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EXHIBITORS

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CONVIRON

The Scientific Program

Sunday, September 4, 2016

15:00 – 20:00 Registration
17:30 Opening (Ganesh Kumar Agrawal, INPPO President)

Public Evening Lecture

18:00 – 18:30 Dominique Job: Plant proteomics, with a special focus on seeds
20:00 – 22:00 Welcome Reception

Monday, September 5, 2016

8:00 – 9:20 Registration, Poster Mounting

Session I: Systems Biology

Chair: Frank Menke

9:20 – 10:00 Wolfram Weckwerth: Green Systems Biology – the need for ecological thinking
10:00 – 10:20 Natalia Bykova: Integration of quantitative proteomics, redox metabolomics and proteogenomics to identify sources of seed dormancy control and pre-harvest sprouting resistance in wheat
10:20 – 10:40 Antoine Champagne: A comprehensive proteome map of glandular trichomes of hop (*Humulus lupulus* L.) female cones: One shot for the entire biosynthetic pathways of terpenoid-related compounds and possible transport proteins
10:40 – 11:00 Julia Svozil: Identification of proteins binding to sense and antisense RNA pairs

11:00 – 11:20 Coffee break

Session II: Posttranslational Modifications

Chair: Jay Thelen

11:20 – 11:40 Ella Nukarinen: The central role of SnRK1 in low energy syndrome
11:40 – 12:00 Ing-Feng Chang: Proteomic and phosphoproteomic profiling of leaves from two natural variants of *Imperata cylindrica*

12:00 – 12:40 Round table I

12:40 – 14:00 Lunch break

Special Session: Joint session of COST and INPPO

Chairs: Vassilis Fotopoulos and Martin Hajduch

14:00 – 14:40 Jay Thelen: Phosphoproteomic resources and technologies – from discovering phosphorylation sites to identifying kinase clients

14:40 – 15:20 Frank Menke: Targeting post translational modifications in plant immunity signaling

15:20 – 15:40 Michel Zivy: Contribution of quantitative proteomics to the analysis of maize responses to drought stress

15:40 – 16:00 Leonor Guerra-Guimaraes: Two resistance inducers relevant in coffee plant protection show distinct metabolic adjustments

16:00 – 16:20 Christine Finnie: Exploring the plant-microbe interface by profiling the surface-associated proteins of barley grains

16:20 – 16:40 Coffee break

16:40 – 17:00 Klara Kosova: Proteomic analysis of wheat and barley response to abiotic and biotic stress factors using gel-based proteomic approaches

17:00 – 17:20 Stephanie Wienkoop: Molecular plasticity during drought recovery is characterized by protein turnover dynamics and translational regulation in *Medicago truncatula*

18:30 – 21:00 Poster Viewing

Tuesday, September 6, 2016

Session III: Sub-cellular Proteomics

Chair: Jenny Renaut

9:00 – 9:30 Ian Max Møller: The plant mitochondrial proteome

9:30 – 9:50 Yann Gohon: Structural proteomics: Use of synchrotron light to map water accessible surface of oil body interacting proteins

9:50 – 10:10 Daisuke Takahashi: Changes of extracellular matrix in response to cold and sub-zero acclimation in *Arabidopsis*

10:10 – 10:30 Coffee break

10:30 – 10:50 Harriet T. Parsons: Dissecting organelles with proteomics; the Golgi cisternae and ER sub-compartments revealed

10:50 – 11:10 Tomáš Takáč: Actin depolymerization-induced changes in proteome of *Arabidopsis* roots

11:10 – 11:30 Kjell Sergeant: The difference 2 Da makes

11:30 – 14:00 Lunch break

Session IV: Growth and Development

Chair: Dominique Job

14:00 – 14:30 Jozef Šamaj: Comparative proteomics of *Arabidopsis* MAPK mutants with developmental defects

14:30 – 14:50 Palak Chaturvedi: Comprehensive pollen proteomics: an evidence for developmental priming

14:50 – 15:10 Chiew Foan Chin: Proteomic analysis of callus proliferation in tissue culture of tropical crops

15:10 – 15:40 Chang-Hsien Yang: A RING-Type E3 ligase controls ovule development by negatively regulating a key enzyme in the process of lignin synthesis in *Arabidopsis*

15:40 – 16:00 Coffee break

Special session: Minute poster presentations

Chair: Maksym Danchenko

16:00 – 17:30 Jazmin Abraham, Namik Rashydov, Reinhard Turetschek, Valentin Roustan, Lucyna Domżalska, Shiori Koga, Thierry Chardot, Sebastian Schneider, Ravi Gupta, Yunqi Wu, Jenny Renaut, Arindam Ghatak, Kristina Majsec, Anna Rita Trentin,

17:30 – 20:00 Poster Viewing

Wednesday, September 7, 2016

Session V: Plant and Pathogen Interaction

Chair: Carla Pinheiro

9:00 – 9:30 Laurence Bindschedler: Exploring the battlefield of an obligate plant pathogen by proteomics and gene silencing: the case of barley powdery mildew

9:30 – 9:50 Letizia Bernardo: Novel insight about plant root interaction with arbuscular mycorrhiza by proteomics approach

9:50 – 10:10 Christof Rampitsch: Redox proteomics identifies redox-sensing protein targets by NADPH oxidase in *Fusarium graminearum*

10:10 – 10:30 Coffee break

10:30 – 11:00 Stephanie Wienkoop: Dissecting microbial and cultivar related influences of the defense response of *Pisum sativum* against *Didymella pinodes*

11:00 – 11:20 Katja Witzel: Comparative proteomic analysis of tomato roots colonized by the soil-borne pathogen *Verticillium dahlia*

Special Session: Use of Proteomics for Regulatory Science

Chair: Martin Hajduch

11:20 – 11:50 Hubert Chassaigne: Proteomics for the safety assessment of nanomaterials from the regulatory science perspective

11:50 – 12:20 Jay Thelen: Absolute quantification of soy allergens using mass spectrometry: Comparison of genetics versus environment on allergen load

12:20 – 14:00 Lunch break

Session VI: Proteomics of Abiotic Stress

Chair: Sebastien Carpentier

14:00 – 14:30 Setsuko Komatsu: Quantitative proteomics reveals initial-response mechanism in soybean under flooding stress

14:30 – 15:00 Luis Valledor: Systems Biology, a novel way for discovering new biomarkers of abiotic stress resistance and wood quality in *Pinus radiate*

15:00 – 15:20 Annelie Gutsch: The cell wall as shield against cadmium toxicity: Proteome changes in the cell wall of alfalfa stems and leaves exposed to cadmium

15:20 – 15:40 Silvia Mazzuca: Exploring leaf proteome of marine plants toward ocean acidification

15:40 – 16:00 Claudia-Nicole Meisrimler: Pea proteomics: Iron deficiency and induced systemic resistance – what are the links?

16:00 – 16:20 Jens Beator: How abiotic factors in growth chambers affect plant growth and experimental results

16:20 – 16:40 Coffee break

Session VII: Proteomics and Breeding

Chair: Michel Zivy

16:40 – 17:10 Erik Andreasson: Potato proteomics for increased pathogen resistance

17:10 – 17:30 Bongani Ndimba: Agricultural proteomic in South Africa and applications of proteomics in agricultural biotechnology research

17:30 – 17:50 Brian P. Mooney: Quantitative proteomics of *Zea mays* hybrids exhibiting different levels of heterosis

19:00 Conference Dinner and Poster Awards

Thursday, September 8, 2016

9:00 – 10:00 Round table II

10:00 – 10:30 INPPO Scientific Committee Meeting

10:30 Closing, Poster dismounting, Farewell

Abstracts

Oral Presentations

Public Evening Lecture

Plant proteomics, with a special focus on seeds

Dominique Job

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Studies on cellular proteomes started in the years 95, coinciding with the tremendous progress in the identification of proteins by mass spectrometry. By this time plant scientists recognized the importance of these new approaches. To date, more than 18,000 papers have been published on plant proteomics. Furthermore, the plant proteomic community structured, leading to the establishment of the International Plant Proteomics Organization (INPPO) and the Oceania Agricultural Proteomics Organization (AOAPO), the organization of international meetings, the publication of special issues devoted to plants or the publication of a new journal named Frontiers in Plant Proteomics.
Since 1998 our team has focused on the proteomic characterization of seed development and their vigor[1]. In particular, we showed that germination is strictly dependent on the proteins stored in the mature seeds and on the proteins neosynthesized from mRNAs also stored in the mature seeds, thus justifying the use of a proteomic approach. I will describe the main results regarding the mechanisms governing the accumulation of seed storage proteins, the restart of metabolism during germination, the impact of the environment on seed vigor, the mechanisms of seed dormancy exit, or those accounting for the exceptional survival of embryo in a dry state.

References

[1]Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C, Job D. 2012. Seed germination and vigor. Annual Review of Plant Biology 63, 507-533

OrMo09:20

Green Systems Biology – the need for ecological thinking

Wolfram Weckwerth[1,2]

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Plants have shaped our human life form from the outset. With the emerging recognition of world population feeding, global climate change, and limited energy resources with fossil fuels, the relevance of plant biology and biotechnology is becoming dramatically important. Thus, in-depth understanding of the physiology of single plant species for practical applications as well as the translation of this knowledge into complex natural as well as anthropogenic ecosystems is needed. Latest developments in biological and bioanalytical research will lead into a paradigm shift towards trying to understand organisms at a systems level and in their ecosystemic context: (i) shotgun and next-generation genome sequencing, gene reconstruction and annotation, (ii) genome-scale molecular analysis using OMICS technologies and (iii) computer-assisted analysis, modeling and interpretation of biological data. Green Systems Biology combines these molecular data, genetic evolution, environmental cues and species interaction with the understanding, modeling and prediction of active biochemical networks up to whole species populations [1]. The ambitious aim of these non-targeted 'omic' technologies is to extend our understanding beyond the analysis of separated parts of the system, in contrast to traditional reductionistic hypothesis-driven approaches. The consequent integration of genotyping, pheno/morphotyping and the analysis of the molecular phenotype using metabolomics, proteomics and transcriptomics will reveal a novel understanding of plant metabolism and its interaction with the environment. An integrated proteomics/metabolomics platform as part of the Vienna Metabolomics Center (VIME) (http://metabolomics.univie.ac.at/) suited for functional genomics and systems biology is presented. The strategy includes MAPA (mass accuracy precursor alignment) [2-5], MASS WESTERN [6, 7] and the toolbox COVAIN [8] for functional data integration and interpretation [3, 8-12]. Further a novel algorithm for structural elucidation of metabolites from untargeted LC/MS based metabolomics data is presented [9]. Knowledge exchange of ecosystems research and green biotechnology merging into green systems biology is anticipated based on the principles of natural variation, biodiversity and the genotype–phenotype environment relationship as the fundamental drivers of ecology and evolution.

References

[1] Weckwerth, W. J Proteomics 2011, 75, 284-305. [2] Chen, Y., et al. The Plant Journal 2010, 63, 1-17. [3] Doerfler, H., etr al. Metabolomics 2013, 9, 564-574. [4] Egelhofer, V., et al. Nat. Protoc. 2013, 8, 595-601. [5] Hoehenwarter, W., et al. Proteomics 2008, 8, 4214-4225. [6] Lehmann, U., et al. The Plant Journal 2008, 55, 1039-1046. [7] Wienkoop, S., et al. Molecular Biosystems 2010, 6, 1018-1031. [8] Sun, X., Weckwerth, W. Metabolomics 2012, 8, 81-93. [9] Doerfler, H., et al. Plos One 2014, 9, E96188. [10] Bellaire, A., et al. New Phytol. 2014, 202, 322-335. [11] Valledor, L., et al. Mol. Cell. Proteomics 2013, 12, 2032-2047. [12] Nägele, T., et al. Plos One 2014, 9, E92299.

OrMo10:00

Integration of quantitative proteomics, redox metabolomics, and proteogenomics to identify sources of seed dormancy control and pre-harvest sprouting resistance in wheat

Natalia Bykova[1], Mark Jordan[1], Junjie Hu[1,2], Natasa Radovanovic[1], Michelle Rampitsch[1]

[1]*Morden Research and Development Centre, Agriculture and Agri-Food Canada, MB, Canada*
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In this work, we analyzed dormancy-imposing processes in aleurone and embryo tissue-specific proteomes and redox metabolomes using redox proteomics, fluorescent labeling, and 2-DE, comparative iTRAQ-based quantitation, and redox metabolite measurements. The iTRAQ-based approach resulted in over 6400 high-confidence protein identifications, of which 62 and 115 showed significant differential expression in dormant phenotypes, and 368 and 1034 were dormancy genotype-specific in embryo and aleurone, respectively. In dormant embryos, significant alterations were found in protein translation, folding, transport and degradation, DNA-repair, and mRNA surveillance, oxidative and nitrosative stress response. Potentially critical for imposing dormancy and after-ripening regulation, changes were found in cell cycle control, epigenetic regulation of gene expression, arrest of development and growth. Proteins responsible for natural defences against pathogens were up-regulated in dormant aleurone. Corresponding genes on chromosome arms where QTL for sprouting tolerance had been previously identified were further analysed to compare their location in the QTL region. The level of total glutathione was significantly higher in dormant embryo tissues, and the capacity for GSSG disulfide regeneration decreased dramatically upon after-ripening. In dormant embryo, the concentration of total and reduced ascorbate increased 2-3 fold during after-ripening indicating high capacity for ascorbate regeneration.

Acknowledgements
This work was supported by Agriculture and Agri-Food Canada Growing Forward funding AAFC AgriInnovation project 1203.

OrMo10:20

A comprehensive proteome map of glandular trichomes of hop (*Humulus lupulus* L.) female cones: One shot for the entire biosynthetic pathways of terpenoid-related compounds and possible transport proteins

Antoine Champagne, Marc Boutry

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Female flowers of hop (*Humulus lupulus*) are an essential source of terpenoid-related compounds mainly used as flavoring in the beer brewing process. The compounds involved are bitter acids, terpenophenolics, as well as mono- and sesqui-terpenoids. In this work, we analyzed the proteome of purified glandular trichomes (lupulin glands), which produce and accumulate these compounds in female flowers. A deep 2DLC-MS/MS analysis identified 1,015 proteins in a single shot. Of these, most correspond to housekeeping and primary metabolism-related proteins, while 75 were classified as involved in specialized (secondary) metabolism. No less than 40 enzymes are involved in the synthesis of terpenoid-derived compounds and 21 are predicted transporters possibly involved in the transport of secondary metabolites. We discuss the possible routes involved in the intra- and intercellular translocation of terpenoids and their precursors. This comprehensive proteomic map of glandular trichomes of hop female flowers represents a valuable resource to improve knowledge on glandular trichome functions.

Acknowledgements
This work was partly supported by the European Commission (grant no. 222716,), the Belgian National Fund for Scientific Research and the Interuniversity Poles of Attraction Program (Belgian State, Scientific, Technical and Cultural Services).

OrMo10:40

Identification of proteins binding to sense and antisense RNA pairs

Julia Svozil, Katja Baerenfaller, Wilhelm Gruissem

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Natural antisense transcripts (NATs), a class of long-noncoding RNAs, are complementary to transcripts from protein-coding genes on the opposing DNA strand at the same locus. Its role is only understood for few examples including the mouse UChl1 and rice PHO1;2 proteins, where an enhancing effect on translation is suggested[1, 2].

We performed an RNA sequencing and proteomics experiment of rice under phosphate starvation and its phosphate sufficient controls. Several cis-NAT levels were increased in one condition, while the coding mRNA level remained stable and the protein level remained stable or increased. For these examples and PHO1;2 we want to assess the RNA binding proteome and identify common features, which provide insights into the role and function of an increased cis-NAT expression.

RNA binding proteins which specifically bind sense or antisense RNA can be purified after immobilization of the respective RNA to streptavidin beads by fusing a hairpin aptamer to the respective RNA and incubating protein extracts with the immobilized RNA[3].

In a different approach, we purify the translation complex of PHO1;2. For this, we crosslink proteins and RNA in vivo. Subsequently, we extract and enrich polysomes and use an antibody specific for the N-terminal domain of PHO1;2 to immunoprecipitate the whole translation complex of PHO1;2 at the time of its translation. This should indicate which proteins are involved in the translation itself and which proteins were recruited by the NAT.

References

[1]Carrieri et al., 2012
[2]Jabnoune et al., 2013
[3]Leppek et al., 2014

OrMo11:20

The central role of SnRK1 in low energy syndrome

Ella Nukarinen[1], Thomas Nägele[1,2], Lorenzo Pedrotti[3], Bernhard Wurzinger[1], Andrea Mair[1], Ramona Landgraf[4], Frederick Börnke[4,5], Markus Teige[1], Wolfgang Dröge-Laser[3], Wolfram Weckwerth[1,2]

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Various stress conditions negatively influence on cellular energy homeostasis. The ability to adjust metabolism is crucial for survival in unfavourable conditions. The full diversity of adjustments depending on the stress conditions can be included in a term low energy syndrome (LES) which finally leads to an adaptation. A central player in LES is a sucrose non-fermenting related protein kinase 1 (SnRK1; SnRK1α1 and SnRK1α2). To mimic low energy conditions we performed an extended night time-course experiment utilizing an inducible *Arabidopsis thaliana snrk1α1/α2*mutant. Changes in phosphoproteome revealed that the activation of SnRK1 is vital for repression of highly energy demanding processes. Ribosomal protein S6 (RPS6) is a target of target of rapamycin (TOR) signalling pathway and its phosphorylation positively correlates with proteins synthesis and growth. Interestingly, RPS6 phosphorylation levels in *snrk1α1/α2* mutant were not reduced by energy deprivation in the extended night. This suggests across talk of SnRK1and TOR-mediated signalling pathways. Further evidence for a potential SnRK1 and TOR interaction, was demonstrated by the *in vivo* interaction of SnRK1α1 and RAPTOR1B and by phosphorylation of RAPTOR1B by SnRK1α1 in kinase assays. In addition, we found a correlation between SnRK1 and a phosphorylation status of some chloroplastic proteins, indicating an unexpected link to the photosynthesis, the main energy source in green plants.

OrMo11:40

Proteomic and phosphoproteomic profiling of leaves from two natural variants of *Imperata cylindrica*

Min-Chieh Tsai[1,4], Ting-Ying Wu[2,4], Ping Kao[2], Chia-Lun Chang[1], Chang-Hung Chou[3], Ing-Feng Chang[1,2,]

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Cogon grass (*Imperata cylindrica* L. Beauv. var. *major*) is one of the top-ten weeds. In particular, an ecotype from mangrove forest was found to be salt tolerant. A proteomic analysis using 2D-difference in gel electrophoresis (DIGE) was carried out to identify salt-responsive leaf proteins in salt-tolerant and salt-intolerant populations. Eight proteins were found with increased abundance in response to 150 mM salt stress. These include photosynthesis proteins *i.e.* OEE1, OEE2, ATP synthase, and an antioxidant protein Mn-superoxide dismutase (Mn-SOD). In addition, a phosphoproteomic analysis also identified unique phosphopeptides in the microsomal fractions isolated from leaves of *Imperata*. Out of these phosphopeptides, 2 belong to sugar transporters, including a sucrose transporter SUT1 which contains a novel phosphorylation site. Whereas the other 3 belong to photosynthesis proteins of which 2 are C4 enzymes. Our study identified differentially expressed targets which may play important roles in the salt tolerance of *Imperata*.

References

[1]Wu TY, Kao P, Chang CL, Hsu PH, Chou CH, Chang IF. 2015. Phosphoproteomic profiling of microsomal fractions in leaves of Cogon grass (*Imperata cylindrica*). Plant OMICS 8: 595-603.

[2]Kao P, Wu TY, Chang CL, Chou CH, Chang IF. 2011. Decreasing of Population Size of *Imperata cylindrica* Mangrove Ecotype & Sea-Level Rising, Global Warming Impacts - Case Studies on the Economy, Human Health, and on Urban and Natural Environments, Stefano Casalegno (Ed.), ISBN: 978-953-307-785-7, InTech.

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OrMo14:00

Phosphoproteomic resources and technologies - from discovering phosphorylation sites to identifying kinase clients

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Protein phosphorylation is the most studied post-translation modification in plants. The Plant Protein Phosphorylation Database (P^3DB, www.p3db.org) was developed as a community repository, containing data for both model and crop plants. Using this database of phosphorylation sites as a training set we developed a phosphorylation prediction tool called MUSite (http://musite.sourceforge.net/). The sensitivity and reliability of this prediction algorithm are unmatched, and application to whole plant proteomes such as *Arabidopsis* indicates greater than 17,000 phosphorylation sites at the 99% confidence interval. Clearly, experimental and bioinformatic prediction of phosphorylation sites is rapidly becoming a facile task. However, confirmation and identification of cognate protein kinases responsible for these events remain challenging. To address this problem we developed an assay called the Kinase Client or KiC Assay, which was validated using the pyruvate dehydrogenase kinase. We applied this validated approach to identify kinase(s) responsible for phosphorylating a type one protein phosphatase inhibitor (PPI-2) and recently to compare substrate specificities for two families of protein kinases involved in metabolic regulation, the SnRK2, and CDPK superfamilies. Screening of a library of 2100 peptides comprising over 3500 *in vivo* phosphorylation sites in *Arabidopsis* revealed high specificity for these kinases and a surprisingly low level of overlap among family members.

OrMo14:40

Targeting post-translational modifications in plant immunity signaling

Frank Menke

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Innate immune systems in animals and plants have to recognize pathogens in the extracellular space. For this, they rely on plasma membrane localized pattern recognition receptors (PRRs) that bind pathogen-derived molecular patterns (PAMPs). Upon binding of PAMPs to PRRs, signalling networks are triggered that ultimately results in PAMP-triggered immunity (PTI). The signaling events that are triggered by an activated PRR depend on various types of post-translation modifications (PTM), of which protein phosphorylation is the most extensively studied. To overcome PTI pathogens have evolved secreted effector proteins that can modulate perception by the host and, when delivered intracellularly, block downstream signal transduction events. Pathogen effectors target specific host proteins and change their PTM status. We use targeted proteomics approaches to study different PTMs including protein phosphorylation[1,2] and acetylation[3] in plant-pathogen interaction and examples will be presented of effectors targeting host proteins to affect their PTM status and overcome immunity.

References

[1]Mithoe, S. C. et al. EMBO reports 17, 441-454, (2016).
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[3]Sarris, P. F. et al. Cell 161, 1089-1100, (2015).

OrMo15:20

Contribution of quantitative proteomics to the analysis of maize responses to drought stress

Mélisande Blein-Nicolas[1], Thierry Balliau[1], Llorens Cabrera-Bosquet[2], François Tardieu[2], Claude Welcker[2], Michel Zivy[1]

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Response to water deficit is a complex character, and its genetic variability is likely to involve a large number of mechanisms. In this context, a greater knowledge of these mechanisms and of the involved proteins can be useful to accelerate the genetic improvement of drought tolerance in maize. While the "candidate protein" strategy classically involve the analysis of proteome variations in populations obtained from a cross between two inbred lines, we will describe a new strategy based on genome-wide association study (GWAS). We analyzed the proteome of 251 genotypes grown in the PhenoArch high-throughput phenotyping platform (Montpellier) under normal irrigation or moderate water deficit. 1008 leaf samples were collected at the pre-flowering stage and analyzed by shotgun label-free proteomics. Proteins were identified by using X!Tandem and the X!TandemPipeline (http://pappso.inra.fr/bioinfo/xtandempipeline/), and quantification was performed by using MassChroQ[1]. GWAS performed with 700.000 SNP for about 2000 protein x treatment combinations. Our results evidenced general proteome responses to water deficit as well as genetic variations that may be related to the variation of physiologic or agronomic traits. GWAS allowed the identification of cis and trans protein quantity loci (PQLs). Their distribution on chromosomes and the use of this strategy to identify candidate proteins will be discussed.

References

[1]Valot et al, 2011, Proteomics, 11, 3572–3577

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OrMo15:40

Two resistance inducers relevant in coffee plant protection show distinct metabolic adjustments

K. Possa[1,2], J. A. G. Silva[1], R. Tenente[2], C. Pinheiro[3,4], I. Chaves[3,5], S. Planchon[6], A. C. A. Monteiro[1], A. C. Souza[7], J. Renaut[6], M. A. F. Carvalho[8], C. P. Ricardo[3],M. L. V. Resende[1], L. Guerra-Guimarães[2,9]

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A good strategy in plant protection is to take advantage of the plant immune system by eliciting the plant's constitutive defenses. Based on this concept resistance inducers have been developed and are commercially available, such as Bion®. An alternative formulation Greenforce CuCa was developed by UFLA partners in Brazil which showed promising results for the control of coffee rust (*Hemileia vastatrix*). We established as working hypothesis that resistance inducers impose metabolic adjustments at the cellular level, mainly on photosynthesis and its regulation. A physiological (leaf gas-exchange) and proteomic (2DE-MALDI/TOF/TOF MS) analysis was performed in *Coffea arabica* leaves sprayed with GreenForce CuCa, Bion® or water (control), followed by the inoculated with *H. vastatrix.* Our results showed that GreenForce CuCa and Bion® triggered opposite responses in leaf stomatal conductance and instantaneous photosynthetic rate. While application with GreenForce CuCa increased leaf-gas exchange, application with Bion® caused a decrease in photosynthesis and stomatal conductance. The proteomic data obtained revealed changes at photosynthetic and respiratory metabolism. Additionally, proteins involved in hormonal signaling were also observed. Taken together, our data support a role for the primary metabolism in defense responses, but the two resistance inducers seem to operate in different ways. This opens new perspectives for the research of plant induced resistance.

Acknowledgements
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OrMo16:00

Exploring the plant-microbe interface by profiling the surface-associated proteins of barley grains

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Objective: Cereal grains are colonized by a microbial community that actively interacts with the plant via secretion of various enzymes, hormones, and metabolites. Microorganisms decompose plant tissues by a collection of depolymerizing enzymes, including β-1,4-xylanases that are in turn inhibited by plant xylanase inhibitors. To gain insight into the importance of the microbial consortia and their interaction with barley grains, the surface-associated proteins and xylanolytic activities of two barley cultivars were profiled.
Method: A washing procedure was implemented to isolate the surface associated proteome from barley grains. A combined gel-based (2-DE coupled with MALDI-TOF-TOF MS) and gel-free (Orbitrap LC-MS/MS) proteomics approach complemented with xylanase activity assays were used.
Results: The surface-associated proteome was dominated by plant proteins with roles in defense and stress-responses, while the relatively less abundant microbial (bacterial and fungal) proteins were involved in cell wall and polysaccharide degradation, and included xylanases. The surface-associated proteomes showed elevated xylanolytic activity and contained several xylanases.
Conclusions: Integration of proteomics with enzyme assays is a powerful tool for analysis and characterization of the interaction between microbial consortia and plants in their natural environment.

References

J Proteome Research (2016) 15: 1151–1167

OrMo16:40

Proteomic analysis of wheat and barley response to abiotic and biotic stress factors using gel-based proteomic approaches

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Abiotic and biotic stresses induce an active plant response resulting in stress acclimation and enhanced stress tolerance. Proteins are directly involved in stress acclimation. Gel-based proteomics using two-dimensional differential gel electrophoresis (2D-DIGE) approach represents a complementary method to gel-free approaches and provides a visual representation of proteome based on pI and MW values. 2D-DIGE analysis enables protein relative quantification leading to an identification of protein spots revealing an altered abundance in stress-treated varieties which can be further tested as potential markers of stress tolerance. A summary of our main results aimed at 2D-DIGE proteomic analyses of stress-treated wheat and barley varieties subjected to cold, drought, salinity and Fusarium head blight disease is provided. Special attention is paid to the proteins revealing differential abundance between different stress treatments or genotypes revealing differential stress response such as spring versus winter genotypes or winter genotypes revealing differential levels of frost tolerance determined as lethal temperature for 50% of the samples. The results of proteomic analyses are interpreted with respect to other physiological data such as parameters related to stress tolerance, phytohormone levels, water regime-related characteristics, and others. The role of gel-based proteomic analysis in understanding plant stress response is discussed.

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Acknowledgements

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Molecular plasticity during drought recovery is characterised by protein turnover dynamics and translational regulation in *Medicago truncatula*

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Plants are continuously exposed to extreme environmental changes. Especially water availability is subjected to a considerable fluctuation; several days of drought are often followed by periods of sufficient rain. This requires a molecular plasticity that enables plants to regulate drought acclimation and deacclimation processes for recovery and continuous growth. A complex network, including proteomic and metabolomic turnover dynamics, is part of this regulatory process. To study this, a partial 15N-metabolic labelling strategy in planta was introduced. Nitrogen incorporation was analysed over a drought-recovery experiment determining the relative isotope abundances (RIA) by using our software tool Protover[1]. Severe drought stressed plants (10 days of water withhold) were re-watered over a period of 4 days until full physiological recovery. The RIA of metabolites and proteins was monitored using mass spectrometry from samples taken 2, 24, 48, 72 and 96 hours after re-watering. The data reveal independent regulatory mechanisms for stress recovery with different dynamic phases that during the course of recovery define the plants deacclimation from stress. An early transition phase that seems key for recovery initiation through water re-supply was observed. Furthermore, the data indicate that plasticity may also be related to the nutritional status of the plant prior to stress initiation.

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OrTu09:00

The plant mitochondrial proteome

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Plant mitochondria are well known to oxidize respiratory substrates typically pyruvate, the end product of glycolysis, via the tricarboxylic acid cycle and oxidative phosphorylation and provide the cell with energy and metabolic intermediates. Bioinformatic and experimental evidence now indicate that plant mitochondria contain a proteome of more than 2000 different proteins expressed in some cells under some conditions. Using various gel-based and non-gel-based methods for separating the mitochondrial proteins and mass spectrometry for their identification, more than 1000 different proteins have been identified in *Arabidopsis* and potato mitochondria. Only 63% of these proteins were recognized as mitochondrial by any of five of the most widely used prediction algorithms. Functional analyses of the identified proteins have greatly improved our understanding of basic mitochondrial functions, have led to the discovery of new mitochondrial metabolic pathways and are helping us towards appreciating the dynamic role of the mitochondria in the responses of the plant cell to biotic and abiotic stress.

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OrTu09:30

Structural proteomics: Use of synchrotron light to map water accessible surface of oil body interacting proteins

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Oil bodies (OBs) are organelles consisting of a core of neutral lipids surrounded by proteins embedded in a specific hemi membrane. Due to their lipophily, the structure of these insoluble proteins is poorly known[1,2]. We have used radiolytic footprinting based on synchrotron radiation to oxidize the solvent accessible surface of proteins[3]. We will present how the approach was implemented at SOLEIL METROLOGY beamline. Location of oxidized residues was determined by proteomic analysis. This allowed determining an original insertion map of proteins embedded in OBs at the level of individual amino acid residues.

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OrTu09:50

Changes of extracellular matrix in response to cold and sub-zero acclimation in *Arabidopsis*

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Low above-zero temperature treatment increases plant freezing tolerance (cold acclimation, CA). CA followed by sub-zero temperature treatment results in further enhancement of freezing tolerance (Sub-zero acclimation, SZA). In global gene expression analysis in *Arabidopsis*1, extracellular matrix (ECM)-related genes were consistently up-regulated during SZA. However, the effect of CA and SZA on ECM is not yet fully understood in *Arabidopsis*. We, therefore, aimed to investigate the proteomic response of ECM to CA and SZA in combination with cell wall analysis. The accession Col-0 and N14, which are intermediate and highly freezing tolerant after CA and SZA, were subjected to shotgun proteomic analysis. Among 396 ECM proteins identified, 74 and 40 proteins significantly changed during CA in Col-0 and N14, respectively. Besides, 39 (Col-0) and 28 (N14) ECM proteins including cell wall-modifying enzymes showed SZA-specific responses. CA and SZA resulted in considerable changes in cell wall polysaccharide and monosaccharide compositions. Therefore, changes of ECM proteins are considered to be accompanied by cell wall changes and important for enhancement of freezing tolerance during CA and SZA.

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OrTu10:30

Dissecting organelles with proteomics; the Golgi cisternae and ER sub-compartments revealed.

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A gradient of surface charge exists across the secretory pathway, which can be exploited when separating components of this complex system of membrane compartments by electrophoresis. Free Flow Electrophoresis is a continuous-flow, liquid separation system which can be used to separate Golgi cisternae, as well as other vesicles. Application of this technique to a membrane enrichment from *Arabidopsis* cell-suspension culture has resulted in the secretory system being fractionated from early (ER) to late (trans-Golgi and TGN). Shotgun proteomics identified approximately 1200 proteins per fraction. Label-free quantification and principal component analysis revealed a distinct clustering of proteins that, when compared to immunogold-localization data, were found to correspond to cis-, medial and trans-Golgi compartments. Interestingly, ER proteins were also observed to resolve into two clusters, each functionally distinct. The trans-membrane (TM) span and surrounding region of different protein groups were extensively analyzed and compared. Results validated known associations with distance through the secretory pathway, such as TM span length, and revealed novel ones likely to affect protein-lipid interactions. Average abundance profiles were produced for each group by performing hierarchical clustering on spectral count data. Clear differences were observed between cargo and resident profiles, as well as between different groups of residents, that were verified using Multiple Reaction Monitoring (MRM), showing that different recycling pathways existed within the secretory system and that they could be mapped. These results constitute the first sub-organelle resolution proteomic analysis of the secretory pathway, in any experimental system, and will contribute much to our understanding of it.

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Stephen C. Fry, Janice G. Millar, Yves Verhertbruggen, Henrik Scheller, Purbasha Sarkhar

OrTu10:50

Actin depolymerization-induced changes in proteome of *Arabidopsis* roots

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Actin cytoskeleton is a vital cellular structure primarily known for controlling cell integrity, division and expansion. Regulation of cytoskeleton is still not fully understood. Proteomics is used to identify new protein candidates regulating organization and dynamics of cytoskeletal arrays as well as for detection of downstream cytoskeleton-dependent processes. Employment of specific inhibitors of cytoskeleton in proteomic studies proved to be effective for such aims (Takáč et al. 2016). Here we present a proteomic dissection of *Arabidopsis* roots treated by actin depolymerizing agent latrunculin B. Pharmacological disintegration of the actin cytoskeleton by latrunculin B caused downregulation of several proteins involved in the actin organization and dynamics. Moreover, this approach helped to identify new protein candidates involved in gene transcription, due to the altered abundance of proteins involved in mRNA nuclear export. Finally, latrunculin B negatively affected the abundance of abscisic acid (ABA) responsive proteins.

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OrTu11:10

The difference 2 Da makes.

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While doing a study on the cell wall proteome of alfalfa stems[1], a remarkable mass shift of -2Da was observed on a phenylalanine of the β-subunit of polygalacturonase. Reanalysis of datasets generated in-house and from other research groups confirmed the observation. All identified phenylalanines in the sequence FxxY of the active protein are modified to didehydrophenylalanine (ΔPhe). However, the alteration was not observed for other proteins.
Although rare in nature, didehydroamino acids are known as components of bioactive peptides. They are furthermore intensively studied for their conformation-directing properties and the applications this could have for the production of recombinant proteins with a custom-made fold. However, our data are the first evidence that an enzymatic function, inherent to plant cells, produces these amino acid derivatives.
The known fold-determining properties of didehydroamino acids also give a clue on the functional significance of this modification. The protein is inherently disordered without modification but known to interact tightly with the catalytic subunit of polygalacturonase and pectin nonetheless. The repetitive occurrence of ΔPhe might be the way by which the protein's fold is stabilized to ensure these interactions.

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Acknowledgements

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OrTu14:00

Comparative proteomics of *Arabidopsis* MAPK mutants with developmental defects

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Shot gun comparative proteomic analysis was performed on *Arabidopsis* mpk4 and mpk6 mutants. In the mpk6 mutant, we have found two proteins, namely CDC48A and phospholipase D alpha 1, which are important modulators of plant development. In the case of the mpk4 mutant, we detected changed the abundance of mRNA decapping complex VCS. Comparison of mpk4 and mpk6 differential proteomes showed differences in defense-related proteins. Especially the mpk4 mutant showed altered abundances and activities of antioxidant proteins including catalase and superoxide dismutase. We proposed some developmentally important proteins as either directly or indirectly regulated by MPK4 and MPK6. These proteins most likely participate in distinct developmental phenotypic features of mpk4 and mpk6 mutants (Takáč et al. 2016).

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Acknowledgements

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OrTu14:30

Comprehensive pollen proteomics: an evidence for developmental priming

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We have generated a cell-specific reference-proteome of tomato pollen development from the ecotype Red Setter which includes microsporocytes, tetrads, microspores, polarized microspores and mature pollen[1]. Each stage showed a specific reprogramming of the proteome, these specific responses in pollen development process was termed as "Developmental priming" as a contrast to "Defense priming". The hypothesis reveals that genetic or epigenetic program causes protective proteins such as HSPs to occur in the non-stressed state, in order to compensate for sudden changes in temperature during the maturation of the pollen[1,2]. A novel approach was introduced for peptide quantification based on mass accuracy precursor alignment (MAPA), considering a target list of "proteotypic peptides" in the ecotype Hazera cv.3017[3].

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OrTu14:50

Proteomic analysis of callus proliferation in tissue culture of tropical crops

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Callus formation represents the initial and vital stage of somatic embryogenesis in plant tissue culture. Many commercial tissue culture laboratories in crops such as oil palm utilize somatic embryogenesis technology to mass produce clonal elite materials for the establishment of plantations. However, one of the main obstacles in somatic embryogenesis is the initiation and proliferation of callus and the subsequent conversion of the callus into embryos. In order to elucidate the fundamental mechanisms underlying callus initiation and proliferation at the cellular and molecular levels, proteomic approaches were used. This presentation demonstrates the use of 2D-PAGE coupled with MALDI TOF-TOF mass spectrometry to analyze callus proliferation on commercial crops, particularly oil palm[1] and vanilla orchids[2]. Differential proteins associated with callus proliferation will be discussed.

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OrTu15:10

A RING-Type E3 ligase controls ovule development by negatively regulating a key enzyme in the process of lignin synthesis in *Arabidopsis*

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In this study, an *Arabidopsis* gene DAFL1 that encodes an E3 protein ligase was investigated. DAFL1 was closely related to DAF; however, DAFL1 was expressed in the pistil and the ovule whereas DAF was expressed in stamen. Transgenic *Arabidopsis* plants that ectopically expressing DAFL1 RNAi, antisense, and dominant negative mutation DAFL1-H135A caused similar indehiscent anthers phenotype. When the stigmas of sterile transgenic plants were pollinated with wild-type pollen, siliques contained about a half of amount seeds to that in wild-type siliques were observed. Transgenic plants that specifically expression of DAFL1-H135A in the pistil and the ovule driven by DAFL1 promoter produced dehiscent anthers. However, about a half of amount seeds to that in wild-type were observed in the siliques. One DAFL1 interacting protein CAD9, a key enzyme in the process of lignin synthesis, was identified through yeast-two-hybrid analysis. Ectopic expression of CAD9 also produced siliques with about a half amount of seeds to that in wild-type siliques. Since DAFL1 was expressed during the early stage of ovule development, our results revealed that DAFL1 might play a role to positively control early stage of ovule development by degrading CAD9 and preventing lignin biosynthesis. Ectopic expression of CAD9 caused the lignin biosynthesis during the early stage of ovule development and resulted in the abortion of the ovule formation as seen in our result.

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Exploring the battlefield of an obligate plant pathogen by proteomics and gene silencing: The case of barley powdery mildew.

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Powdery mildews are economically important obligate fungal pathogens. *Blumeria graminis*is the causal agent of barley powdery mildew. Like most biotrophs, *Blumeria* possesses haustoria which are involved in nutrient uptake and secretion of virulence factors or effectors.
A large scale proteogenomics assisted the annotation of the *Blumeria* genome in validating ORF models[1], such as63 *Blumeria* Effector Candidates (BECs) and 100 out of the 500+ Candidate secreted effector proteins (CSEPs) predicted by bioinformatics[2-4] or proteins associated with repetitive elements/ transposons[5].
Virulence function was validated by RNAi and biolistic or the new and GM free method "Silencing Targeted Effectors in Planta" (STEP) using short antisense oligonucleotides delivered to excised leaves. Eight effectors were confirmed, including BEC1011 and BEC1054 RNAse-Like proteins in Haustoria (RALPHs), and a "universal" BEC1019 metalloprotease like protein[6]. Barley proteins interacting with BEC1054, identified by pull-down and Y2H, included an elongation factor, a malate dehydrogenase, a pathogen-related protein PR5 and a GST[7].

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OrWe09:30

Novel insight about plant root interaction with arbuscular mycorrhiza by proteomics approach

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Mycorrhizal interaction with plant roots supports plant nutrition and development, inducing changes at the biochemical level. A spring bread wheat cultivar was investigated under controlled drought condition in association to mycorrhizal fungus. The effects of arbuscular mycorrhiza (AM) interaction with wheat roots under either drought and watered condition were investigated at proteome level, by a gel-fractionation followed by nano LC/MS approach, using a hybrid quadrupole-time-of-flight (QTOF) mass spectrometer. A label-free quantitation was carried out after protein identification inferred from validate peptides (auto-thresholds, FDR 1%), followed by fold-change analysis. The cluster analysis highlighted a positive interaction between AM and roots, allowing enhanced tolerance to drought. Several proteins related to environmental stress response and to ROS detoxification (PAL, superoxide dismutase, peroxidase, and glutathione transferase) were down accumulated in AM-treated plants under drought, like for watered plants. The sulphur metabolism (e.g. cysteine synthase) was also affected by the symbiotic treatment. Furthermore, a pivotal protein in fructans biosynthesis and the 14-3-3 protein were down-represented in the AM-plant association. The proteomic evidenced were consistent with physiological measures on plant biomass and water content.

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Acknowledgements

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OrWe09:50

Redox proteomics identifies redox-sensing proteins targeted by NADPH oxidase in *Fusarium graminearum*.

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Fungal NADPH oxidases (NoxA and NoxB) are regulated to generate superoxide and ultimately hydrogen peroxide as an intracellular signal to target specific proteins. Susceptible sulfhydryl groups can be oxidized reversibly and their redox state can act as a molecular switch to modulate protein function. We have used two strategies to label reactive cysteines in *Fusarium graminearum*, either with biotin to facilitate affinity enrichment, or with monobromo bimane, a fluorescent label, to facilitate their detection on 2D gels. We then used LC-MS/MS to identify proteins and their modified cysteines in comparative analyses with a wild-type strain of *F. graminearum* and a ΔnoxAB deletion mutant. *F. graminearum* is a multicellular fungus that causes serious economic losses in cereal crops worldwide. The ΔnoxAB mutant lacks NoxA and B and is non-pathogenic. The level of oxidized cysteines in target proteins should be lower in the mutant as it produces less H2O2. The labelling approaches yielded candidate redox-sensing proteins which are putative targets of redox signalling originating from NoxAB. To confirm their roles we constructed both deletion and substitution mutants (C to S) of six of these candidates and examined their phenotypes in vitro and in planta. One mutant (FGSG 10089, a protein of unknown function) is phenotypically similar to ΔnoxAB. The most recent results will be presented.

Acknowledgements
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OrWe10:30

Dissecting microbial and cultivar related influences of the defense response of *Pisum sativum* against *Didymella pinodes*

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Pisum sativum vitally benefits from the association with rhizobia and mycorrhiza. The symbiotic effects on plant growth, metabolism and potentially also the immune system have widely been studied, however, they are still controversially discussed. *D. pinodes*is a major pathogen of field pea. One aspect of our project assesses the influence of different compositions of associated microbes on the leaf metabolism upon pathogen infection. Another focus lies on the comparison of these symbiotic effects on cultivars, differing in the pathogen susceptibility. We aimed to elucidate the symbiotic and pathogenic effects on Pisum cultivars by integrating morphological traits with the leaf metabolism. Our data indicate that different compositions of associated microbes affect leaf metabolism on different levels under healthy conditions[1]. Infection with *D.pinodes* instead caused a more pronounced metabolic response. Interestingly, the Rhizobia treatment showed the most enhanced resistance. This suggests that the extent of Pisum´s pathogen defense response depends on the prevailing symbiotic associations[1]. However, this effect was less significant in the more resistant cultivar. Lower infection rates compared to the susceptible cultivar seemed to rather cause of higher inherent levels of the secondary metabolism and stress response than of the symbiotic interaction. This implies that genotypic plasticity has a higher impact on pathogen resistance than symbiont induced phenotypic effects.

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Acknowledgements

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OrWe11:00

Comparative proteomic analysis of tomato roots colonized by the soil-borne pathogen *Verticillium dahliae*

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Verticillium species are destructive vascular wilt fungi with worldwide distribution, causing severe losses in crop yield and quality. These soil-borne pathogens colonise the plant root surface in response to root exudates, penetrate the cortex and endodermis, and spread systemically through conidia transported by the transpiration stream in the xylem. While a large body of physiological and biochemical alterations in the host are reported, the cellular effects of pathogen colonisation on the host's root are still not fully clarified. Here, we report on the time-resolved analysis of the tomato root proteome in response to fungal colonisation. Tomato (*Solanum lycopersicum* cv. Hildares) was grown in quartz sand and inoculated with *Verticillium dahliae* at the two-leaf stage. Roots were harvested at seven, 14 and 21 days after inoculation. In order to identify proteins related to the fungal spread at the different time points, a subsequent proteome analysis by the two-dimensional differential in-gel electrophoresis (2D-DIGE) was initiated on samples from three independent experiments. Hierarchical clustering and principal component analysis were applied to interpret the data set. First results of protein identifications using MALDI-TOF MS/MS from the comparison of treated and non-treated plants as well as from the time-course analysis of fungal spread are presented.

OrWe11:20

Proteomics for the safety assessment of nanomaterials: from the regulatory science perspective

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Emerging approaches in the area of the safety assessment of nanomaterials combine the use of *in vitro* systems and post-genomics techniques. Our work should contribute to a better understanding of nanomaterials' effects at the cellular level as well as propose alternative test methods to standardised regulatory methods when required[1].
The modification that occurs in the proteome profile of the human colon adenocarcinoma (Caco-2) cell line when exposed to nanosilver was investigated using two quantitative proteomic approaches. Both 2D gel-based and label-free MS-based proteomic approaches include extensive bioinformatics and data mining procedures. Confidence in protein identification and quantification, repeatability, instrument time, and cost are all important factors that need to be taken into account when comparing the two approaches.
To date, however, no established guideline has yet been made available regarding the generation and assessment of the quality of proteomics data that could be reviewed by EU regulatory bodies for decision making. The aim of this work is to support and anticipate EU policy needs in the area of human exposure to nanomaterials by contributing to the development of new testing and predictive methods.

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Acknowledgments

The research described in this work was supported by the European Commission Joint Research Centre (JRC) within the Molecular Biology and Genomics and Nanobiosciences Units through the JRC Multiannual Work Programme.

OrWe11:50

Absolute quantification of soy allergens using mass spectrometry: comparison of genetics versus environment on allergen load

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Soybeans contain approximately 20 protein allergens. Regulatory and toxicology safety practices require equivalency testing of endogenous allergenic proteins in GM crops. I present an accurate and robust experimental approach for assessing endogenous allergens. Absolute quantification was performed for the major allergens using multiple reaction monitoring, whereby signals from an endogenous tryptic peptide are compared to those from a heavy-labeled peptide standard. This method has proceeded from proof-of-principle to a validated, multiplexed assay that is now commercially available through PepPro Analytics. Soy seed composition is affected by breeding and environmental conditions. The objective of this study was to evaluate the influence of genotype and environment on the accumulation of allergens in soybean. To address genetic and environmental effects, four varieties of non-GM soybeans were grown in six geographically distinct regions of North America. A follow-up study was performed two years later within the central US to compare the robustness of the data acquisition method and address the issue of genetics versus environment on allergen accumulation for a smaller region. Statistical analyses show that diverse environmental conditions exert a greater impact on allergen variability than conventional breeding. Understanding the natural variation in soy allergen concentrations in the existing food supply provides a framework for assessing unintended changes in GM crops.

OrWe14:00

Quantitative proteomics reveals initial-response mechanism in soybean under flooding stress

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Climate change causes abiotic stresses, which lead to the great threat for crops. Soybean is sensitive to flooding stress, which reduces its growth. In particular, at initial flooding stress, plant growth, and ATP content are suppressed in soybean. To uncover the response mechanism of soybean at initial flooding, proteins were analyzed using gel-free/label-free proteomic technique. In soybean, initial flooding stress led to the decrease of mRNA transport/boxCD snoRNAs and histone variants related proteins. The decrease of these nuclear proteins caused suppression of mRNA export/pre-ribosomal biogenesis and change of chromatin structure. These changes happened in nucleus continuously regulated cytoplasmic events including inhibition of protein synthesis, alteration of energy metabolism, and suppression of cell wall formation. Ethylene weakened flooding tolerance of soybean through promoting plant weight and energy consume; however, abscisic acid enhanced flooding tolerance of soybean through retarding plant growth and controlling energy conservation. Furthermore, assembly of newly synthesized proteins, scavenging of reactive oxygen species, and inhibition of cell wall loosening might play a key role through protein phosphorylation in soybean for producing initial flooding tolerance.

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Systems Biology, a novel way for discovering new biomarkers of abiotic stress resistance and wood quality in *Pinus radiata*

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Despite some advances towards more tolerant varieties have been achieved in edible crops, the knowledge about the specific mechanisms mediating stress adaptive responses in Conifers are scarce. Knowing these pathways is crucial for designing new strategies focused on maintaining forest productivity since globally expected changes in environmental conditions, mainly increased temperatures, irradiation, and droughts threaten plant productivity. These advances should cover, at the same time, strategies for increasing wood quality.
In our laboratory we are focused on the study of abiotic stresses (UV and temperature) and wood quality/tree growth traits, since together with drought, are the most important challenges that Mediterranean forests will face in the next decades. We studied the effect of these stresses employing realistic intensities aiming to mimic future scenarios based on current models in a time course in greenhouse experiments. Furthermore, the availability of a common garden, ten origins covering North-South clonal variation and Mediterranean and Atlantic basins, allowed us to have a field system to exploit natural variation towards deciphering how Pine can adapt to different environments and provide different wood qualities. Current technology for high-throughput phenotyping at the different -omic levels and its integrative analysis will revolutionize the way we how we understand tree biology and forest management.

OrWe15:00

The cell wall as shield against cadmium toxicity: proteome changes in the cell wall of alfalfa stems and leaves exposed to cadmium

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In this proteome study, we use alfalfa (*Medicago sativa*) stems and leaves to investigate the influence of Cd-exposure on cell wall (CW) structure. Plants were grown on contaminated (10mg Cd/kg soil) and uncontaminated soil. Un-targeted and CW-targeted protocols were used to extract proteins, and quantitative analyses performed based on 2-D DIGE.
Current results show changes in the abundance of a high number of CW and soluble proteins in both tissues. A variety of proteins involved in plant defense is up-regulated (e.g. chitinase, BSP-family protein, thaumatin, cysteine-rich secretory protein). Several proteins involved in carbohydrate metabolism were identified, most prevalent glucan endo-1,3-β-glucosidase which has a higher abundance of Cd-exposed plants. Proteins involved in CW remodeling either show a lower (e.g. fasciclin-like arabinogalactan protein, polygalacturonase β-protein) or higher abundance (e.g pectinesterase, xyloglucan endotransglucosylase/hydrolase, non-classical arabinogalactan protein) as a response to Cd. Further, we identified a large number of peroxidases being of higher abundance in the Cd-treated samples. Our findings support the hypothesis that Cd exposure influences the CW structure and that the CW acts as a primary defense barrier against Cd stress.

Acknowledgement
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OrWe15:20

Exploring leaf proteome of marine plants toward ocean acidification

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The study concerned the acclimation responses of marine plants living in the undersea volcanic sites producing carbon dioxide vents that induce an acid pH gradient around the bauble emission. As it is well known, water acidification may negatively affect some species while favouring other invasive ones (Hall-Spencer J. M. et al., Nature 454, 96-99, 2008). *Cymodocea nodosa* living in the Vulcano island and *Posidonia oceanica* living in Ischia were sampled at noon in meadows exposed to CO_2 vents and in nearby meadows with normal CO_2 condition. The team of researchers coming from the "HighGrass" project, applied at these sites ecophysiology, genomic and proteomic approaches, thus integrating the required know-how and research capacities to successfully accomplish the goals. A label-free proteomic approach was applied to both species at the sites and the differential accumulation of proteins in leaves was investigated. At the same time ultrathin leaf sections from normal and acidified sites were analyzed by the image analysis software to get the mean measurements of cell length, cell volume, and epidermis thickness among samples. Proteomic results will be also discussed in the light of the expression of target genes (Lauritano et al., Biogeosciences, 12, 4185–4194, 2015). Our results revealed a good correspondence between the metabolic pathways induced by the acidification and the cytological features thus lead us to understand whether seagrasses may mitigate the climate change.

Acknowledgements
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OrWe15:40

Pea proteomics: Iron deficiency and induced systemic resistance – what are the links?

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Pisum sativum L. (garden pea) is part of the economically important legume family. Pea has been investigated as a model with respect to several physiological aspects, unfortunately, the genome has not been completed to date. Regions of pea cultivation often overlap with iron deficient (-Fe) soils, affecting productivity and seed quality. Proteomic approaches were used to elucidate differences in common pea cultivars, their adaptation processes to long-term -Fe and effector treatment. -Fe efficiency was always related to proteins playing a role in the reorganization of the mitochondrial electron transport chain, ROS scavenging, and protein refolding or recycling. Proteins related to similar processes were also identified after chitosan treatment under -Fe. Chitosan, which induces similar to flagellin systemic resistance in plants, showed a strong effect on quantity and activity of plasma membrane-bound peroxidases. Recently, it became apparent that induced systemic resistance (ISR) is co-regulated with –Fe, e.g. transcription factor MYB72 and FIT in A.thaliana[1]. To date, the role of MYB72 and FIT in context with ISR are not clear in legumes. Proteomic data for pea were integrated into latest models for -Fe regulations and set in context with ISR. Co-regulations, possible cross-talks, and prospects using targeted proteomic approaches will be discussed.

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OrWe16:00

How abiotic factors in growth chambers affect plant growth and experimental results

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Generation of comparative quantitative data e.g. for different cultivars, genotypes, or different experimental treatments requires growth of plants in uniform environments. Plants in growth chambers need highly specific conditions e.g. for evaporation of water, heat dissipation of irradiant energy, and gas exchange to achieve normal and consistent growth and physiological performance. Data will be discussed showing the importance of uniform abiotic factors for plant growth and gene expression at multiple levels in different model plants. Factors crucial for achieving homogeneous and consistent plant growth will be presented and discussed.

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OrWe16:40

Potato proteomics a base for increased pathogen resistance

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We first investigated compatible and incompatible interactions between *Phytophthora infestans* and potato (*Solanum tuberosum*). This interaction can cause one of the most important crop diseases worldwide. Quantitative label-free proteomics of 51 apoplastic secretome samples in combination with genome-wide transcript analysis by 42 microarrays was used to capture changes in protein abundance and gene expression after inoculation with *P. infestans*. To aid mass spectrometry analysis we generated cultivar-specific RNA-seq data, which increased peptide identifications by 17%. Half of the differentially abundant proteins showed a corresponding change at the transcript level. For precision plant breeding, DNA based markers have been used extensively, but the potential of protein biomarkers has not been exploited. In this work, we developed an SRM marker panel with assays for 104 potato (*Solanum tuberosum*) peptides. Thereafter, the prediction markers were identified for *Phytopthora infestans* resistance in leaves, *P. infestans* resistance in tubers, and for plant yield in potato leaf secretome samples. The results suggest that the marker panel has the predictive potential for three traits, two of which have no commercial DNA markers. We have also investigated samples collected from different field conditions.

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Current issues in molecular biology 19:73-88 (2016); BMC Genomics (2014) 15:497; Journal of Proteome Research (2015) 15: 638–646

Agricultural Proteomics in South Africa & Application of Proteomics in Agricultural Biotechnology Research

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Agricultural products are generally used for food/meat, animal feed, biofuels, clothing, fibre, medicine and building materials. Crops, therefore remain the main artery/vein for the survival, productivity, growth and development of the world's food, health, and economic systems. As such, agricultural products are a central pillar of modern bioeconomy. For example, in Europe, the knowledge-based bioeconomy has an estimated worth of more than 2 trillion Euros, an industry that supports more than 21 million families. Agriculture, a cornerstone of human survival and development in less industrialised countries, particularly in Africa and Asia, with the majority of the developing world's human populations dependent exclusively on subsistence farming. Proteomics, which is generally defined as the simultaneous and high throughput study of protein expression profiles in cells, tissues, organs and organisms, is now recognised as one of the most important tools used in the identification and characterisation of proteins (and genes) of biological and biotechnological interest. Over the past 15 years, the agricultural proteomics field has grown notably, contributing positively to global biotechnology research, particularly in new knowledge generation, medical and agricultural applications. This talk will give an African perspective of Agricultural Proteomics with examples and particular attention to applications in South African Research and Development.

OrWe17:30

Quantitative proteomics of *Zea mays* hybrids exhibiting different levels of heterosis

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Maize hybrids exhibiting heterosis (hybrid vigor) were generated from inbred parents with increasing genetic distance. B73 was used as the common female parent in crosses with N192 (low heterosis), MO17 (high-heterosis 1), and NC350 (high-heterosis 2). Total and mitochondria enriched proteomes were analyzed from ear shoots of field-grown hybrids and their inbred parents. GeLCMS (1D SDS-PAGE fractionation, trypsin digestion, LTQ Orbitrap nano-RP-LC MS/MS) was used to analyze proteins and spectral counting was used for quantitation. In total, 3,568 proteins were identified and quantified in hybrids including 2,489 in the mitochondria-enriched fraction and 2,162 in the total-protein fraction. Sixty-one proteins were differentially abundant ($p<0.05$) in one or both of the high-heterosis hybrids compared with the low-heterosis hybrid. For the total proteome, 8 of these showed similar trends in abundance in both of higher-heterosis hybrids. Nine proteins showed this heterosis-correlated pattern in the mitochondrial proteome, including a mitochondria-associated target of rapamycin (TOR) protein. Although differentially abundant proteins belong to various pathways, protein and RNA metabolism, and stress responsive proteins were the major classes changed in response to increasing heterosis.

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Abstracts

Poster Presentations

P-1

Proteomic and transcriptomic analysis of narrow odd dwarf (nod), a novel maize mutant affected in development

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Maize is an important agronomic crop as well as a genetic model in plant biology. Identification of the genetic basis of mutant phenotypes is helpful in breeding unique varieties. We are characterizing a mutant affected in a number of important traits for maize yield. The narrow odd dwarf (nod) is an EMS mutant and shows severe pleiotropic defects in vegetative and reproductive organs. The affected gene is an *Arabidopsis thaliana* MCA1 ortholog, which complements a Ca2+ transport defective yeast mutant and is a plasma membrane-localized protein. To know the pathways within which NOD functions, we are doing identification of NOD protein interactors by immunoprecipitation (IP), followed by LC-MS/MS and Y2H assays. In addition, we have carried out an RNA-seq analysis comparing wt and nod siblings. Results from the IP show proteins related to Ca2+ transport and hormone signaling, which is being confirmed by BiFC. Analysis of differentially expressed genes from the RNA-seq show overrepresentation of hormone metabolism, signaling, stress and cell wall related genes, which suggests a multifaceted role of NOD in maize developmental processes. All together, the project will help identify the pathways in which NOD is involved, and elucidate its relationship with plant architecture and environmental response.

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P-2

How einkorn (*Triticum monoccocum*) deals with variable nitrogen (N) and sulphur (S) supply during grain development? Exploration through proteome investigation

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The concentration and composition of storage proteins in mature wheat grain and consequently its quality are strongly affected by nitrogen (N) and sulphur (S) supply1. Einkorn (*Triticum monoccocum*), a wheat diploid species was used as a model of bread wheat (*T. aestivum*). Einkorn plants were grown in a greenhouse under controlled conditions with different N and S amounts. Grains were harvested at different stages from 300 to 600°Cd corresponding to the filling phase of grain development. Proteomics approach by gel-free mass spectrometry analysis was used to study effects of N and S nutrition on metabolic proteins (albumins-globulins).
The accumulation of storage proteins was affected by N and S supply, with significant changes in the grain composition at maturity. In particular, the accumulation of S-rich and S-poor storage proteins were differentially impacted by S supply. Some metabolic proteins (155 albumins-globulins) were also differentially accumulated in response to the nutrition. A number of proteins involved in key biological functions were either up- or down-regulated. We focus on the data set obtained in response to S supply in order to highlight some proteins and underline mechanisms occurring in response to nutrition in wheat grain.

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P-3

Comparative proteomics of male sterile *Arabidopsis* mutant with silenced RING-type E3 ubiquitin ligase

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Proteins undergo multiple post-translational modifications which considerably influence their functions. Ubiquitination usually marks proteins for degradation by a 26S proteasome pathway; notably, this process is involved in the regulation of plant development. *Arabidopsis* RING-type E3 ubiquitin ligase DAF-like gene 1 (DAFL1) expresses during early stages of flower development, both in a pistil and an ovule. Transgenic plants with silenced DAFL1 produce indehiscent anthers, causing male sterility. We performed comparative proteomic analysis to highlight the proteins affected by the DAFL1 mutation. Upon protein extraction and digestion, we used a long-gradient single-step chromatographic profiling on a reverse phase column. Mass spectra were acquired in a data independent mode. Relative protein quantification was done by peak area integration in the Progenesis QI software. Functional implications from this study will be visualized in a poster.

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P-4

Proteomic studies of somatic embryogenesis phenomenon in the tree fern *Cyathea delgadii*

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Presented results concern proteomic analysis of an unique system of somatic embryogenesis (SE) in fern. Protein profiles of stipes and internodes explants that manifest a different model of SE induction, single and multi-cell respectively, were compared. For this purpose 2D-PAGE coupled with LC-MS/MS was used. Differentiating protein spots between control and treated material as also types of explant were identified. Proteins associated with the cell organization as also defense and stress response were more numerous internodes. In stipes, proteome was a greater share of proteins related with protein metabolism also more proteins related to amino acid metabolism were induced. Single identified proteins were categorized as universal for both or specific for one type of explant and induction path. This allows for a broad interpretation of the changes leading to a different way of SE induction in the same plant species.

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P-5

An analysis of protein phosphorylation in immature soybeans harvested from radio-contaminated Chernobyl environment

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Post-translational modifications have a key role in many biological processes. Herein we performed an initial study of protein phosphorylation using soybeans harvested from plants grown in radio-contaminated Chernobyl area for five generations. For this purpose, proteins were isolated from immature soybeans harvested at five weeks after flowering (WAF) from plants grown in the radio-contaminated and non-radioactive Chernobyl areas. Isolated proteins were comparatively analyzed using two-dimensional gel electrophoresis (2-DE) phosphoproteomics approach which included ProQ Diamond staining, computer-assisted 2-DE gel analysis, and tandem mass spectrometry. This study showed that the abundances of six putative phosphoproteins, namely, two β-subunits of β- and one α-conglycinin seed storage protein, acyltransferase, peroxiredoxin, and sucrose synthase were significantly altered in 5WAF Chernobyl soybeans.

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P-6

Proteomic studies in pearl millet (*Pennisetum glaucum* (L.) R. Br.) under drought stress condition

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Pearl millet [*Pennisetum glaucum* (L.) R.Br] is the fifth most important cereal crop in the world after rice, wheat, maize, and sorghum. Shotgun proteomic approach[1] was applied for the identification and quantification of proteins from different tissues under drought and control conditions. Drought stress was measured using sensors (ML3 Theta Probe)[2]. Drought stressed plants showed significant changes in stomatal conductance and increased root growth compared to the control plants. Root, leaf, and seed tissues were harvested and 2281 proteins were identified and quantified in total. Leaf tissue showed the largest number of significant changes (120), followed by roots (25) and seeds (10). Increased root length impaired shoot-root communication under drought stress. Proteins with a high correlation to the harvest index (HI) were identified using sparse partial least square (sPLS) analysis[2].

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P-7

Label free quantitative proteomic approach highlights the involvement of a novel cystein protease in rice - *Xanthomonas oryzae* interaction

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Bacterial blight disease, caused by *Xanthomonas oryzae* (Xoo), is one of the most serious threats to rice productivity. First interaction between the rice and Xoo takes place in the host apoplast and is mediated by secretion of various proteins from both the partners1. Here, we employed a label-free quantitative proteomics approach to identify the secreted Xoo proteins isolated from in vitro and in planta culture conditions. In total 766 Xoo secreted proteins were identified wherein 441 and 314 were from in vitro and in planta conditions, respectively. Gene Ontology analysis revealed that the identified proteins were majorly involved in catalytic, transporter, and ATPase activities. In particular, a Xoo secreted cysteine protease (XoCP) showed drastically increased abundance during in planta infection conditions. Knock-out mutants of XoCP showed reduced pathogenicity on rice, highlighting the involvement of XoCP in Xoo virulence. In parallel, 186 rice secreted proteins, mainly associated with the catalytic, antioxidant, and electron carrier activities were also identified during in planta analysis. Taken together, our quantitative secretome analysis provided new clues of rice-Xoo interaction, which contribute to the understanding of Xoo pathogenicity.

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Acknowledgements

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P-8

Arabidopsis thaliana gonidialess A (AtGlsA) are essential for maintenance of meristem integrity

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Because the SAM is the engine of plant growth that provides all of the cells to make organs, the behavior of genes such as AtGlsA is of great interest in plant developmental biology. We functionally characterized the two genes encoding GlsA orthologs annotated in the *A. thaliana* genome. Patterns of expression showed that AtGlsA genes are strongly expressed in SAMs and RAMs and developing embryos in double mutants showed multiple changes in morphology. Double mutants showed stunted growth of aerial and root tissue, the formation of multiple ectopic meristems and effects on cotyledons, leaves, and flowers. The genes STM and BP were upregulated in double mutants whereas CLV3, WUS and AS1 were repressed and lack of AtGlsA expression was also associated with changes in localization of auxin and cytokinin. These results suggest that GlsA is an essential component of the machinery that maintains the integrity of SAM and RAM. The next goal is to understand the function of AtGlsA in the establishment of the *Arabidopsis* SAM. In order to do this, we will find what proteins physically interact with AtGlsA, to can construct a network in which AtGlsA works. This project will help to know how different plant species develop.

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P-9

Chloroplast proteome of *Malus x domestica*

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Recent genome sequencing of important crop species of the Rosaceae family, such as *Malus x domestica* (apple), *Prunus persica* (peach), *Fragaria vesca* (woodland strawberry),has made it easier to analyze the proteomes of these species. The subcellular localization of proteins can be predicted in silico from known transit peptide sequences[1] and sequence similarity to model species such as *Arabidopsis thaliana*[2]. However, there are limited experimental data on subcellular localization of proteins in these species.
We purified chloroplasts from *Malus x domestica* spp. 'Golden Delicious', by centrifugation in percoll gradient[3]. The purity of the chloroplast fractions was evaluated by microscopy and immunoblotting. The proteins were pre-fractioned using SDS-PAGE, in-gel trypsin digested and analysed by nano-LC-MS/MS. Of the identified proteins, 5% did not have annotated subcellular localization and 25% were not predicted to localize to the chloroplast.

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P-10

X-ray induced protein footprinting in oleosines: “Seed solvent and sun“

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Radiolytic footprinting based on synchrotron radiation is a very powerful technique to access the solvent accessible surface of biopolymers[1].
In this poster, we present the approach we set up on Metrology beamline at synchrotron Soleil. This technic has been used to study interactions of different protein interactions: a human soluble protein from the innate immune complement[2] (protein-protein interactions), and from oil bodies (OBs) associated plant seed proteins (protein-lipid interactions).

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P-11

Proteomic and small RNA characterization of two coffee leaf rust races with different pathogenic behavior

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The obligate biotrophic rust fungus *Hemileia vastatrix* (Coffee Leaf Rust), that is the most widespread pathogen of the *Arabica coffee* plants, causes severe yield losses. More than 50 rust physiological races have been detected by means of 23 coffee differentials (Várzea et al. 2009), but the molecular identification has still not been possible. We have now identified in uredospores of two rust races (II and XV) proteins by 2-DE/MS and small RNAs by high-throughput sequencing. The 2-DE gels revealed that 33 polypeptide spots, out of more than 300, changed in abundance between the two rust races. When analyzing the small RNA profiles by the miRPursuit workflow (http://goo.gl/opMkZn) and using The Third Hybrid assembly (Cristancho et al. 2014) as the reference genome for *H. vastatrix*, it was possible to predict the existence of more than 300 tasi-RNA and 700 novel-miRNAs. The small RNA profiles were quite different between the two rust races and about 500 of the classified small RNAs were differentially expressed. The role of the differentially expressed proteins and small RNAs will be further discussed. With this integrated approach, we have identified differences between the two races that will potentially contribute to unveil the pathogenic diversity of these rust races.

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P-12

Effect of nitrogen fertilization and training system in SDS-PAGE protein profiles of almond and hazelnut cultivars

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Almond (*Prunus dulcis* (Mill.) D.A.Webb.) and hazelnut (*Corylus avellana* L.) are among the most important nut crops, both in Europe and in the Portuguese nut industry. In view of the level of protein that this two crop presents the objective of this work was to study the Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) patterns of one almond cultivar ('Masbovera') under 8 different nitrogen treatments and two hazelnut cultivars ('Butler' and 'Segorbe') under 2 training systems. Results obtained show that protein patterns of different hazelnut and almond cultivars revealed by SDS-PAGE and Coomassie Blue were quite similar. However, a different profile in particular of low molecular weight proteins was observed in almonds treated with 50 kg N/ha. The comparison between hazelnut cultivar x training system combinations allows observing a repetition of the protein profiles, in particular, a repetition of number, position, and intensity of bands. A total of 38 bands corresponding to HMW globulins and 29 bands corresponding to LMW globulins were identified in hazelnut.

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P-13

Allelic diversity of wheat storage proteins of varieties grown in Portugal during 2015

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Knowledge of the diversity of storage proteins, which are the major gluten components, will greatly increase our understanding of the quality differences that might exist between wheat varieties. We investigated a collection of 33 bread wheat (*Triticum aestivum* L.) and 10 durum wheat (*Triticum durum* Desf.). The composition of wheat storage proteins, namely high molecular weight-glutenin subunits (HMW-GS), low molecular weight-glutenin subunits (LMW-GS) and □-gliadins were characterized through sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE). In bread wheat, a total of 17, 21, and 16 different patterns were observed forHMW-GS, LMW-GS and ω-gliadins, respectively. For HMW-GS encoded at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, 4, 6 and 3 alleles were observed, respectively. LMW-GS demonstrated similar polymorphism, as *Glu-A3*, *Glu-B3* and *Glu-D3* loci, which comprises 4, 8 and 3 alleles. Sixteen alleles were observed for ω -gliadins found at *Gli-A1*, *Gli-B1* and *Gli-D1* loci with, 4, 9 and 3 alleles respectively. In durum wheat, a total of 19 alleles were identified for seven loci studied: *Glu-A1* (1), *Glu-B1* (4), *Glu-A3* (2), *Glu-B3* (2), *Glu-B2* (2), *Gli-A1* (2) and *Gli-B1* (6). The most common patterns for HMW-GS of was 2*-7+8-5+10 and N-7+8 for bread and durum wheats, respectively.

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P-14

A 1D LC-MS/MS investigation of the *Arabidopsis* root response to cytokinin

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Cytokinins (CKs) have a profound effect on plant development and the development of CK responses is achieved through the direct regulation of CK-sensitive, primary target genes, but also through their interactions with other signal pathways, including those of light perception, Ca^{++} and other hormonal signalling pathways.
Although the main molecular components involved in the multi-step phosphor-relay pathway (MSP) of CK signalling are known, the molecular mechanisms by which CK interacts with other signalling pathways have not been fully explored.
To address this, we are developing 1D LC-MS/MS (Orbitrap) studies of early CK-sensitive changes in total and kinase-enriched (2h) and phosphoproteomic samples (30 min, 2 h) prepared from roots of WT and mutant lines deficient in the B-type regulator ARR1, a transcription factor which, together with other B-type regulators, transmits the final step of the CK MSP to primary, CK target genes. The mutant line *arr1-3* demonstrates an altered root morphology and a reduced sensitivity to exogenous CK (BAP). Comparative studies with WT and *arr1-3* enable us to identify exogenous CK-sensitive changes in WT which require a full function MSP, but also to identify CK-sensitive changes in response to endogenous CK signalling.
We will be presenting our latest results and progress to date.

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P-15

A combination of gel based and shotgun proteomics approaches to identify biomarker(s) involved in salt stress in *Panax ginseng*

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Salt stress is one of the major abiotic stresses affecting the yield of ginseng[1]. The objective of this study was to identify bio-marker involved in salt stress. Total leaf and secreted proteins were isolated from 3, 4 and 5 years old ginseng plants exposed to 5 ds/m salt concentration at 0, 24, 72 and 120 hours. A total of 75 secreted proteins were identified using a shot-gun proteomic approach and 10 leaf proteins were identified using a 2-DE based approach. Identified proteins were majorly involved in diverse functional categories including metabolic process and oxidation-reduction process. Western blot analysis revealed that SOD (superoxide dismutase) was accumulated only in 5 years old ginseng leaves exposed to salt stress, indicating SOD as a potential biomarker for salt stress. Screening of SOD in 5 different cultivars of ginseng showed expression pattern of the SOD was consistent with the salt stress tolerance capacity of different ginseng cultivars, further suggesting the potential role of SOD in the early response to salt stress in ginseng leaves.

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P-16

Exploring a method to identify proteases that degrade gluten proteins in wheat

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Large variation in breadmaking quality of Norwegian wheat has been a serious problem for the milling industry. Our resent study suggested that exogenous proteases derived from microorganisms, such as *Fusarium spp*., may cause a degradation of gluten proteins, which are important for breadmaking. Our goal is to identify proteases that have the ability to degrade gluten proteins and identify their respective origin.
Wheat grains that were infected with *F. graminearum* were exploited for establishing a method to identify the proteases. Salt-soluble proteins were extracted from Fusarium infected grains and protease activities were analyzed with zymography. Salt-soluble proteins were also separated with gel-based methods and size-exclusion chromatography followed by LC-MS/MS to identify candidate proteases.
High protease activities were observed with Zymography using SDS-PAGE gels containing copolymerized gluten or gelatin. However, a very weak or almost no band was visible at corresponding regions in silver stained SDS-PAGE gels, which made it difficult to identify candidate proteases by LC-MS/MS analysis. Therefore, proteins were separated by chromatography and fractions with high protease activities were analyzed by LC-MS/MS. Our current results showed some difficulties to identify proteases derived from *F. graminearum* due to: 1) the amount of wheat proteins dominate compared to fungal proteins and 2) the amount of proteases derived from *F. graminearum* appears to be very low.

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P-17

Revealing metabolic pathways of barley seedlings during initial stages of growth using a gel-based proteomics approach

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Hordeum vulgare, commonly known as barley, is an important cereal crop worldwide. Young barley seedlings are under rapid and complex biological process that gives rise to a new generation. However, the molecular mechanisms, especially during initial stage of seedlings growth, are still not fully understood. Here, we utilized a gel-based proteomics approach to investigate the changes in barley proteome during early stages of barley seedlings growth. Proteins from a different stage of barley seedlings were isolated using Tris-Mg/NP-40 buffer and resolved on high-resolution 2D gels. A total of 63 differentially modulated spots were identified in 2 to 4 days germinated seedlings compared to that of one day germinated seedlings. Among those proteins, 21 proteins were up-regulated and 42 proteins were down-regulated. Gene ontology analysis revealed that proteins involved in methionine metabolism, amino acid biosynthesis, energy metabolism, and defense response were up-regulated; while over than half of down-regulated proteins were associated with the metabolic processes, and cell division. Taken together, these results enriched our knowledge of the biological process in young seedlings of barley.

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P-18

Proteomic responses of *Nicotiana tabacum* seedlings exposed to differently coated silver nanoparticles

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Interest in small sized (1-100 nm) nanoparticles (NPs) is growing rapidly. However, the same characteristics making them so attractive for exploitation in new products have led to concerns that NPs may pose a risk to the environment and human health. Among different available NPs, silver nanoparticles (AgNPs) are of particular interest because of their well-known antibacterial and antifungal properties and can be found in a variety of consumer products. Since plants play a significant role in accumulation and biodistribution of many environmentally released substances, they could be influenced by AgNPs, serving as a potential pathway for AgNP-transport and bioaccumulation into food chains. Current proteomics-based techniques have been scarcely used on plants to analyse the effects of AgNPs. Our research goal was to determine the influence of differently coated AgNPs on tobacco seedlings proteome. Seedlings were grown from seeds in a liquid ½ MS medium supplemented with 100 µM citrate-, PEG- and bPEI-AgNP. After 10 days proteins were extracted from whole seedlings and analysed with MALDI-TOF/TOF. MS spectra were identified with MASCOT and analysed with R statistical and proteomics packages. Here we present changes in proteomes of tobacco seedlings upon AgNP treatments and discuss differences in proteome response dependent on different AgNP-coatings used.

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P-19

Factors affecting cysteine availability in seeds: a possible role of apoplastic gamma-glutamyl transferases ggt1?

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In animals, glutathione (GSH) reportedly functions in cys delivery to organs and tissues demanding it. In plants, this function is not documented, but the existence of a gamma glutamyl cycle also in plant cells, together with the observation that GSH is abundant in phloem, suggests that a similar function could be operating in plants too.
A key component in the gamma-glutamyl cycle is the extracellular enzyme gamma-glutamyl transferase (GGT). We speculated that these isoforms could be involved in cysteine delivery to the seed, and to demonstrate that, we carried out an iTRAQ labelling experiment for relative peptide quantification by LC-MS-MS analysis. Following iTRAQ labelling, identification and relative quantification of proteins were achieved by LC-MS/MS analysis.
Based on our results indicating that the cys rich 2S albumin expression is reduced in the GGT1 mutant compared with the wildtype, we propose that apoplastic GGTs are involved in the delivery of cysteine to the seed. Another major alteration was observed in the amount of glutathione S-transferase (GSTF2 and GSTF6) and peroxidase (PER34), which reportedly play a role in response to different stress situations; hence, GGT may be assumed to be involved in redox signalling or sensing to the cell[1].

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P-20

Gel-free proteomic analysis of soybean seed (*Glycine max*) under artificial aging storage conditions

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Soybean seed deterioration (or aging) is negatively affected their economic value and quality for soy-food quality, seed longevity and vigor. However, the complex behavior of soybean seeds during aging is poorly understood. Soybean seeds were stored under artificial aging condition (42°C, RH 99.9%) for 3 and 7 days were used for label free quantitative proteome analysis. Proteins were extracted using Tris Mg/NP-40 followed by precipitation of storage proteins (SPs) by protamine sulfate (PS) to enrich the low-abundance proteins to better understand the seed deterioration physiology. A total of 1368 redundant proteins were identified using Q-Executive Orbitrap of which abundance of 115 proteins (35 and 80 with increased and decreased abundance respectively) changed significantly (>1.5 fold change, $p< 0.05$) during aging condition. Gene ontology and KEGG analyses revealed that proteins with increased abundance were mainly associated with the glycan biosynthesis and metabolism, response to abiotic stimulus whereas proteins related to amino acid metabolism, lipid metabolism and metabolism of terpenoids, polyketides, cofactors, vitamins and developmental process showed decreased abundance during aging conditions. Moreover, Gene ontology results revealed that proteins with decreased abundance were mainly associated with the cellular developmental process and stress responsiveness. Western blot analysis revealed degradation of antioxidant enzymes (DHAR, APX, MDAR, SOD) during aging conditions. Overall, our results suggest an alteration of primary metabolism in aging negatively affects the protein composition of soybean seeds.

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P-21

Proteome changes in tobacco infected by Plum pox virus strains with different pathogenicity

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Plant viruses are a major problem for agriculture since they reduce yield as well as food quality. Stone fruit trees-infecting Plum pox virus (PPV, genus *Potyvirus*, family Potyviridae) represents a typical epidemiologically important case. Among multiple PPV strains, only PPV-C and PPV-CR are able to infect cherries under natural conditions. Interestingly, although sharing this unique feature, their genomes differ by 16.5%[1]. In this work, we studied a response of the host—model plant *Nicotiana benthamiana*—to individual infection caused by both cherry-attacking PPV strains. ELISA test showed a faster PPV-CR accumulation comparing to PPV-C. This finding was in line with observed phenotypic symptoms on leaves. To understand molecular details of the host response to viral strains with different pathogenicity, total proteins from leaves were isolated by phenol extraction/ammonium acetate precipitation protocol. Following that, we quantified changes in relative abundance of proteins, caused by viral inoculation; by the label-free method, using an ultra-high performance liquid chromatography coupled to a tandem mass spectrometry.

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P-22

In clean soil again. Proteome analysis of soybeans harvested from plants grown in clean soil after eight years in Chernobyl area

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Since 2007, our experiments are focused on plant adaptation in the radio-contaminated area from proteomics perspective. The aim of this study is to comparatively investigate seed proteome of soybean (*Glycine max* [L.] Merr. variety Soniancha) transferred to clean soil after eight years of growth in radio-contaminated Chernobyl area (eight generation). For this purpose, proteins isolated from mature soybeans are analysed using two-dimensional electrophoresis (2-DE) proteomics approach. This approach includes 24cm pH 4-7 IPG strips, SYPRO Ruby gel staining, computer-assisted 2-DE gel analysis, and tandem mass spectrometry. This on-going study will provide the characterization of the recovery of soybean's seed proteome in the clean environment after growth in radio-contaminated Chernobyl area for eight generations.

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P-23

Tips and tricks of antibody production and validation process – how to obtain good results?

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Antibodies are a popular tool used in plant cell biology research. They can be either custom made or purchased from a commercial supplier. In either case, their production is a complex process, consisting of three very important components which have to be carefully considered. These are: Antigen-Animal-Testing. Which source of antigen is most optimal for your project: a peptide, recombinant protein or a native protein isolated from tissue? Which animal species to choose? Are certain species making better antibodies compare to others? Do I have any controls to validate produced antibody? What controls should be used? What to do if my antibody is not giving any signal in a western blot? Is there a protocol for not working antibody?

P-24

Systems biology explains how agricultural plants withstand chronic ionizing radiation

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Our group focuses on the plant adaptation towards a low-dose chronic ionizing radiation. This environmental stress factor is common, since (i) there are natural radioecological anomalies, (ii) some lands were polluted from nuclear weapon tests, (iii) other territories were affected by radioactive waste leakages or nuclear power plant accidents (for instance at Chernobyl and Fukushima). Plants which were exposed to radionuclide contamination show characteristic "adaptation symptoms": a decreased yield, an earlier flowering/senescence, and multiple changes in metabolic pathways. The aim of this project was to use different temporary approaches to evaluate transgenerational changes of metabolic pathways, as a result of soybean and flax adaptation to the environment in the Chernobyl alienation zone. We used a combination of traditional biological methods (as radiation mutagenesis) and molecular analyses (as genomic screening, cytogenetics, and proteomics) for systemic evaluation of plant's response to chronic ionizing radiation. In our hands proteomics proved to be efficient for the elucidation of mechanisms of plant's reaction to low-dose chronic ionizing radiation, improving understanding of metabolic network adjustment.

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P-25

Proteome changes induced by cold acclimation in red clover (*Trifolium pratense* L.) populations recurrently selected for improved freezing tolerance

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Cold acclimation (CA) allows improving plant freezing tolerance. Red clover is a leading forage legume in Northern hemisphere but compared to alfalfa, it is characterized by rapid spring establishment and superior performance on acid and wet soils[1]. Red clover is however considered as a short-lived perennial partly due to poor winter hardiness. In this study[2], the freezing tolerance (FT) was assessed and the proteome of non-acclimated and cold-acclimated plants of 2 cultivars of red clover compared: Endure (E-TF0) and Christie (C-TF0) and of populations issued from these cultivars after 3 (TF3) and 4 (TF4) cycles of phenotypic recurrent selection for superior freezing tolerance. FT (LT50) increased markedly from approximately −2 to −16°C following CA. Recurrent selection allowed a significant increase of the LT50 after 4 cycles. PCA on 2D-DIGE results revealed that the largest variability was related to the CA treatment and that the 2 genetic backgrounds had differential protein composition in the acclimated state only. In conclusion, recurrent selection performed indoor is an effective approach to improve the FT of red clover. This significant improvement of FT was associated with differential accumulation of a small number of cold-regulated proteins playing an important role in the determination of the FT level.

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P-26

Phosphoproteome dynamic is involved in *Chlamydomonas reinhardtii* adaptation to nitrogen depletion and recovery.

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Nitrogen (N) deprivation and replenishment induce massive changes at the physiological and molecular level of the green algae *Chlamydomonas reinhardtii*. We performed a label-free in vivo phosphoproteomics approach to enable integration of our dataset with proteomics, metabolomics, and physiological ones. For that, we followed our recent setup of nitrogen depletion and recovery experiment[1]. Uni- and multi-variate analysis revealed a very rapid and highly dynamic adaptation of the phosphoproteome to nitrogen depletion and recovery. Proteins involved in intracellular signaling and adaptation such as kinases and phosphatases, transcription factors and stress signaling-related proteins seems to be phosphorylated during the early N stress adaptation. To reveal interconnections between physiological, proteome, metabolome and phosphoproteome dataset, both sPLS and Pearson correlation analysis were performed. Additionally, we focused on the pattern observed between proteins (from proteomic analysis) and their corresponding phosphopeptide(s) highlighting the potential effect of phosphorylation events on proteins. Here, we provide the first experimental phospho-dataset for chlamydomonas N availability adaptation.

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P-27

Quantitative phosphoproteomics data need to be interpreted in relation to the corresponding protein abundances

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Phosphoproteomics techniques are used to decipher signalling processes and *in vivo* phosphorylation in diverse biological processes. However, it is not always clear whether the detected site-specific phosphorylation changes originate from changes in kinase/phosphatase activities or changes at the protein level. Therefore, it is important to discuss whether phosphoproteomics data can be interpreted without information about protein abundances. Firstly, we performed a literature survey of how many of the phosphoproteomics analyses included the quantification of total proteins within the four highest impact journals in the proteomics field in 2015 and 2016. Secondly, we analysed the overlap of protein IDs between phosphoproteomics and proteomics results in those studies, and finally picked examples to highlight different possibilities to interpret the data and to reveal potential errors, which result from the separate analysis of the phosphorylated peptides. We found that only a few studies had performed total protein measurements together with phosphoproteomics and even fewer compared these datasets. Nevertheless, several examples underline the weaknesses of conclusions drawn only from the phosphoproteomics analyses. In conclusion, it can be suggested that we should aim to analyse phosphoproteomics data in combination with total proteome measurements.

P-28

Comparative proteomics on *Arabidopsis* MAP2K overexpressor line and MAP3K mutants

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Modern proteomics provides new information about mitogen-activated protein kinases (MAPKs) in plants. Here we provide shot-gun proteomic analyses performed on *Arabidopsis* plants overexpressing *Medicago sativa* stress-induced MAPKK (SIMKK) and on two loss- and gain-of-function *Arabidopsis* mutants yda1 and ΔNyda1 defective in the MAPKKK4 gene called YODA. Comparative proteomic analysis of *Arabidopsis* SIMKK-YFP overexpressor line versus wild type showed decreased abundances of proteins involved in salt-induced oxidative stress. On the other hand, auxin-related phenotypes of yda and ΔNyda1 mutants were accompanied by changes in abundances of auxin biosynthetic proteins. When supported by appropriate biochemical and cell biological validation we consider this proteomic approach feasible for functional characterization of respective kinases including molecular interpretation of phenotypes and stress responses in related transgenic and mutant plants.

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P-29

Establishment of a Mass Western approach linking quantitative subcellular protein and organelle distribution analyses of *Pisum sativum* cultivars upon pathogen attack

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The aim of the present study was to analyze the subcellular distribution of the leaf proteome of *Pisum sativum* cultivars upon pathogen infection (*Didymela pinodes*). A combination of subcellular non-aqueous fractionation (NAF)[1] and LC-MS/MS was applied to assign proteins to cell compartments.
Based on subcellular and proteotypic distinctions, marker peptides of the chloroplast, mitochondria and vacuole were selected and synthesized as heavy isotope labelled standards. The Mass Western approach[2] for accurate stoichiometry targeted absolute quantification, allowed for the determination of the proportional organelle abundances measure. Confocal Laser Scanning Microscopy was used to verify these results. The more sensitive Mass Western additionally allowed for a cultivar specific discrimination of the mitochondria to vacuole relation. Furthermore, multivariate statistics enabled the identification of several significant changes of the proteome upon pathogen attack and across cell compartments.

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P-30

Comparison between proteome and transcriptome response in potato (*Solanum tuberosum* L.) leaves after PVY infection

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Molecular mechanisms underlying plant defense response can be studied on different molecular levels (transcripts, proteins, metabolites). Linking of different levels provides a better understanding of the whole system. We have investigated the proteome and transcriptome response in potato (*Solanum tuberosum* L.) leaves the following infection with potato virus Y (PVY) as described in 1. To compare proteome and transcriptome data each protein was mapped to the corresponding potato transcript according to StNIB putative paralogue grouping (2). Transcriptomic results were designated to each protein whose concentration was statistically significantly influenced by PVY infection. The expression profiles on transcriptome and proteome level correlate only partly. We conclude that the reason for the discordance between transcript and protein abundance lies partly in complexity and structure of biological regulation of proteome and transcriptome and partly in technical issues.

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P-31

Identification of proteins from tobacco seedlings (*Nicotiana tabacum*) exposed to silver nanoparticles from 2-D gel electrophoresis using AssayMAP technology

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Nanoparticles research is currently an area of intense scientific surveys. Among different NPs, silver NPs are of particular interest because of their antibacterial and antifungal properties. AgNPs can be found in many different consumer products. Since plants play a significant role in accumulation and biodistribution of many environmentally released substances, they could serve as a potential pathway for AgNP-transport and bioaccumulation into food chains. Current proteomics-based techniques have been scarcely used on plants to analyse the effects of AgNPs. Our research goal was to develop the method to identify proteins from 2-D-gels using liquid handling chromatography. Tobacco seedlings were treated with 100µM citrate-AgNP. 10th day of treatment, proteins were extracted and separated on IEF, pH 3-10 NL gradient strips, followed by 2-D-PAGE. Proteins were detected with Coomassie BB. Tryptic peptides were separated and purified using Bravo AssayMAP instrument. 50 protein spots were identified using MALDI-TOF/TOF analyzer. 11 protein spots which were not identified in the first instance were repeated and subjected to 1-D strong cation-exchange fractionation at pH10/pH2.5 and C18 2-D fractionation using AssayMAP instrument. Until now, we successfully fractionated and identified several important but difficult-to-identify proteins especially ones with glycine-rich domains. With this method, we will be able to identify proteins which usually remain unidentified with conventional proteomics approach.

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P-32

Deciphering the roles of nitric oxide in the acclimation of banana to drought stress at the proteome level

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Water deficit caused by drought is the main threat to crop growth and production[1]. An understanding of the molecular mechanisms that underpin plant stress adaptation is crucial to counter these challenges. Nitric oxide (NO), an important signal molecule, plays diverse roles in plant growth and defensive responses. In this study, we investigated the role of NO in alleviating drought stress in banana. We found that PEG significantly reduced plant growth. The application of SNP, however, significantly alleviated the inhibiting effect of the drought, whereas the NO scavenger reversed the effect, suggesting the involvement of NO in the process. The effect of NO is a dose-dependent manner as the enhancement effect was reduced at the higher concentration of NO (≥20 μM). We applied a gel-based proteomic technique to investigate the response to drought stress to define the role of NO. Ten from 72 differentially expressed protein spots were identified using LC-MS/MS analysis. The majority of these proteins were classified as defensive and stress-response proteins. These findings suggest that exogenously applied NO can appreciably improve drought tolerance in banana, affirming its role in this stress-survival mechanism.

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P-33

Protein composition readjustment in *Arabidopsis thaliana* roots following sulfadiazine treatment

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The application of manure, slurry, and biosolids to fertilize arable lands is one of the major means through which pharmaceuticals enter the environment. The presence of antibiotics in arable soils could lead to accumulation in food crops and impact plant growth[1]. Studies on the toxicity of these substances towards plants are rarely studied through biochemical and proteomic approaches. Aim of this experiment is to study the response of *Arabidopsis thaliana* to Sulfadiazine (SDZ), a sulfonamide antibiotic compound, by analysing some parameters related to oxidative stress (ascorbate, glutathione and MDA contents), and measuring the activity of some enzymes involved in xenobiotic detoxification (glutathione S-transferase) and glutathione metabolism (gamma-glutamyl-transferase). Moreover, the combined iTRAQ and LC-MS/MS-based quantitative proteomics approach was applied. Biochemical parameters do not indicate that SDZ induces oxidative stress in *Arabidopsis*, whereas among the two enzymes studied, only GST activity shows an increase in treated plants. Instead, when we compared treated with control plants a clear upregulation of specific peroxidases (PER22, PER32, PER39, PER34) was highlighted. This result is in line with some findings in other biological systems, claiming at a role of peroxidases in the conjugation of SDZ with natural substrates, acting as a detoxification process.

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P-34

Differential priming of *Pisum sativum* cultivars by AMF and Rhizobia

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Because of their nutritional value to man and life stock, legumes account for a big part of agricultural production. Due to their symbiotic interactions (Rhizobia & arbuscular mycorrhizal fungi - AMF) which enhance nutritional uptake, they substantially contribute to sustainable agriculture. Each legume is capable of forming a symbiosis with particular Rhizobia and commonly several species of AMF. The interaction with Rhizobia is to a great extent controlled by the plant and each species shows different nodule morphology. With regard to breeding strategies, agronomy is interested in the effect of below ground parts on above-ground traits (e.g. biomass, pathogen resistance levels, and yield)[1]. We tested the effect of single and co-inoculation with Rhizobia and AMF on the plants´ morphology as well as the leaf proteome and metabolome in two cultivars of *P. sativum*. The nodulation profile (weight and number of nodules) is remarkably distinct among cultivars and the proteome shows predominantly cultivar related effects rather than effects of the symbionts. However, we found that single Rhizobia or AMF inoculation showed the utmost effect on the proteome in a cultivar-specific manner. As the intensity of the host-symbiont interaction over a plants´ lifespan usually varies between cultivars, we further aim to elucidate the nodules´ morphology as well as its proteome in a time series. These insights about cultivar specific symbiotic interaction provide knowledge for advanced sustainable breeding strategies.

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P-35

Increased expression of ClpD1 protease is correlated with increased drought tolerance in rice

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Rice is the major staple food for more than half of the world's population. As global climate changes, we are observing more floods, droughts, and severe heat waves. Two rice cultivars with contrasting genetic backgrounds and levels of tolerance to drought, Nipponbare and IAC1131, were used in this study. Four-week-old seedlings of both cultivars were grown in large soil volumes and then exposed to moderate and extreme drought for 7 days, followed by 3 days of re-watering. Mature leaves were harvested from plants from each treatment for protein extraction and subsequent shotgun proteomic analysis, with validation of selected proteins by western blotting. Gene Ontology (GO) annotations of differentially expressed proteins provide insights into the metabolic pathways that are involved in drought stress resistance. Our data indicate that IAC1131 appears to be better able to cope with stressful conditions by up-regulating a suite of stress and defence response related proteins. Nipponbare, in contrast, lacks the range of stress responses shown by the more stress tolerant variety, and responds to drought stress by initiating a partial shutdown of chlorophyll biosynthesis in an apparent attempt to preserve resources.

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The Common Bean (*Phaseolus vulgaris* L.) Shoot Proteome under Mineral Stress

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Common bean (*Phaseolus vulgaris* L.) is a nutritionally as well as a medicinally valuable legume food crop. Common bean is considered as a legume model to unravel the responses and adaptation to Fe, Zn and P deficiencies.

The current study reports the impact of Fe deficiency and excess Zn on the leaf proteome of 15-days-old common bean seedlings (variety VLR-125). Physio-morphological parameters revealed that Fe deficiency and excess Zn had a similar impact. In the proteomics analysis, 46 proteins were found up-regulated and 26 proteins down-regulated in Fe deficiency compared to control. Similarly, 9 proteins were observed up-regulated and 6 proteins were observed down-regulated in excess Zn condition compared to control. Further, 7 proteins were observed up-regulated and 5 proteins down-regulated under both Fe deficiency and excess Zn conditions indicating the possibility of cross talk under such conditions. This work provides a comprehensive model to understand, the adaptive mechanism used by common bean shoots under Fe deficiency/excess Zn conditions. However, further, in-depth proteome analysis is required to understand the cross-talk under Fe deficiency and excess Zn in common bean.

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Minimisation of the inter-user variation of differential expression analysis using SameSpots software for 2D gel analysis

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Differential expression analysis using 2D gel electrophoresis is a staple technology in plant proteomics. SameSpots image analysis software was developed to provide a reproducible, fast and simple solution to the challenges of 2D gel analysis. Quality checking features throughout the analysis, its alignment based approach which solved the problem of missing values and the inbuilt multivariate statistics features make SameSpots an ideal solution to the challenge of 2D analysis. The inter-user variation of the software was assessed in this analysis. Analysts with a range of experience from a novice with minimal training to a technical expert analysed the same data set and results were compared between users. The rank position of the top spots with statistically significant changes (ANOVA P value <0.05) between conditions were identified and compared between users.

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COST P6

Multiple MS methodologies for the identification of proteolysis resistant proteins

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We demonstrate that chickpea seed proteins are able to resist cooking and in vitro simulated human digestion, and therefore these proteins and peptides have the potential to influence human health. We hypothesized that proteolysis resistant proteins could represent a challenge in MS identification and we made use of different methodologies in order to increase the identification success and confidence. Following 1D/SDS-PAGE, the efficiency of several proteases (trypsin, AspN, chymotrypsin and LysC) was tested, and two MS technologies were employed (MALDI-TOF/TOF, LC-nanoESI-TripleTOF). Using this strategy we are able to identify 312 proteins resistant to in vitro simulated digestion: 237 by LC-MS/MS; 75 by MALDI-MS/MS. Trypsin was found to be the most efficient protease when using LC-MS/MS (n=232) in sharp contrast with AspN (n=8). AspN was shown to provide better results when using MALDI-MS/MS (n=44), but even so lower than trypsin (n=64). The identified proteins corresponded to 115 unique proteins, represented by 124 UniProt accessions. The proteins were found to belong to 31 distinct super families, and the use of LC-ESI-MS/MS allowed us to detect proteins other than seed storage proteins. An in silico digestion of the chickpea genome was performed and it was found that only a small fraction of the proteolysis resistant proteins (experimentally validated) were predicted to resist protease digestion: 13% proteins had been predicted to resist either pepsin or chymotrypsin digestion; 16% proteins had been predicted to resist to trypsin digestion. Our approach proves to be suitable for the analysis of proteins able to resist gastrointestinal digestion, which allows to validating genomic data and provides tools for the refinement of the genomic models.

COST P8

Proteome analysis of crop response to drought stress

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The two-dimensional differential gel electrophoresis (2D-DIGE) analysis enables protein relative quantification leading to an identification of the protein spots revealing an enhanced abundance in stress-treated or stress-tolerant varieties which could be further tested as potential markers of stress tolerance. Proteomic experiments aimed at crop (barley, melon) proteome response to drought were analyzed. The aim of these analyses was to identify protein spots revealing differential abundance between different stress treatments or differently tolerant genotypes that could be potentially used for abiotic stress phenotyping. The majority of potential proteins for phenotyping belong into energy-, stress- and defence-related proteins. The results of proteomic analyses were interpreted with respect to other physiological data such as parameters related to stress tolerance (membrane stability, LT50), water regime-related characteristics (water saturation deficit, osmotic potential), and others. The role of gel-based proteomic analysis in understanding plant stress response and acquisition of plant stress tolerance is discussed.

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COST P13

The effect of cold hardening on leaf protein profile in barley DH lines varying in frost tolerance

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Barley (*Hordeum vulgare* L.) belongs to the most important cereal grains, however, in Polish climatic conditions, its poor winter hardiness results in significant yield losses from cold injury every year. The aim of the presented study was the identification of factors that determine freeze tolerance and could improve selection efficiency of genotypes of potential value for future breeding programmes.
The plant material consisted of 8 doubled haploid (DH) lines produced by anther culture method from Polish breeding materials (F_2 generation). Selected DH lines have been characterized as significantly different in freezing tolerance estimated according to Rapacz et al. (2011). The changes in abundance in protein species were analysed after cold treatment (3 weeks at 4°C) in barley leaves using gel-based proteomics. The proteins were isolated according to the phenol-based procedure (Hajduch et al. 2005) and examined by 2-D electrophoresis and PDQuest Software. Quantitative and qualitative differences in protein expression have been detected. Chosen proteins highly differentiated across examined DH lines were identified by MALDI TOF/TOF MS/MS analysis.

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COST P19

Functional roles of ht1 and mpk12 kinases in co2-induced stomatal responses

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Plants are immobile organisms and to survive they need to respond to the constantly changing environment. The communication between plants and surroundings is mediated by guard cells, highly specialized cells that form stomatal pores. Guard cells sense various signals from the environment and adjust the stomatal pore size to maximize CO2 uptake for photosynthesis and to minimize the water loss by transpiration. The opening and closure of stomatal pores are regulated by dynamic changes of protein phosphorylation in guard cells. Plants possessing several kinase mutants have been shown to be defective in regulating guard cells movement but the direct substrates and roles of these kinases are often unknown.
We have been studying two protein kinases, HT1 and MPK12, as key regulators in CO2-induced stomatal responses[1,2]. Using in vitro kinase assays we show that protein kinase HT1 phosphorylates well-known proteins associated with stomatal pore movement. Additionally, we revealed that HT1 kinase activity is inhibited by MAP kinase MPK12. In the light of these findings, we provide a new model for CO2 signalling in guard cells.

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