow rates.

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PRESENTATIONS:

Poster

Poster 104

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Multi-Omic Analysis Of Seed Dormancy And Reserve Mobilisation Mechanisms In Wheat Varieties With Late Maturity Amylase

Late maturity amylase (LMA), unlike pre-harvest sprouting (PHS), is a genetic defect in specific wheat genotypes caused by cold stress and temperature shocks during post-anthesis, yet the underlying mechanisms remain unknown. We acquired transcriptomics, global proteome, central carbon metabolite (CCM) and micronutrient quantitation at three-time points (dormant, 2h imbibed, and 48h germination) for LMA-resistant, susceptible and control cultivars. The comparison between constituent expresser and LMA-resistant varieties at zero-timepoint showed the 710 upregulated and 1403 downregulated genes (Fold change=2; Adj. p value=0.05), where the upregulated genes were associated with galactose metabolism and increased ion transporter activity, evidencing the initiation of vacuolation and α-amylase activity. Notably, the resistant cultivar retained the functions associated with nutrient reservoir activity, while the constitutive and intermediate expresser cultivars were high in carbohydrate metabolism and positive regulation of nutrient levels during the 48-hour germination period. Pairwise comparisons of the proteomics datasets between the wheat cultivars revealed the enrichment of peroxidase, antioxidant and oxidoreductase activities in the resistant varieties, suggesting their role in blocking starch catabolism and hydrolase activities. The Weighted Gene Network Correlation Analysis on the proteomics dataset reveals that the LMA-resistant cultivars have a significantly higher abundance of endopeptidase inhibitors, peroxidases and nutrient reservoir proteins, explaining the role of protease inhibitors and the peroxidases in maintaining dormancy. LMA-susceptible varieties have enriched CCM pathways like starch metabolism and Ca2+ signalling, while resistant varieties have high protein synthesis. Our multi-omics analysis improves our understanding of starch breakdown, hydrolase activities, and nutrient mobilisation beyond alpha-amylase in LMA-susceptible cultivars.

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PRESENTATIONS:

Poster

Poster 105

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Trees to Targets: Uncovering the depths of forest resilience against Mountain Pine Beetle using quantitative proteomics

Mountain Pine Beetle (MPB; Dendroctonus ponderosae) is an invasive pest species affecting Lodgepole Pine (LP; Pinus contorta) in Canada. Along with its fungal associate Grosmannia clavigera, a necrotrophic pathogen, MPB has devastated LP forests in Western Canada. Amongst the devastated tree stands, survivor trees leave the potential to study and understand the resilient traits. Using samples collected from F1 Progeny of MPB-killed and healthy LP trees, a custom SNP-probe & RNA-seq analyses found candidate gene targets for further characterization. To aid in rapidly screening these candidate target genes, corresponding Arabidopsis thaliana orthologs were identified and cloned, and gene-deficient plant lines were acquired. In parallel, efforts have been made to clone putative MPB resilient gene targets from LP for complementation assays involving the model plant Arabidopsis thaliana and the necrotrophic pathogen Botrytis cinerea. With these targets involved in various plant cell processes, we undertook phosphoproteome and proteome analyses to better understand the potential molecular mechanisms underpinning MPB resilience to enable future breeding efforts.

Contributing Authors
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PRESENTATIONS:

Poster

Poster 106

Lin, Kuan-Hung
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The landscape of protein phosphorylation dynamics under heat stress in Marchantia Polymorpha

Climate change-induced heat stress poses a significant threat to economically important plants, impeding growth and crop productivity. To address this challenge, understanding the molecular mechanisms underlying plant responses to high temperatures is essential. The bryophyte Marchantia polymorpha has emerged as a valuable model organism for studying heat stress (HS) responses due to its minimal genetic redundancy, easy cultivation, and representation of terrestrial adaptation. This study proposes to investigate HS signaling networks and heat shock transcription factor A (HSFA) in Marchantia, focusing on the time- and temperature dependent responses to HS. In this pioneer study, we have generated a quantitative atlas of proteomics and phosphoproteomics responses in wild-type (Tak-1) and HSFA-knockout (hsfa1) Marchantia exposed to various temperature and time course treatments. Our preliminary results identified over 20,000 phosphosites. Notably, we reported about 20% of regulated phosphosites, alongside less than 1% of regulated protein abundance within the first hour of heat exposure, suggesting that the post-translational modifications played a major role in the early response to HS. Further functional analysis revealed the regulated phosphoproteins are primary associated with kinase activity, chromatin remodeling, and cell wall biogenesis. Additionally, we applied Weighted Correlation Network Analysis (WGCNA) for phosphosites clustering and identified 16 distinct modules. These modules demonstrated the different expressed patterns across the various treatments. However, the phosphoproteomics landscape showed no significant difference between Tak-1 and hsfa1 plants, indicating that HSFA1 might play a major role in in heat memory.

Contributing Authors
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PRESENTATIONS:

Poster

Poster 107

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Resurrection plants—a valuable source of natural bioactive compounds involved in inhibition of breast cancer cellular processes

Resurrection plant species are a rare group of higher plants whose vegetative tissues are able to withstand long periods of almost full desiccation and recover quickly upon rewatering. Apart from being a model system for studying desiccation tolerance, resurrection plant species appear to be a valuable source of bioactive compounds with various areas of application. On the other hand, breast cancer cases account for 12% of all cancer cases and affect women worldwide, although about 1% of breast cancer patients are men. Long-term use of drugs in anticancer therapy can lead to the emergence of resistance to them in cancer cells (multidrug resistance, MDR). The MDR of cancer cells results from molecular mechanisms related to the inability of administered drugs to penetrate into the cells and trigger a mechanism of cancer cell elimination. The probability of the emergence of multidrug resistance in cancer patients, together with the strong impact of the side effects of chemotherapy drugs on the treated organism, makes the search for new anticancer therapeutics so necessary and significant. Possible substances that could be expected to have little or no side effects are certain substances of plant origin. Intensive studies of plant secondary metabolites and bioactive peptides are being carried out on cancer cell lines, a model system that enables the testing of plant derivatives on cell lines with characteristics of different types of breast cancer. The subject of our study is to evaluate the potential of application of bioactive compounds from resurrection plant H. rhodopensis for breast cancer treatment. We applied preparative fractionation of plant extracts and evaluate effects of fractions on survival and proliferation rates of two breast cancer cell lines and control normal cell line. Active compounds were identified by NMR and LC-MS and their mechanisms of action were determined by shotgun based quantitative proteomics and phospho-proteomics analyses of treated breast cancer cells.

Contributing Authors
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PRESENTATIONS:

Poster

Poster 108

Hau, Bryan
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Identification and characterisation of the molecular drivers of malting performance in Hordeum vulgare

The end goal of barley malting is the degradation of storage proteins and carbohydrates, which provides yeast its essential nutrients and sugars for beer brewing. The process relies on synthesis of various enzymes during the germination process in different parts of the grain. Although malting has been around since Ancient Egypt, there are still improvements to the process and the molecular regulation of what occurs that still contains key unknown processes. Investigations into the barley proteome throughout the malting process has yet to be thoroughly investigated thoroughly. Here, protein-level differences between malting and feed barley cultivars and identify molecular drivers for the malting process. A comparison of barley grains for 17 barley cultivars showed differentially abundant proteins in significant functional groups. To assess the functionality of these, protease activity was measure via Activity-Based Protein Profiling, which identified a major storage protein degrading enzyme. Tissue-specific proteomics was performed to determine the localisation of proteins, which suggested that proteins are differentially synthesised/transported between tissues inside the grain. To follow up, protein turnover experiments determining the ageof proteins, as well as which proteins are most rapidly synthesised can be performed. Additionally, these turnover experiments will investigate changes in the degradation rate for proteins in the starchy endosperm during germination and show protein activity dictated by polypeptide age and post-translation modifications. These proteomic investigations into the major differences between malting and non-malting cultivars aims to advance barley research, breeding, and the malting process leading to improved process sustainability.

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PRESENTATIONS:

Poster

Poster 109

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Opening Moves and Endgames: Proteomic Insights into Host-Pathogen Interaction During Fusarium Head Blight Infection

Fusarium graminearum, the main cause of Fusarium head blight (FHB), threatens global wheat yields while producing mycotoxins. Climate change accelerates FHB spread, highlighting the need for innovative control strategies. Our research employed mass spectrometry-based proteomics to compare the host proteome remodelling of FHB-resistant (Sumai#3) and -susceptible (Norwell) spring wheat cultivars at 24- and 120-hours post-infection (hpi) and profiled the pathogen proteome of a highly aggressive F. graminearum strain. Our analysis revealed a high abundance of conserved pathogenesis-related proteins (e.g. chitinase) at 24 hpi. This finding was supported by an endochitinase activity assay, which confirmed significantly higher activity in Sumai#3 than in Norwell. Simultaneously, fungal proteome revealed putative effector LysM, which sequesters chitin oligosaccharides to evade host recognition. Temporal dynamics revealed distinct cultivar-specific responses: Norwell reduced the abundance of photosynthesis-related proteins by 120 hpi, whereas Sumai#3 decreased it by 24 hpi. We further tested the grain integrity at 120 hpi using an anti-gluten dot-blot, which indicated that infected Sumai#3 contained significantly more gluten than Norwell, suggesting the grain development was heavily impaired in the susceptible cultivar. Based on our findings, we propose a new model: Sumai#3 responded to the infection with early and effective defence responses, thus improving grain integrity compared to Norwell. For example, when fungal effectors suppressed chitin detection, Sumai#3 produced more chitinases as a defensive mechanism, thereby reducing the disease severity. These results indicate resource allocation between growth and defense against F. graminearum, crucial for breeding FHB-resistant wheat in a changing climate and supporting sustainable food security.

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PRESENTATIONS:

Poster

Poster 110

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A Proteomics-Based Screen to Determine Potential Regulators of Thermosensitive Plant Immunity

Rising global temperatures negatively impact plant immune systems, for example, elevated temperatures suppress the biosynthesis and signalling of the plant defence hormone salicylic acid (SA). Heat-mediated suppression of SA biosynthesis is due to the downregulation of CBP60g, which encode master transcription factors of plant immunity. When constitutively expressed, CBP60g expression can restore SA levels at elevated temperatures, showing its importance in plant immune resilience under warmer conditions. However, we currently do not know the detailed mechanisms of how CBP60g can restore immunity at elevated temperature. Here, we shed light on CBP60g-mediated immune resilience by determining changes in the Arabidopsis thaliana plant proteome after pathogen infection at different temperatures using mass spectrometry (MS/MS). In both thermosensitive Col-0 and thermoresilient CBP60g-overexpressing plants, we observed a core group of temperature-sensitive proteins in terms of abundance, which could suggest novel thermosensing mechanisms upstream of CBP60g gene expression. One of these proteins was PHOSPHOETHANOLAMINE METHYLTRANSFERASE3 (NMT3), which is known to encode a S-adenosyl-L-methionine-dependent phosphoethanolamine N-methyltransferase. Remarkably, nmt3 mutants exhibited temperature-insensitive disease susceptibility after infection with the model plant pathogen Pseudomonas syringae pv. tomato (Pst) DC3000, suggesting that it may be functioning as a potential regulator of thermosensitive plant immunity. Further functional analyses of NMT3 will reveal how it plays a role in plant immune responses under changing temperatures. Overall, this work has unveiled the global proteome landscape of Arabidopsis immune responses under changing temperatures. The identification of NMT3 and other thermosensitive proteins can inform us of potential targets for genetic engineering of climate-resilient, disease-resistant plants.

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PRESENTATIONS:

Poster

Poster 111

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Assessment Of Fusarium Mycotoxin Detoxification By Prioritized Proteins In Canadian Wheat Varieties

During fungal infections of cereal crops, such as Fusarium Head Blight (FHB) caused by Fusarium graminearum, the fungus produces harmful mycotoxins, which decrease crop quality and yield and contaminate wheat grains, causing detrimental effects on livestock and humans. In response, cereal crops express diverse proteins that facilitate mycotoxin detoxification and associated plant defense, including cultivar-specific strategies. Previous proteome remodeling of over 100 proteins within FHB-resistant (Sumai#3) and -susceptible (Norwell) wheat varieties inoculated with high and low 15-Acetyldeoxynivaol (A-DON) concentrations over a temporal scale suggested that wheat activates diverse time and resistance-dependent defense mechanisms against DON. Our current study hypothesizes that these wheat proteins with increased production in the presence of DON have a role in detoxification or defense response. To assess DON detoxification capacity in vitro, we prioritized 38 proteins based on their putative and confirmed DON detoxification roles. We selected five candidates (three glutathione-associated, two oxidases) and designed yeast strains for in-vitro protein expression. We assessed the growth of yeast expressing the candidate proteins and positive (UDP-glycosyltransferase; TaUGT6) and negative (empty vector) control via a DON resistance assay. The candidates and the positive control showed better growth in the presence of DON compared to the negative control at 24- and 48 hours post-incubation by optical density measurements and colony-forming unit counts. Next, we will characterize these candidates and their DON detoxification metabolites by mass spectrometry and assess their toxicity on animal cells. Overall, the study aims to identify and characterize novel DON detoxification-associated wheat proteins to selectively breed varieties with improved mycotoxin tolerance.

Contributing Authors
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PRESENTATIONS:

Poster

Poster 112

Molasiotis, Athanasios
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Dissecting Chilling Injury Mechanisms in Peach Fruit Using Multi-Omics Strategies

Cold storage is widely used to preserve peach fruit quality and delay deterioration. However, prolonged exposure to low temperatures can lead to chilling injury (CI), a physiological disorder manifesting as undesirable softening, mealiness, flavor loss, and internal reddening. Although the genetic diversity of peaches is somewhat limited, cultivars exhibit varying degrees of CI tolerance, suggesting a complex genetic and molecular basis for cold stress responses. To elucidate the molecular mechanisms underlying CI, we employed a multi-omics approach, integrating transcriptomics, epigenomics, proteomics, and metabolomics, to analyze mesocarp tissues of a CI-sensitive peach cultivar at different pre-conditioning stages. This comprehensive analysis identified key genes, proteins, and metabolites associated with cold stress responses and CI symptom development. Among these, we focused on specific cold-responsive transcription factors (TFs) and assessed their expression at both pro-symptomatic and symptomatic stages in 30 peach cultivars with distinct CI phenotypes. To further investigate the genetic basis of CI tolerance, we screened 118 cultivars from the Peach Reference Collection (PeachRefPop) in Naoussa, Greece, to identify potential CI-related donor germplasm. Whole-genome resequencing of these cultivars enabled the identification of small genetic variants (SNPs and InDels), facilitating genome-to-phenome association studies. This analysis revealed putative genetic markers linked to CI susceptibility, providing valuable targets for marker-assisted breeding. Overall, our findings contribute to a deeper understanding of the genetic and molecular determinants of CI in peach, offering new avenues for breeding strategies aimed at improving postharvest fruit quality in this important perennial crop.

Contributing Authors
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PRESENTATIONS:

Poster

Poster 113

Mouliom Ntapnze, Awa Marina
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Finding the right balance : influence of a far-red supplement gradient on biological processes related to growth and stress in bell pepper plants (Capsicum annuum L.).

Far-red supplementation to artificial lighting has emerged as a prevalent strategy for enhancing plant growth and yield production in controlled environments. However, far-red supplementation often results in increased plant susceptibility to pathogens and stress. A comprehensive understanding of the optimal threshold for far-red supplements would facilitate the use of far-red supplements to lighting by the greenhouse producers and the augmentation of production yields without compromising their resilience to stress. In this study, it was examined how far-red supplement gradient influences biological processes related to growth and stress. For this sweet bell pepper plants were grown under white light supplemented with a far-red intensity gradient. A quantitative analysis of the leaf proteome was combined with a survey of agronomic parameters and specific metabolites to decipher the effects of the light quality on the plants. We observed that far-red supplementation accelerated plant height, photosynthesis, sugar levels and fruit yield. Our proteome analyses revealed that either proteins involved in growth-promoting biological processes (sugar and protein metabolism, root growth), or proteins involved in stress, inhibition of sugar metabolism, and abscisic acid signalling will be activated depending on the intensity of far-red supplement used. These results indicate that there is an optimal threshold of far-red supplementation to artificial lighting of bell pepper plants that must be used to avoid plant stress. It remains to be clarified whether this threshold varies from one variety to another or from one species to another.

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PRESENTATIONS:

Poster

Poster 114

Uhrig, Richard
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Shining light into the daily growing cycles of Arabidopsis with proteomics

To date, our understanding of diel plant chronobiology has been driven by a combination of genetic and transcriptomic technologies, which have together powered a revolution in our understanding of plant and crop systems. However, this view has overlooked more complex protein-level events that directly impact plant physiology and agronomic traits of interest (e.g. climate resiliency). Arabidopsis thaliana (Arabidopsis) represents the most widely used model plant system to study the molecular responses governed by diel changes (day / night changes) in sunlight and temperature. In this work we show protein and phosphorylation changes across the Arabidopsis day/night cycle by quantifying changes in the proteome and phosphoproteome every 2 hours, providing the most in-depth diel proteome dataset available to date.

Contributing Authors
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PRESENTATIONS:

Poster

Poster 115

Battisiti, Ilaria
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Proteomic alterations in response to flooding in Solanum commersonii plants with different ploidy levels

Polyploidy is a well-established phenomenon commonly occurring in plants, resulting in changes at the genomic, epigenetic, transcriptional, and metabolic levels. Polyploid species are generally more vigorous and can better tolerate both biotic and abiotic stresses. While there is information regarding the beneficial effects of ploidy, the biological processes involved in adaptability to stress conditions, especially at the proteomic level, are not well understood to date. To elucidate the molecular mechanisms underlying the metabolic response to flooding stress, we performed a label-free quantitative proteomic analysis on leaves of diploid (2n=2x=24) and oryzalin-induced tetraploid (2n=4x=48) plants of a wild potato species, Solanum commersonii. Unstressed plants were used as a control. A total of >3600 proteins were reliably quantified. 785 and 507 differentially abundant proteins (DAPs) were identified in response to flooding stress in diploid and tetraploid samples, respectively, of which 381 were in common. Functional categorization of DAPs in diploid plants indicated that the most represented biological processes were related to energy metabolism and nucleotide processing. Concerning the tetraploid plants, the most affected processes were related to energy, amino acid, and chlorophyll metabolism. The comparison between stressed diploid and tetraploid plants outlined that most of the DAPs were involved in protein synthesis, folding, and maturation. Altogether, these results indicate potentially different adaptation mechanisms of diploid and tetraploid plants against flooding and contribute to elucidating the pathways involved in the stress tolerance in S. commersonii.

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Poster 115 continued

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Proteomic alterations in response to flooding in Solanum commersonii plants with different ploidy levels

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PRESENTATIONS:

Poster

Poster 116

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Integrative Proteomics and Metabolomics Reveal Key Pathways and Compounds for Enhanced Tolerance Against Ascochyta Blight in Pea Cultivars

Ascochyta blight is a growing disease in agriculture. Didymella pinodes (syn. Mycosphaerella pinodes) is the major fungal pathogen threatening pea cultivation. To date, no substantial plant resistance mechanism has been found to better tolerate or even prevent the disease. Systemic acquired resistance (SAR) is a signaling pathway involved in the response to the pathogen. Salicylic acid triggers the production of phytoalexins such as Pisatin. This isoflavone is known to be produced by pea plants during infection. However, through methylation, the pathogen rendering it inactive. Previous studies have indicated that more tolerant pea cultivars produce higher levels of secondary metabolites other than pisatin. In order to fully understand higher tolerance, further investigation are necessary in order to explain when, how, where and what compounds are formed upon the course of infection. Here, we integrated proteomics and metabolimics to tackle the major pathways and compounds, crucial for higher tolerance against D. pinodes in different Pisum sativum cultivars. Using a time course approach, we were comparing old (infected) and young leaves (formed after infection), that enabled us to better understand SAR and identify metabolites crucial for enhanced tolerance against the pathogen. Our findings reveal key pathways and compounds associated with higher tolerance against D. pinodes, providing valuable insights for developing resistant pea cultivars.

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PRESENTATIONS:

Poster

Poster 117

Lu, Jiaxi
University of Guelph

Investigating Proteomic Modulations in Canadian Triticum aestivum Cultivar Under Fusarium-Derived Deoxynivalenol Contamination

Fusarium graminearum is the leading causative fungal pathogen of Fusarium head blight (FHB), which leads to significant economic losses in wheat production. F. graminearum produces mycotoxin, such as deoxynivalenol (DON), which contaminates cereal-based and livestock food. When humans consume DON-contaminated food products, such as pasta or bread, it causes vomiting, nausea, or gastrointestinal inflammation due to cytotoxicity. To improve current FHB management methods and reduce the accumulation of harmful mycotoxins, it is important to study mechanisms of DON detoxification to mitigate its detrimental effects on plants, animals, and humans. In this study, we performed mass spectrometry-based proteomics profiling of the Canadian hard red spring wheat cultivar, AAC Tenacious, which demonstrates resistance towards F. graminearum infection in the field. To simulate FHB infection in AAC Tenacious at varying levels of DON accumulation, we point inoculated spikelets at heading with the mycotoxin. Inoculation groups included mock (Tween-20), 0.1 mg/mL DON, and 1.0 mg/mL DON with sample collection at 24 hours post-inoculation (hpi) and 120 hpi. This experiment allows for evaluation of host plants respond to different concentrations of DON, mimicking the natural gradient of toxin accumulation during F. graminearum infections in agricultural settings. The findings reveal anticipated activation of defense response pathways in DON detoxification, cell wall thickening, and response to oxidative stress to limit the fungal damage and inhibit DON accumulation in plants. The aim of this research is to uncover connections between FHB resistance and DON tolerance in AAC Tenacious and to identify mechanisms of resistance to DON to enhance crop yields, grain quality, and food safety.

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PRESENTATIONS:

Poster

Poster 118

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Proteomic Insights into Host Defense Mechanisms of AAC Tenacious Against Fusarium graminearum Infection

Fusarium head blight (FHB) is a severe fungal disease affecting grain quality and yield in wheat production, which leads to severe economic losses in Canada. The fungal pathogen, Fusarium graminearum, is the primary causative agent of FHB symptoms in wheat. F. graminearum produces mycotoxins, such as deoxynivalenol (DON), the most devastating secondary metabolite that directly influences human and livestock health. AAC Tenacious is an FHB-resistance red spring wheat cultivar developed in Canada. Defense mechanisms against FHB in AAC Tenacious remain to be clearly elucidated. In this study mass spectrometry-based proteomics was used to profile host defense responses to F. graminearum infection in AAC Tenacious. We examined two infection time points, 24- and 120-hours post-inoculation, highlighting the dynamic responses based on a time course of disease progress. Moreover, we compared F. graminearum- and mock-inoculated samples to distinguish growth regulators of defense from bona-fide disease-specific responses. Our study reveals that AAC Tenacious dynamically shifts its metabolic processes from growth-related pathways to defense responses during infection. On a temporal scale, during early infection, energy production was prioritized to trigger an immune response, whereas when infection progresses, wheat defense responses focused on mycotoxin detoxification, stress management, and limiting pathogen spread. This metabolic shift underscores the ability of AAC Tenacious to balance survival and growth, strongly emphasizing resisting fungal proliferation and reducing toxin damage. Key words: Fusarium Head Blight (FHB), Fusarium graminearum, AAC Tenacious, Defense pathways, Mass spectrometry-based proteomics

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PRESENTATIONS:

Poster

Poster 119

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Quantitative proteomics of plant receptor kinase signaling

Cellular responses to external stimuli are governed at the molecular level by signal transduction networks. In this context, endogenous and exogenous signals are perceived by receptors that activate signaling to regulate the activity of downstream effectors to reprogram cellular states. In plants, plasma membrane-localized receptor kinases (RKs) serve as the primary receivers of extracellular molecular signals and activate signal transduction through cytosolic protein kinase domains. While considerable effort has been made to understand how RK-mediated signaling is regulated, a quantitative understanding of RK complex composition and regulation in living plant cells is lacking. To address this knowledge gap, we developed quantitative proteomics workflows to characterize RK complex composition and dynamics. Analysis of several immune-related RKs by affinity-enrichment mass spectrometry reveals novel facets of RK signaling: 1) Ligand perception results in recruitment of co-receptors but has little effect on RK complex composition; 2) Activated RK complexes are likely present at relatively low stoichiometries; and 3) RKs may associate in functionally related receptor clusters. In addition to the analysis of RK complexes, we have established a simple, quantitative phosphoproteomics workflow allowing for the identification and quantification of more than 30,000 and 27,000 phosphorylation sites, respectively, in a single experiment, which opens avenues for the identification of receptors for orphan ligands and novel signaling components. Collectively, our results provide insights into RK signaling in an in vivo context and provide a framework to address critical questions in RK biology, including a path forward to understand mechanisms governing signaling specificity in RK-activated pathways.d

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PRESENTATIONS:

Poster

Poster 120

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FERONIA/LLG1 as a membrane microdomain organizer impacting multiple signaling pathways

Sterol- and sphingolipid-rich microdomains in the plasma membrane play a vital role in signaling, yet the mechanisms coordinating these signals remain poorly understood. Our research identifies the FERONIA (FER) receptor kinase and the LRE-LIKE GPI-ANCHORED PROTEIN 1 (LLG1) coreceptor as key organizers of these specialized membrane microdomains. We have determined that loss of FER-LLG1 microdomain constituents are severely impacted. To further investigate the proteomic composition of the FER-LLG1-anchored microdomains, we employed a mass spectrometry-based proteomics approach, comparing the proteomes of wild-type (WT) Arabidopsis thaliana seedling microdomains with those from fer and llg1 mutants. Our proteomic data revealed a significant reduction in many membrane microdomain-associated proteins, including many key regulatory proteins, enzymes critical for sterol and sphingolipid biosynthesis and pectin remodeling in fer and llg1 mutants compared to WT. The findings presented here support that FER/LLG1 is a microdomain organizer crucial for maintaining microdomain integrity and regulating the abundance of numerous regulatory molecules in these specialized membrane subdomains.

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PRESENTATIONS:

Poster

Poster 121

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The Proteomes that Feed the World: Decoding Plant Proteins and Peptides

Introduction Despite extensive research on crop genomes, plant proteomes remain underexplored. This study aims to comprehensively characterize and quantify the proteomes of the 100 most essential crops for human nutrition. Beyond larger proteins, plants produce numerous native peptides that are critical in stress response, pathogen defense, and molecular signaling. However, peptidomics is often overlooked due to challenges in sample preparation and data analysis. To address this, the peptidomic response to biotic stress in Arabidopsis thaliana was investigated. Methods Plant proteomes were mapped across various tissues using an optimized workflow, including TCA/acetone precipitation, SDS solubilization, phenol extraction, SP3 cleanup, high-pH peptide fractionation, and microflow LC-MS/MS. Peptidome analyses were conducted on apoplast and leaf samples using 10 kDa filters followed by reduction, alkylation, and nanoflow LC-MS/MS. Data were analyzed using an unspecific search in FragPipe with MSBooster for enhanced sensitivity. Results On average, the plant proteomics workflow enabled the quantification of up to 20,000 proteins per crop, with around 10,000 proteins per tissue type. Peptidome analyses showed clear peptide modulations upon bacterial infection, particularly in the apoplast, with enriched peptides from apoplast-associated and extracellular matrix proteins. Outlook All data will become publicly accessible via ProteomicsDB, providing valuable insights into tissue-specific protein expressions and the plants native peptide response to infection, potentially aiding the discovery of novel antimicrobial peptides.

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PRESENTATIONS:

Poster

Poster 122

Bourassa, Francis
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MapRT: combining real-time mass spectrometry analysis and proteogenomics for a deeper protein identification

Bottom-up proteomics by data-dependent acquisition mass spectrometry (MS) is among the prime approaches for high-throughput protein identification. However, technical limitations still hinder the identification of all proteins in complex biological samples. First, since only the most abundant precursors are fragmented by the instrument, the MS solely identifies the most abundant peptides. Second, all proteins absent from selected databases will not be visible. By enriching protein databases with genomic and transcriptomic information, proteogenomic approaches allow the detection of novel proteins and novel proteoforms. Still, because unique peptides tend to be of lower abundance compared to shared ones (originating from more than one protein), we often lack the information to unequivocally identify such novel proteins. We propose that a real-time control of MS data acquisition using MealTime-MS can favor detection of less abundant peptides and would greatly benefit proteogenomic approaches. However, previous simulations with MealTime-MS have shown that its speed and its ability to assess protein identification confidence in real-time is greatly affected by the redundancy and the size of a proteogenomic database. Thus, we are currently developing MapRT, a real-time MS algorithm specifically designed for proteogenomics, by optimizing MealTime-MS architecture and dependancies, and by integrating new features in the algorithm's classifier. The latest simulations showed an important speed increase (4-fold faster peptide identification rate) and increased performance (0.74 vs 0.78 ROC-AUC). Ultimately, MapRT will greatly benefit plant research by allowing the large-scale study of plants at protein-level independently of the quality of their annotation and collect information at an isoform resolution.

PRESENTATIONS:

Poster

Poster 123

Ankita Sehrawat
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Exploring the role of Nitric Oxide (NO) priming in stress tolerance and analyzing biotechnological use of NO in increasing secondary metabolites production

Nitric oxide (NO) regulates various processes of plant growth and development, including seed dormancy, root growth, flowering, fruit ripening and senescence. Priming is an immunity-like response in stress conditions by prior stimulation. Prominent function of “NO priming” in providing resistance from biotic stress is known. However, little information is available regarding the role of “NO priming” in abiotic stress and in mitigating stress. NO performs its signaling function by protein post-translational modification (PTM), S-nitrosylation. S-nitrosylation is a reversible modification which involves the formation of a covalent bond between NO and sulfhydryl groups of reactive cysteine residues. Collectively, the accumulating data suggest that NO via S-nitrosylation play a vital role in providing stress tolerance. Moreover, NO treatment enhances ginseng production, tannins, saponins, phenols, flavonoids, taxol, and catharanthine, indicating the role of NO in inducing secondary metabolites production.

PRESENTATIONS:

Poster

Poster 124

Maywald, Niels Julian
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Next-Generation Intercropping: Synergistic Nitrogen Fixation for Sustainable Agriculture

The overuse of synthetic nitrogen fertilizers in agriculture has led to severe environmental consequences, including soil degradation, water contamination, and greenhouse gas emissions. To address these challenges, this research project focuses on genetically modifying common bean plants (Phaseolus vulgaris) to enhance the release of biologically fixed nitrogen from their root nodules. By making this nitrogen available to neighboring non-nitrogen-fixing crops, this approach aims to reduce reliance on synthetic fertilizers and promote more sustainable agricultural practices. To achieve this goal, we employ advanced molecular techniques, including proteomics, to identify key genes involved in nitrogen release from root nodules. Once these genes are identified, targeted genetic modifications will be introduced to optimize nitrogen transfer. The effects of these modifications will then be systematically evaluated under controlled conditions, assessing their impact on plant growth and nitrogen uptake by associated crops. This research holds significant potential for improving agricultural sustainability. By enhancing the natural nitrogen cycle, it could reduce fertilizer dependency, lower environmental pollution, and support ecosystem health. Additionally, increased nitrogen availability may improve soil fertility and boost crop yields while mitigating the ecological damage associated with conventional fertilization methods. Ultimately, our findings will provide valuable insights into plant-microbe interactions and the genetic regulation of nitrogen release, paving the way for innovative strategies in environmentally friendly agriculture.

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PRESENTATIONS:

Poster

Poster 125

Reyes, Andres
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Next-Generation Mapping of the ACINUS-Mediated Alternative Splicing Machinery and Its Regulation by O-glycosylation in Arabidopsis

Alternative splicing (AS) is a key mechanism of gene regulation, but the full repertoire of proteins involved and the regulatory mechanisms governing this process remain poorly understood. Using TurboID-based proximity labeling coupled with mass spectrometry (PL-MS), we comprehensively mapped the Arabidopsis AS machinery, focusing on the evolutionarily conserved splicing factor ACINUS, its paralog PININ, and the stable interactor SR45. We identified 298 high-confidence components, including both established and novel interactors, providing strong evidence that alternative splicing is coupled to transcription and that multiple RNA processing steps occur simultaneously in plants. Bioinformatic analysis reveals high redundancy, conserved mechanisms, and unique plant-specific features. Selected known and novel interactors were validated by AS readouts and phenotypic analysis, which also revealed a coordinated influence on splicing. Furthermore, a systematic evaluation of O-glycosylation double mutants revealed that SECRET AGENT (O-GlcNAc transferase) and SPINDLY (O-fucose transferase) modulate AS through both ACINUS-dependent and -independent pathways. Our results reveal the conserved as well as plant-specific AS regulatory network and highlight the global role of sugar modification in RNA processing. To further investigate the impact of alternative splicing (AS) on the proteome, we conducted large-scale quantitative proteomics and data mining. Our results demonstrate that AS enhances proteome diversity, while retained introns primarily lead to nonsense-mediated decay, reducing protein abundance.

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6th InPPO Conference Maps

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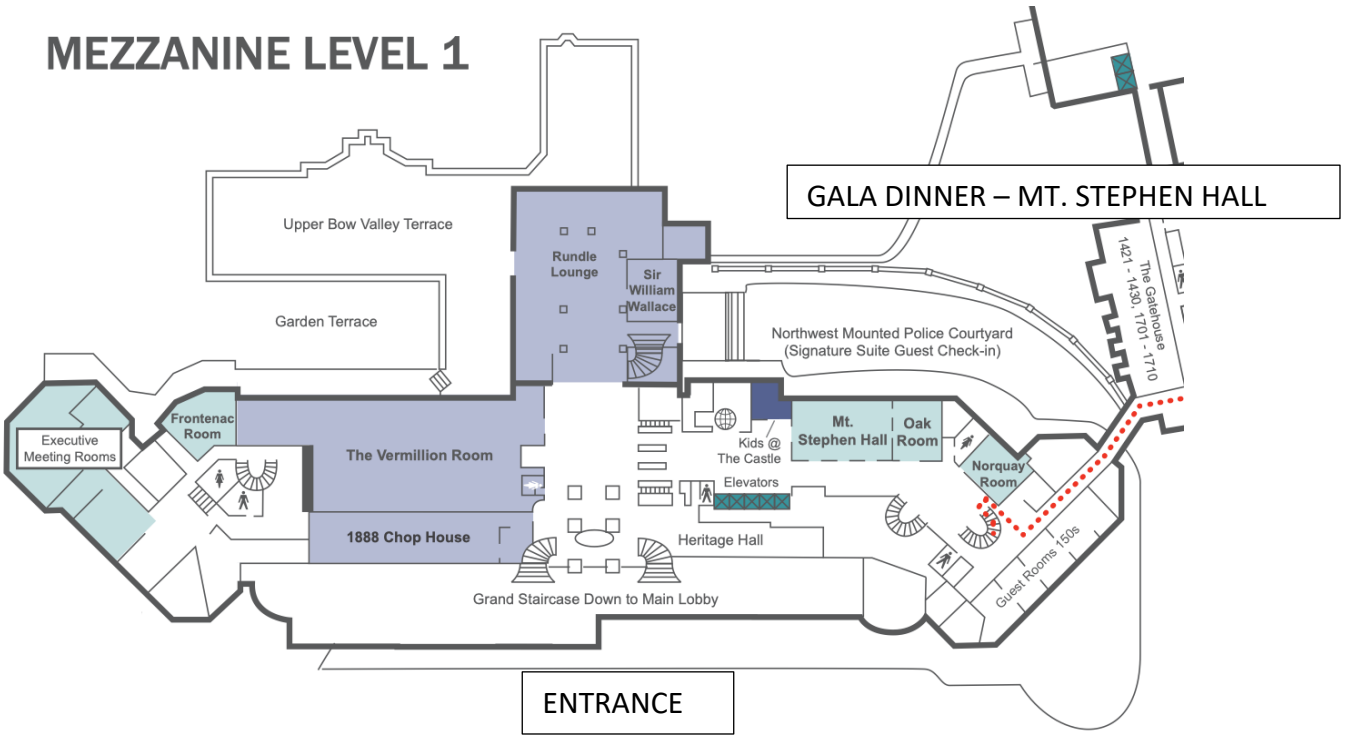

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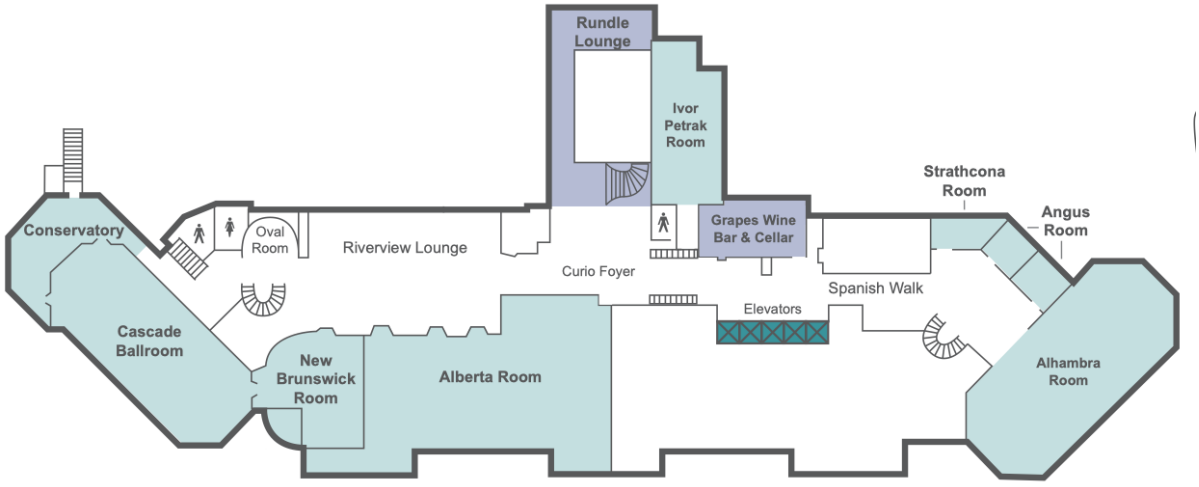

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