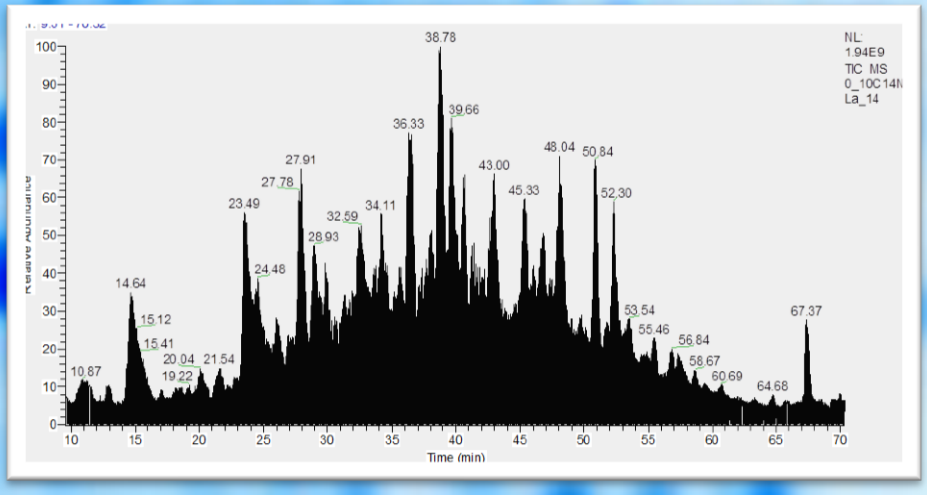
1st INPPO World Congress on Plant Proteomics: Methodology to Biology

August 31st to September 4th, 2014
Hamburg, Germany



Hosted by the University of Hamburg, Germany

The 1st INPPO World Congress on

Plant Proteomics: Methodology to Biology

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Hosted by

Universität Hamburg
DER FORSCHUNG | DER LEHRE | DER BILDUNG

Germany

Dear colleagues,

on behalf of the Scientific Committee of the INPPO World Congress and the local organizing committee it is a great pleasure to welcome you in Hamburg for the 1st World Congress on Plant Proteomics!

INPPO2014 is hosted by the University of Hamburg. About 150 attendants from all over the world will discuss their recent work and projects with leading experts, install collaborations and expand their networks on plant proteomics. About 25% of the participants are students, 12 young researchers were awarded with travel grants. Grantees come from Australia, Austria, Brazil, Canada, Mexico, Portugal, Spain, and South Africa. We would like to thank our sponsors for this support.

The scientific program will offer 12 sessions with over 100 scientific contributions (52 oral presentations and 59 posters). Two round tables will discuss perspectives of plant proteomics and two Doctoral Offices will answer most frequent questions on mass spectrometry and gel-based techniques by experts in the field.

Finally, we would like to thank all contributors for their efforts and their willingness to share their latest results and their success.

We wish you a great and successful scientific meeting and plesant days in Hamburg!

The organizers

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SCIENTIFIC COMMITTEE

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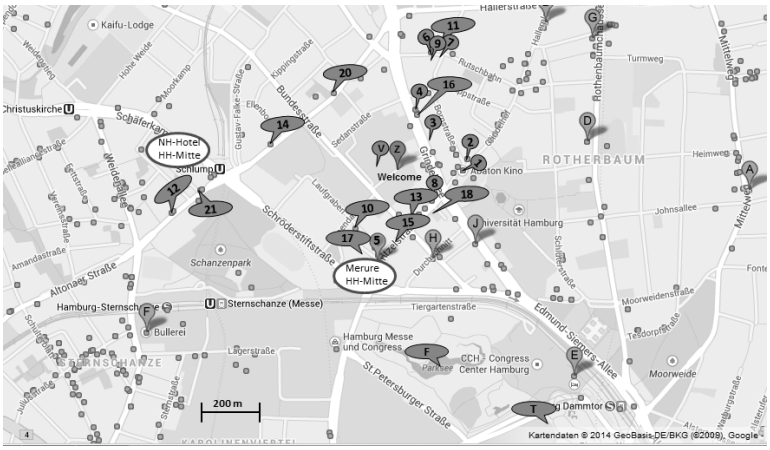
GENERAL INFORMATION

- **Registration and INPPO Office**
  Registration and the INPPO Office will be open on Sunday 4 p.m. to 9 p.m. and during the week from 8 a.m.
- **Contact Persons**
  In case you have any question, do not hesitate to ask the organizers of the conference (red labelled badges).
- **Internet Access**
  Free wireless internet acess (WiFi) will be available at the conference venue. Individual User names and passwords are given in your map and on the back of your badge.
- **Speaker Information**
  Speakers are asked to upload presentations during the breaks, preferently on the evening before the presentation. Presentations should be compatible with Microsoft Office 2007. The duration of oral presentations is 25 min (20 min + 5 min discussion) for invited speakers and 20 min (15 min + 5 min discussion) for short papers. Due to the time schedule of the congress speakers are reliant not to overrun these time limits. Session chair persons will enforce the time schedule.
- **Poster Information**
  All posters have been assigned a poster number. The numbers are indicated on the bottom of each abstract page. Posters should be mounted at the beginning of the conference and will be on display throughout the conference.
- **Conference Dinner**
  To get to the conference dinner you may take the bus **lane 5** from Grindelhof. The bus is going every 5 min. and will take 10 min to Rathausmarkt.
  The restaurant “DAS PARLAMENT” is on the left of the historical building.
- **Lunch**
  Besides small bistros, several restaurants are located around the congress venue. These are indicated on the map on the next page. Additionally, Campus Mensa offers cheap lunches (Von-Melle-Park 2, 20146 Hamburg) Actual menue plan (http://speiseplan.studierendenwerk-hamburg.de/en/340/2014/36/). Vegetarian Link http://www.happycow.net/



Venue, Hotels and Restaurants:

1) Abatin ..... Grindelhof
2) Arkadasch ..... Grindlhof 17
3) Arrabiata ..... Grindelallee 134
4) Backwahn ..... Grindelallee 148
5) Bekaa ..... Rentzelstraße 50
6) Benvenuto ..... Heinrich-Barth-Straße 15
7) Café Nuestro ..... Rutschbahn
8) Curry Grindel ..... Rentzelstraße 2
9) Dieze ..... Heinrich-Barth-Straße 15
10) Dwaraka ..... Rentzelstraße 38
11) Elbfood ..... Heinrich-Barth-Straße 17
12) El Torro ..... Kleiner Schäferkammp 21
13) Falaffelshop ..... Rentzelstraße 16
14) Geo 53 ..... Beim Schlump 53
15) Han ..... Rentzelstraße 36
16) Manu (Vegetarian) ..... Grindelallee 148
17) Peking Enten Haus ..... Rentzelstraße 48
18) Portugal ..... Renzelstraße 11
19) Roxie ..... Rentzelstraße 6
20) Rucola e Parma ..... Beim Schlump 27
21) Sapa Cuisin de Vietnam ..... Kleiner Schäferkamp 14
22) Gopalam Vegetarische Indische Küche ..... Grindelallee 159
23) Golden Temple Teehaus (Vegan) ..... Grindelallee 26
24) Qrito (Vegetarian) ..... Grindelallee 79
F) Water Fountain ..... Parksee, Planten un Blomen
T) Tropical Houses ..... Jungiusstraße 6
V) Venue ..... Martin-Luther-King-Platz 6
Z) Zoological Museum ..... Martin-Luther-King-Platz 3

SCIENTIFIC PROGRAM

Sunday, August 31st 2014	
15:00-17:00	Registration
17:15-18:00	*Opening Ceremony* Sabine Lüthje **1st INPPO World Congress** /Welcome, facts and acknowledgements Dominique Job*, Ganesh Kumar Agrawal, Abhijit Sarkar, and Randeep Rakwal **The First Steps of Establishing INPPO** Ganesh Kumar Agrawal, Abhijit Sarkar, Raj Agrawal, Jenny Renaut, Dominique Job, Randeep Rakwal **INPPO Initiative: Global Platform for Involving, Gathering and Disseminating Plant Proteomics Knowledge**
18:00-19:00	*Public Evening Lecture* Pier Giorgio RIGHETTI (Milano, Italy) **The Monkey King and Pigsy ferrying The Proteomic Sutras into the 3rd Millennium: A Chronicle**
19:00-21:00	Welcome Reception

Monday, September 1st, 2014	
8:00-9:00	Registration, Poster Mounting
9:00-11:00	*Session I: Bioinformatics & Mass Spectrometry* *Chair: Wolfgang Schröder (Umeå, Sweden)*
9:00-9:25	Kathryn Lilley **Characterisation of the Arabidopsis sub-cellular proteome**
9:25-9:45	Kim Buts, Maarten Hertog, Bart Nicolai and Sebastien Carpentier **Label free quantitative proteomics of a complex eukaryote via LC MSMS: data independent analysis (DIA) or data dependent analysis (DDA)?**
9:45-10:05	Christof Rampitsch, Guus Bakkeren, Brent McCallum **Custom database searching to reveal differences in the haustoria proteomes of three races of wheat leaf rust, Puccinia triticina.**
10:05-10:25	Isa Catarina Ribeiro, Sébastien Planchon, Céline Leclercq, Nuno Simões, António Toureiro, Isabel Duarte, Miguel Costa, João Bengala Freire, Manuela Chaves, Jenny Renaut, Carla Pinheiro **Identification of potentially bio-active proteins in chickpea (Cicer arietinum L.) seed**
10:25-10:45	Stefanie Wienkoop **Novel strategy for SRM based protein absolute quantification**
11:00-11:30	*Coffee break*
11:30-13:00	*Session II: Systems Biology & Mass Spectrometry* *Chair: Wolfram Weckwerth (Vienna, Austria)*
11:30-11:55	Waltraud Schulze **Phosphoproteomic approaches in studying functions of plant kinases**
11:55-12:15	Alexander Graf, Anna Flis, Stitt Mark, Gruissem Wilhelm **Orchestrated responses in protein abundance tune plant metabolism to different photoperiods**
12:15-12:35	Lilian Carmo, Andre M. Murad, Ana Luiza M. Lacerda, Elibio Rech, Renato O. Resende, Simone G. Ribeiro, Angela Mehta **Identification of host proteins modulated in response to Tomato chlorotic mottle virus (ToCMoV)**

12:35-12:55	Mathilde Decourcelle, Jérémy Duval, Hajar Chouiki, Valérie Rofidal, Véronique Santoni, Sonia Hem **Variations of carotenoid pathways in transgenic maize endosperms highlighted by a label-free quantitative proteomics approach**
13:00-14:30	*Lunch & Coffee break* *COST-Meeting*
14:30-16:00	*Session III: Integrated Proteomics* *Chair: Dominique Job (Lyon, France)*
14:30-14:55	Kentaro Tamura, Yoichiro Fukao, Ikuko Hara-Nishimura **Interactive proteomics dissects structural dynamics of plant nuclear pore complex**
14:55-15:15	Marc GALLAND, Romain HUGUET, Erwann ARC, Gwendal CUEFF, Dominique JOB, Loïc RAJJOU **Dynamic proteomics reveals that selective mRNA translation is a key regulatory step during Arabidopsis seed germination**
15:15-15:35	Alfredo Simone Negri, Bhakti Prinsi, Luca Espen **An integrated proteomic and metabolomic approach reveals peculiar traits in the exocarp of four grape cultivars characterized by a different anthocyanin accumulation**
15:35-15:55	Pingfang Yang **Proteomic analysis on rice seed germination**
16:00-16:30	*Coffee break*
16:30-17:30	**Doctoral´s Office — Mass spectrometry**
17:30-19:30	Poster Viewing with Wine & Chees Even numbers

Tuesday, September 2nd, 2014	
8:00-9:00	*Registration open*
9:00-11:00	*Session IV: Quantitative Proteomics* *Chair: Stefanie Wienkoop (Vienna, Austria)*
9:00-9:25	Sacha Baginsky **Functional proteomics offers a new perspective on chloroplast biogenesis and the function of posttranslational modifications**
9:25-9:45	Natalia V. Bykova, Junjie Hu, Natasa Radovanovic, Christof Rampitsch, Frank You, Tao Fan **Quantitative proteomic approaches to revealing mechanisms of seed dormancy control and pre-harvest sprouting resistance in wheat**
9:45-10:05	Madalina Oppermann, Claire Dauly **Multiplexed protein quantification deploying high resolution, accurate mass strategies**
10:05-10:25	Antoine Champagne, Marc Boutry **Proteomics as a tool for monitoring plant secondary metabolism and identifying candidate proteins for unattributed functions**
10:25-10:45	Iniga Seraphina George, Robert D Willows, Paul A Haynes **Quantitative label-free shotgun proteomic analysis of a red grapevine variety exposed to hot and cold temperature stresses.**
11:00-11:30	*Coffee break*
11:30-13:00	*Session V: Post-translational Modifications (Part 1)* *Chair: Thomas Kieselbach (Umeå, Sweden)*
11:30-11:55	Renu Deswal, Ankita Sherawat **Bird's eye view of Nitric Oxide Signaling in cold stress via nitrosylation in Brassica juncea seedlings**
11:55-12:15	Christina Mark, Barba-Espín Gregorio, Zor Kinga, Dedvisitsakul Plaipol, Heiskanen Arto, Hägglund Per, Svensson Birte, Dufva Martin, Emneus Jenny, Christine Finnie **Secretory proteins and programmed cell death: insights from the barley grain aleurone layer**

12:15-12:35	Iris Finkemeier **Exploring the role of lysine acetylation in the regulation of plant metabolism.**
12:35-12:55	Julia Svozil, Wilhelm Gruissem, Katja Bärenfaller **Proteasome targeting of proteins in Arabidopsis leaf mesophyll, epidermal and vascular tissues**
13:00-14:30	*Lunch & Coffee break* Committee Meetings
14:30-16:00	*Session V: Posttranslational Modifications (Part 2)* *Chair: Renu Deswal (Delhi, India)*
14:30-14:55	Magali Di Pietro, Jerome Vialaret, Guo Wei Li, Sonia Hemm, Karine Prado, Christophe Maurel, Veronique Santoni **Quantitative proteomics unravels novel post-translational regulatory mechanisms of plant aquaporins**
14:55-15:15	Niklas Hausmann, Mirita Franz-Wachtel, Boris Macek, Rüdiger Hampp **SILAC for the analysis of phosphorylationevents under micro-gravitation in Arabidopsis thaliana cell cultures**
15:15-15:35	Elisabeth Stes, Kun Yue, Elisabeth Williams, Priyanka Sandal, Evan Murphy, Eveline Van De Slijke, Daniel Van Damme, Geert De Jaeger, A. Gururaj Rao, Tom Beeckman[1], Kris Gevaert, Ive De Smet **Systems approaches to identify ACR4 interactors and substrates in Arabidopsis**
15:35-15:55	Claudia-Nicole Meisrimler, Alexandra Schwendke, Sabine Lüthje **Native 2D-PAGE for comprehensive analysis of native phospho-proteins**
16:00-16:30	Coffee break
16:30-18:30	*Round table I - Plant proteomics and international organizations/initiatives* Jesus Novo-Jorrin, Joshua Heazlewood, Jenny Renaut, Ganesh K. Agrawal, Wolfram Weckwerth
18:30-19:30	Member gathering

Wendsday, September 3ed, 2014	
9:00-11:00	*Session VI: Sub-cellular Proteomics* *Chair : Sabine Lüthje (Hamburg,Germany)*
9:00-9:25	Susana González Fernández-Niño, Harriet Parsons, Roberto de Michele, Joshua Heazlewood **Exploring the plant endomembrane by free-flow electrophoresis**
9:25-9:45	Norbert Rolland, Martino Tomizioli, Cosmin Lazar, Daniel Salvi, Sabine Brugière, Lucas Moyet, Thomas Burger, Giovanni Finazzi, Daphné Berny, Myriam Ferro **Subcellular and subplastidial proteomics**
9:45-10:05	Stefan Helm, Matthes Zessin, Dirk Dobritzsch, Anja Rödiger, Sacha Baginsky **From validation to application: Quantitative multiplex MS analysis (MSE) of chloroplast development**
10:05-10:25	Harriet T Parsons, Christopher J Petzold, Pragya Singh, Hiren J Joshi, Leanne Chan, Joshua L Heazlewood **Using Multiple Reaction Monitoring (MRM) to determine organelle abundance during plant development and organelle isolation procedures**
10:25-10:45	Silvia Mazzuca, Amalia Piro, Ilia Anna Serra, Antonia Spadafora, Monica Cardilio, Linda Bianco, Gaetano Perrotta **Purification of intact chloroplasts from marine plant Posidonia oceanica suitable for organelle proteomics.**
11:00-11:30	*Coffee break*
11:30-13:00	Poster Viewing Odd numbers
13:00-14:30	*Lunch & Coffee break*
14:30-16:00	*Session VII: Gel-based Proteomics (Part 1)* *Chair: Jenny Renaut (Belvaux, Luxembourg)*
14:30-14:55	Jesus V Jorrin-Novo **Do plant proteomics current publications fit in the MIAPEs and scientific standards requirements?**
14:55-15:15	Kim, Sun Tae; Agrawal, Ganesh Kumar; Rakwal, Agrawal; Gupta, Ravi; Min, Chul Woo; Kim, So Wun; Wang, Yiming; Kim, Yong Chul

	Protamine sulfate precipitation: A reliable, simple and efficient depletion method for high abundant storage proteins in oilseeds
15:15-15:35	Pavel Vitamvas, Klara Kosova, Iva Hlavackova, Jenny Renaut, Milan, Oldrich Urban, Ilja, Tom Prasi **Quantitative analysis of proteome extracted from crops under drought stress**
15:35-15:55	Klara Kosova, Pavel Vitamvas, Sebastien Planchon, Jenny Renaut, Radomira Vankova, Ilja Tom Prasil **Proteome analysis of cold response in spring and winter wheat (Triticum aestivum) crowns reveals similarities in stress adaptation and differences in regulatory processes between the growth habits**
16:00-16:30	*Coffee break*
16:30-18:00	*Session VII: Gel-based Proteomics (Part 2)* *Chair: San Tae Kim (South Korea)*
16:30-16:55	Martin Hajduch **Seven years of plant proteomics in radio-contaminated Chernobyl area**
16:55-17:15	Arun Kumaran Anguraj Vadivel, Sangeeta Dhaubhadel **Regulation of Isoflavonoid Biosynthesis and GmMYB176 interactome in soybean**
17:15-17:35	Jayaseelan Murugaiyan, Christoph Weise, Uwe Roesler **Proteomics analysis of 'Orphan Species' - the case of Prototheca zopfii (family Chlorellaceae), the only known plant-like infectious organism**
17:35-17:55	Joanna Porankiewicz-Asplund **Tips and tricks of antibody production and validation process - how to obtain good results?"**
18:00-19:00	**Doctoral´s Office on *Gel-based Methods***
20:00-24:00	Conference Dinner "DAS PARLAMENT"

Thursday, September 4th, 2014	
9:00-11:00	*Session VIII: Plant Growth and Development* *Chair: Michel Zivy (France)*
9:00-9:25	Dominique Job **Proteomics Highlights Specific Features of Ancestral Amborella Trichopoda Seeds**
9:25-9:45	Maria Angelica G. Duarte[1], Amanda L. Medeiros[1], Ana Paula P Costa[2], Adriana F. Uchoa[1], Katia Castanho Scortecci[1] **Identification of differentially expressed proteins in the shoot apical meristem using contrasting sugarcane cultivars for flowering**
9:45-10:05	Norazreen Abd Rahman, Siti Arija Mad Arif **Analysis of ethephon stimulation effects on B serum proteins from Hevea latex using shotgun proteomics approach**
10:05-10:25	Ahmed Debez[1], Bernhard Huchzermeyer[2], Andreas Pich[3], Hans-Peter Braun[4] **Proteomic approach provides new insights into salt-tolerance of the oilseed halophyte Cakile maritime**
10:25-10:45	Christina Mark[1], Kinga Zór[2], Arto Heiskanen[2], Martin Dufva[3], Jenny Emnéus[2], Christine Finnie[1] **Programmed cell death: The life ambition of the barley aleurone layer**
11:00-11:30	*Coffee break*
11:30-13:00	*Session IX: Interaction with Environmental Factors (abiotic/biotic)* *Chair: Carla Pinheiro (Portugal)*
11:30-11:55	Wolfgang Schmidt, Isabel Cristina Velez Bermudez, I-Chun Pan **Translational fitness of messenger RNAs: Impact of environmental signals**
11:55-12:15	Bhakti Prinsi, Luca Espen **Drought effects on root proteome in two grapevine rootstocks with different susceptibility**
12:15-12:35	Melisande Blein-Nicolas, Balliau Thierry, Cabrera-Bosquet Llorens, Corti Hélène, Welcker Claude, Tardieu François, Zivy Michel **Proteomics analysis of the genetic diversity of drought tolerance in maize**

12:35-12:55	Mélanie Mauriat, Stéphane Claverol, Luc Negroni, Marc Bonneu, Jérôme Bartholomé, Céline Lalanne, Nicolas Richet, Jean-Charles Leplé, Catherine Coutand, Christophe Plomion **A quantitative phosphoproteomic approach to decipher the signaling pathway of tension wood formation in poplar**
13:00-14:30	*Lunch & Coffee break*
14:30-16:00	*Session IX: Interaction with Environmental Factors (abiotic/biotic)* *Chair: Laurence Bindschedler (London UK)*
14:30-14:55	Laurence BINDSCHEDLER **Proteo(genomics) to understand molecular plant-pathogen interactions between barley and its powdery mildew**
14:55-15:15	M. José Martinez-Esteso, M. Teresa Vilella-Antón, Susana Sellés-Marchart, Lorena Almagro, Jaime A. Morante-Carriel, Elías Hurtado-Gaitán, M. Angeles Pedreño-García, Roque Bru-Martinez **An omics exploration of elicitor-mediated stilbenoids accumulation in grapevine (Vitis vinifera L.) cell cultures**
15:15-15:35	Gabriela Villeth, Luciano Silva, Mateus Santos, Osmundo Brilhante, Maria Grossi-de-Sá, Igor Ribeiro, Suelen Tameirão, Rodrigo Fragoso, Daiane Ribeiro, Octávio Franco, Angela Mehta **Brassica oleracea-Xanthomonas campestris interaction: challenges and contributions of proteomic methods**
15:35-15:55	Agnieszka Szuba, Leszek Karliński, Łukasz Marczak **Leaf proteomics of ectomycorrhizal poplars**
16:00-16:30	*Coffee break*
16:30-18:00	Round table II - *INPPO Perspectives* Ganesh K. Agrawal, Dominique Job, Jenny Renaut
18:00	Closing, Poster dismounting
19:00-22:00	Farewell – Water fountain

OPENING CEREMONY

THE FIRST STEPS OF ESTABLISHING INPPO

Dominique Job, Ganesh Kumar Agrawal, Abhijit Sarkar, and Randeep Rakwal*

* Proteomics Platform - CNRS / UCB / INSA / Bayer CropScience Joint laboratory (UMR5240) - Lyon, FRANCE

Plants are the fundamental basis of human and animal nutrition. They also provide invaluable resources for both renewable raw material and energy, and they synthesize a great variety of essential molecules (amino acids, vitamins), lipids and secondary metabolites, including highly efficient pharmaceuticals.

Some years ago, benefiting from the enormous progress in plant genome sequencing and the impressive development of large genetic resources for functional genomics in plants, it became clear that proteomics would be instrumental for unraveling the full potential of plants. Notably, this expectation was supported by the success of MASCP (Multinational Arabidopsis Steering Committee), an initiative established to facilitate the coordination of international proteomic research in the model plant *Arabidopsis thaliana*. However, proteomic studies on crops were still in infancy.

Inspired by the HUPO (Human Proteome Organization) initiative, an international plant proteomics organization, termed INPPO (International Plant Proteome Organization; http://www.inppo.com), was established in October 2008, following discussions between Randeep, Ganesh and me. HUPO was for us an example of utmost importance on the success of scientific cooperation at the world level to tackle the huge question of establishing the complete human proteome. Since the creation of the plant platform, the INPPO family grew globally and steadily with about 700 members as of today, representing 45 countries.

The considerable support and work of INPPO members allowed accomplishment of several important actions, including coordination in the edition of Special Issues on plant proteomics, the writing of viewpoint and review papers, the creation of a NewsLetter (INPPO Express: News and Views), the establishment of strong links with the international journal PROTEOMICS allowing the launching of a new Section in this journal under the heading “Plant Proteomics”, the development of education outreach in plant proteomics, and the organization of our 1st world Congress in Hamburg (Germany) thanks to the support of University of Hamburg and the efficient coordination of Sabine Lüthje and her team.

INPPO INITIATIVE: GLOBAL PLATFORM FOR INVOLVING, ATHERING AND DISSEMINATING PLANT PROTEOMICS KNOWLEDGE

Ganesh Kumar Agrawal[1,2], Abhijit Sarkar[1,2,3,4], Raj Agrawal[4], Jenny Renaut[5], Dominique Job[6], Randeep Rakwal[1,2,7,8]

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Tremendous development in plant proteomics over the last decade led to the establishment of International Plant Proteomics Organization (INPPO) – a non-governmental, non-profit, global interactive platform, consisting of mainly plant biologists (those who are working and / or interested in plant proteomics – the plant proteomers) – to create a physical as well as virtual 'knowledge network' for development, improvement and dissemination of plant proteomic knowledge worldwide. INPPO was officially launched in 2011 via its website (www.inppo.com) and publication of the 'viewpoint paper' discussing the necessity and vision of INPPO under the title 'Time to articulate a vision for the future of plant proteomics – A global perspective: An initiative for establishing the International Plant Proteomics Organization (INPPO)'. Ten initiatives of INPPO were outlined in that viewpoint paper to promote plant proteomics in each and every country. Over the years, INPPO and its initiatives were globally accepted and appreciated by the plant proteomics community; and subsequently recognized as the international platform for plant proteomers. INPPO progresses and upcoming activities are disseminated to the scientific community via INPPO website, newsletter (termed, INPPO Express: News & Views), and highlights (officially published by the PROTEOMICS journal). INPPO initiatives and their implementation has been a driving force for the progress in plant proteomics at the global stage. Numerous country representatives are taking it upon themselves to help promote plant proteomics in their respective countries via gathering and presenting information on plant proteomic research, people, facilities and challenges ahead. The INPPO initiative has also led to international collaborations as is evident from India-Japan, USA and Italy initiatives promoting the visit of researchers to leading laboratories. In this first INPPO World Congress, first the INPPO vision and achievements to date will be presented. Second, some examples of ongoing collaborative works will be highlighted. Third, the focus will be on the current issues and challenges ahead for INPPO as a global organization. INPPO is / will strive to always move forward keeping in sight what is best for plant proteomics and the challenges that go with it, including and most importantly its community. As our vision is global, we sincerely hope the scientific communities around the world will come together to support and join INPPO. We too share the dream of a sustainable green world and believe to reach there united.

PUBLIC EVENING LECTURE

THE MONKEY KING AND PIGSY FERRYING THE PROTEOMIC SUTRAS INTO THE 3RD MILLENNIUM: A CHRONICLE

Pier Giorgio Righetti

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When surveying the deeds of scientists working with mammalian proteomics, one can find that today they can explore to an incredible extent the proteome of any living cell line. For instance, when analysing 11 human cell lines, M. Mann's group identified a total of 11,731 proteins and on average 10,361 ±120 proteins in each cell line, an outstanding catch, indeed. On the contrary, in plant proteomics, it is hard to reveal more than a few hundred species in each experiment. What is wrong with us? We have the same tools, i.e. the Monkey King par excellence, represented by the most advanced mass spectrometry instruments. Our problem is that most of our samples are recalcitrant tissues, in that scarcely represented proteins in vegetable tissues are embedded in a vast sea of plant polymers, insoluble fibres and metabolites. So we need an extra tool to carry our Sutras into the third millennium and this might be Pigsy, the other super natural power that helped the monk Tripitaca to carry back to China the original Buddha's Holy Books, thus contrasting Evil Spirits barring his way. Pigsy, half man, half pig, shows a voracious appetite for any type of foodstuff, just like the technique we have devised, the combinatorial peptide ligand libraries (CPLL), which show a unique affinity for all proteins present in living tissues. CPLLs comprise millions of diverse baits (all of them hexapeptides obtained via combinatorial synthesis from 16 amino acids) grafted onto organic polymer beads. They act by simultaneously shaving the excess of high-abundance proteins (HAP) and substantially enriching the low-abundance ones (LAP), thus bringing them to the lime lights. The concentration increment of LAPs can reach three to four orders of magnitude, thus augmenting the survey of any plant proteome by 5 to 6 folds as compared to untreated samples. I will describe here the proteome of three tropical fruits, belonging to the category of recalcitrant tissues, namely the banana, avocado and mango. In all these cases, whereas up to the present a few dozen proteins had been reported, we could detect and describe from a minimum of 1000 up to 3000 unique gene products. While still far from Mathias Mann's catch, this represents anyhow a remarkable step forward.

Reference

Boschetti, E.; Righetti, P.G. (2013) *Low-Abundance Protein Discovery: State of the Art and Protocols*. Elsevier, Amsterdam, pp. 1-351. ISBN 978-0-12-401734-4.

L 1

Keywords: Arabiddopsis, sub-cellular proteome, LOPIT, Trans Golgi Network

CHARACTERISATION OF THE ARABIDOPSIS SUB-CELLULAR PROTEOME

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Many proteins exist in highly controlled micro-environments, including organelles, suborganellar compartments, clusters of membrane proteins and multi-protein complexes. The components of such micro-environments may vary in a role-dependent manner increasing their functional diversity. Our understanding of the proteins residing in these niches is important to the analysis of protein function and our knowledge of cellular systems. Moreover, the ability to chart the dynamic change in location is vital to the elucidation of the full repertoire of cellular mechanisms.
Localization of Organelle Proteins by Isotope Tagging (LOPIT) is a spatial proteomics method which enables the characterization of subcellular localization using quantitative mass-spectrometry [1]. It relies on the partial separation of subcellular compartments using a biochemical fractionation.
Recently, using quantitative mass spectrometry and the application of semi-supervised machine-learning tools, we have simultaneously assigned the steady state location of thousands of proteins from Arabidopsis root tissue to multiple subcellular compartments in a single experiment [2]. Using this approach, we have been able to assign steady state location of proteins from the Trans Golgi Network (TGN), which has been a difficult organelle to characterise due to its dynamic nature and the necessity to distinguish between full time residents, cargo and contamination within TGN enriched fractions [3]. We also compared and contrast the results of applying LOPIT to define the plant TGN with immuno-isolation approaches.

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[2] Mass-spectrometry-based spatial proteomics data analysis using pRoloc and pRolocdata. Gatto L, et al Bioinformatics. 2014;30(9):1322-4.
[3] Identification of trans-golgi network proteins in Arabidopsis thaliana root tissue. Groen AJ, et al. J Proteome Res. 2014 Feb 7;13(2):763-76

L 2

Keywords: label free shot gun proteomics, DDA, DIA

LABEL FREE QUANTITATIVE PROTEOMICS OF A COMPLEX EUKARYOTE VIA LC MSMS: DATA INDEPENDENT ANALYSIS (DIA) OR DATA DEPENDENT ANALYSIS (DDA)?

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The most frequently applied LC-MS/MS strategy is data-dependent acquisition (DDA). DDA however implies some shortcomings inherent to the serial nature of the cycling. Several co-eluting precursors in complex biological samples lead to reduced reproducibility and loss of quantitative information. Data independent acquisition (DIA) methods apply no or a broad precursor selectivity enabling rapid cycling. So in theory, only one analytical run is needed to obtain an accurate label free quantification and reliable identification. In practice, using complex samples from plants, the chimeric MSE fragmentation spectra usually lead to poor peptide identification. We developed a software to combine DIA runs to DDA libraries to acquire a high identification rate (Buts et al 2014). The development of the next generation mass spectrometers with fast serial cycles and parallel analysis, questions whether DIA still has advantages. In a fast DDA based approach using a QE orbitrap, we calculated from a single UPLC (50cm, 5h gradient) that only 22% of the precursors is selected for MS/MS. We conclude that both approaches are complementaryand that a DIA approach is specifically useful for HRM.

L 3

Keywords: Custom database, leaf rust, haustoria.

CUSTOM DATABASE SEARCHING TO REVEAL DIFFERENCES IN THE HAUSTORIA PROTEOMES OF THREE RACES OF WHEAT LEAF RUST, PUCCINIATRITICINA

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The fungus *Pucciniatriticina* (*Pt*) is an obligate parasite that causes leaf rust on wheat. Rust populations consist of genetically determined races, some of which can overcome the host's defences and cause disease. *Pt* enters the host leaf through stomata and forms intracellular feeding structures called haustoria, which direct the import of nutrients from the host into the fungus and which synthesize and secrete proteins critical for pathogenesis, including avirulence (*avr*)proteins and effectors. We can routinely purify haustoria to near-homogeneity using haustoria-specific monoclonal antibodies, thus gaining access to the haustoria proteome for analysis. The genome of *Pt* Race 1 was sequenced in 2009 and we have access to sequences for Race 9 and Race 161. This has enabled us to create custom databases which we can query with high-resolution MS spectra raw files created from haustoria proteomes of a single unique race of *Pt.* Comparative analysis of the mass spectra from the three races is expected to identify variation at the protein level and provide candidate effectors and possibly *avr* proteins.

L 4

Keywords: grain legumes, proteomics, food safety

IDENTIFICATION OF POTENTIALLY BIO-ACTIVE PROTEINS IN CHICKPEA (CICER ARIETINUM L.) SEED

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Grain legume seeds are crucial staple foods, particularly in underdeveloped countries due to their balanced nutritional composition, constituting essential sources of protein, calories, minerals and vitamins. Chickpea (*Cicer arietinum* L.) has one of the best nutritional compositions and ranks 3rd in world legume production. Chickpea contains a number of bioactive substances with potential health-beneficial effects, still, it is considered a major food allergen in the Mediterranean and India. We aim to characterize potential bioactive proteins in the chickpea seed – proteins with the potential to modulate health after ingestion. To fulfill our objective we will focus on proteins resistant to cooking and simulated digestion, which consequently, might influence consumer's health. Our results show that, after cooking and simulated digestion, up to 22% of the initial protein amount remained undigested and 14 peptide bands on a SDS-PAGE were found to resist cooking and digestion. In this report we present our progresses in identifying proteins resistant to digestion using a combination of different MS techniques (LC-MS/MS and MALDI-TOF-TOF and different proteases).

L 5

Keywords: mass western, SRM

NOVEL STRATEGY FOR SRM BASED PROTEIN ABSOLUTE QUANTIFICATION

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In recent years, absolute protein quantification using the selective reaction monitoring (SRM) technology has become more and more established in many laboratories. Its advantages are fast analysis time and high sensitivity. However some protein biochemical properties, such as solubility, digestion efficiency as well as standard peptide purity and accurate quantity are still challenging. More accurate protein stoichiometry calculations can be obtained by a novel Mass Western approach. Two important properties for improved verification of the quality of the SRM based absolute quantification differing from common strategies will be introduced.

L 6

Keywords: receptor kinase, phosphoproteomics, network, sucrose

PHOSPHOPROTEOMIC APPROACHES IN STUDYING FUNCTIONS OF PLANT KINASES

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Phosphorylation is the most well studied post-translational modification with regulatory function. It can induce changes in protein activity, it may provide docking sites for interaction of other signaling proteins or induce the formation of protein complexes. A proteomic approach was taken to characterize protein modification and to study changes in protein abundance. Starvation-resupply time-course experiments were is designed as a screening approach for nutrient-specific and nutrient-regulated phosphorylation sites. Based on these time-dependent phosphorylationprofiles, a network model was constructed suggesting a layered topology of signaling networks. Key proteins from the network were then functionally characterized based on knock-out mutants involving phosphor-proteomics, enzyme activity measurements, and cell biology.

L 7

Keywords: Plant proteomics, metabolism, photoperiod, growth

ORCHESTRATED RESPONSES IN PROTEIN ABUNDANCE TUNE PLANT METABOLISM TO DIFFERENT PHOTOPERIODS

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The availability of light is a key determinant of plant growth. A complex relationship between primary carbon metabolism, protein synthesis and growth was recently revealed in Arabidopsis plants grown in photoperiods ranging from 6h to 18h of light [1]. We used the same plant material to study how photoperiod length affects the Arabidopsis proteome. We obtained quantitative data on more than 4300 proteins. This is the highest number of proteins quantified in a single plant proteomics study to date and allows in-depth analysis of metabolic pathways with unprecedented enzyme coverage. Our data reveals highly coordinated changes in protein abundance in primary and secondary metabolism pathways. They reflect adaptation to light limiting conditions in short photoperiods and increasing light saturation as photoperiods become longer. An additional study of the transcriptome in all four photoperiods uncovered that responses on the proteome level are guided by transcriptional networks which integrate signals from the circadian clock and carbohydrate metabolism.

Reference

[1] Sulpice R, Flis A, Ivakov AA, Apelt F, Krohn N, Encke B, Abel C, Feil R, Lunn JE, Stitt M, 2013, 7(1), 137-55

L 8

Keywords: Differential expression, Plant proteomics, Plant-virus interaction, ToCMoV

IDENTIFICATION OF HOST PROTEINS MODULATED IN RESPONSE TO TOMATO CHLOROTIC MOTTLE VIRUS (TOCMOV)

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Tomato is an important crop cultivated worlwide, severely affected by begomoviruses such as Tomato chrolotic mottle virus (ToCMoV). The aim of the present study was to identify differentially expressed proteins in the ToCMoV-host plant interaction. The virulence factor AC2 is considered crucial for a successful virus-plant interaction. Therefore, *Nicotiana benthamiana* was inoculated with *Agrobacterium tumefaciens* containing the viral vector Potato virus X (PVX) and PVX-AC2 construction. 2-DE was performed and MALDI TOF-TOF analysis revealed proteins involved in oxidative stress, photosynthesis and host defense. We have also performed 2D-NanoUPLC/MS^E for the analysis of the proteomic profiles of the susceptible (Santa Clara) and resistant (LAM 157) tomato genotypes infected with ToCMoV. The effects caused by AC2 of ToCMoV and the intact virus were similar and resulted in the recruitment of proteins involved in ubiquitination and defense response. RT-qPCR was also performed for selected genes, including 1443 protein, histone H4, SGT1 and ubiquitin. The results showed that during the infection process a highly variable profile was obtained with expression peaks at specific time points.

L 9

Keywords: mass spectrometry, label free, carotenoids

VARIATIONS OF CAROTENOID PATHWAYS IN TRANSGENIC MAIZE ENDOSPERMS HIGHLIGHTED BY A LABEL-FREE QUANTITATIVE PROTEOMICS APPROACH

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b-carotene and astaxanthin that are mainly produced by chemical synthesis constitute typical supplement feed for poultries and fishes. The project CaroMaize (ANR program Plant-KBBE) proposes to use transgenic maize seeds expressing high level of carotenoids, obtained by metabolic engineering of biosynthesis pathway (Zhu *et al.*) as a raw source for industry and animal feeding. In addition to metabolomics analyses including the characterization of carotenoids profiles, transgenic maize lines are subjected to integrated transcriptomics and proteomics approach. As such, the proteomic profile of endosperm from transgenic plants enriched in b-carotene and astaxanthin was compared to that of a control genotype. A quantitative label-free proteomics analysis of total protein extracts allowed us to establish a first endosperm protein directory, to describe its functional characteristics and to characterize proteins with significant variations between genotypes highlighting putative variations of the carotenoids pathway.

Reference

Zhu, C. Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. PNAS; 105 (47),18232-7. 2008.

L 10

Keywords: nucleoporin, nuclear pore complex, nucleus, interactive proteomics, Arabidopsis

INTERACTIVE PROTEOMICS DISSECTS STRUCTURAL DYNAMICS OF PLANT NUCLEAR PORE COMPLEX

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Nuclear pore complex (NPC) is a large protein complex composed of multiple copies of approximately 30 different nucleoporins. Despite of its important role for nucleocytoplasmic trafficking, knowledge of the function of individual components and the overall structure in plant NPC lag behind those of other organisms.

In this study, an interactive proteomic approach was used to identify *Arabidopsis* nucleoporins (1). We firstly generated transgenic plant expressing GFP- RAE1 to visualize NPC. To identify other nucleoporins that make up the NPC, the transgenic plants were subjected to immunoprecipitation using anti-GFP antibody followed by mass spectrometry identification of proteins. A series of five cycles of interactive proteomic analysis was performed using GFP-nucleoporins. We finally found that the plant NPC contains at least 30 nucleoporins, 22 of which had not been previously annotated. Overall, the *Arabidopsis* nucleoporins shared higher homology with vertebrate than with yeast nucleoporins, although it is thought that each organism uses specialized nucleoporins with functions specific to their own NPCs (2).

References

1) Plant Cell (2010) Tamura et al.

2) J. Exp. Bot. (2013) Tamura et al.

L 11

Keywords: Arabidopsis, rice, seed, germination, translation

DYNAMIC PROTEOMICS REVEALS THAT SELECTIVE MRNA TRANSLATION IS A KEY REGULATORY STEP DURING ARABIDOPSIS SEED GERMINATION

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Background and goals. Studies on seeds used transcriptomic or proteomic approaches to discover determinants of germination quality but only a few reports addressed post-transcriptional regulations *i.e.* mRNA translation. To bridge this gap, we radiolabeled ([^{35}S]-Methionine) *de novo* synthetized proteins in *Arabidopsis* germinating seeds to investigate mRNA translation regulations [1].

Results. Translation in Arabidopsis seeds slightly precedes the classical three germination phases with a high activity between 8 and 24 hours after imbibition. It was possible to highlight 8 precise patterns of mRNA translation. Few concordant profiles were found when we crossed mRNA and protein abundances with the *de novo* synthetized proteins data.

Conclusions. mRNA translation during seed germination is very selective and provides time-specific biological insights. These results shed a new light on the classical three phases of seed germination. We are currently working on rice embryo post-transcriptional regulations during germination through the integration of transcriptomic and proteomic results.

References

1. Galland et al, 2014 *Molecular & Cellular Proteomics* **13**(1):252-68

L 12

Keywords: grape berry; ripening; anthocyanin content; 2-DE; GC-MS

AN INTEGRATED PROTEOMIC AND METABOLOMIC APPROACH REVEALS PECULIAR TRAITS IN THE EXOCARP OF FOUR GRAPE CULTIVARS CHARACTERIZED BY A DIFFERENT ANTHOCYANIN ACCUMULATION

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In order to study the biochemical processes relating to secondary metabolism that occur in berry ripening, a proteomic and metabolomic comparative analysis among four vines characterized by different anthocyanin content was performed.

The experiment restricted on skin tissue was conducted by using the 2-DE gels/ LC-ESI-MS/MS for proteomic investigation and GC-MS in Selected Ion Monitoring mode (SIM) for the metabolomic one. Linear discriminant analysis (LDA) pointed out that the cultivars were distinguishable and the order in which they were grouped mainly reflected their relative anthocyanin contents. The hierarchical clustering analysis clearly showed that in addition to proteins directly linked to anthocyanin biosynthesis also many of those relating to primary metabolism increased in abundance. As far as it concerns metabolomic analysis, only the levels of few key intermediates varied among cultivars in a way that may refer to anthocyanin content. Taken together the results showed that the process of grape ripening in the skin differs in some central metabolic traits among cultivars and that these variations could be linked to differences in secondary metabolism.

L 13

Keywords: Rice, proteomics, phosphoproteomics, metabolism

PROTEOMIC ANALYSIS ON RICE SEED GERMINATION

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Rice seeds provide the staple food resource for the world population, and the essential material for agricultural production. Successful germination of rice seeds is very important. To uncover the mechanisms of rice seed germination, proteomic and phosphoproteomic analyses were conducted. Metabolic pathways were constructed based on the proteome profiling data. Through comparative proteomic analyses, we found that there were several GA and ABA responsive elements in the promoters of most of the genes encoding the up and down-regulated proteins respectively, which suggested that germination of rice seed is als regulated by GA and ABA. Starch were quickly degraded in the endosperm. The intermediate (likely to be sucrose) of starch degradation was transferred into embryos either for further degradation or for biosynthesis of starch. Phosphoproteomic analysis showed that the first 12 h after imbibition is a important signal transduction phase for the initiation of germination. Three core components in BR signaling displayed significant changes in their phosphorylation status. BL treatment increased the germination rate. These findings suggest that BR may also regulate seed germination.

L 14

Keywords: MSE, quantification, posttranslational modification

FUNCTIONAL PROTEOMICS OFFERS A NEW PERSPECTIVE ON CHLOROPLAST BIOGENESIS AND THE FUNCTION OF POSTTRANSLATIONAL MODIFICATIONS

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Our research is focused on the characterization of basic chloroplast functions with functional proteomics tools. Two lines of research are currently prevalent: First, it is our goal to understand the assembly of the chloroplast proteome. To this end, we quantify chloroplast proteins (e.g. Helm et al., 2014, J Proteomics 98, 79-89), characterize the plastid protein import machinery, assess its dynamic subunit composition and aim at identifying new interaction partners (e.g. Bischof et al., 2011, Plant Cell 23, 3911-28, Grimmer et al., 2014, Front Plant Sci, doi: 10.3389/fpls.2014.00258). On the other hand, our research aims at deciphering the chloroplast phosphoproteome network entailing the identification of protein kinase substrates and the analysis of phosphorylation dynamics (e.g. Reiland et al., 2011, PNAS 108, 12955-60; (Schönberg and Baginsky, 2012, Front Plant Sci 3, 256)). The tools we are using comprise comparative quantitative phosphoproteomics and the peptide chip ChloroPhos1.0 that allows the multiparallel analysis of *in vitro* phosphorylation activity on more than 900 substrate peptides.

L 15

Keywords: Quantitative profiling, iTRAQ, Blue Native PAGE, seed dormancy control, Triticum aestivum

QUANTITATIVE PROTEOMIC APPROACHES TO REVEALING MECHANISMS OF SEED DORMANCY CONTROL AND PRE-HARVEST SPROUTING RESISTANCE IN WHEAT

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We have recently shown changes in the redox-sensitive proteome upon seed dormancy release and accumulation of stress-protective proteins in high dormancy genotypes. Here, iTRAQ-based proteomic analysis in conjunction with wheat EST database was used for quantitative monitoring of after-ripening mediated changes in aleurone and embryo tissue-specific proteomes from hybrid spring wheat genotypes. A total of 2724 proteins were identified with high confidence, of which 85 and 212 showed significant differential expression in dormant embryos and aleurones, respectively, and 387 were expressed in a tissue-specific manner. In dormant embryos, significant increase was found for protein translation, folding, transport and degradation, DNA-repair, and mRNA surveillance, oxidative and nitrosative stress response, both lactic acid fermentation and aerobic respiration resulting in a greater flux through pyruvate under hypoxic conditions. Using Blue Native PAGE and label-free quantitative analysis changes in 23 membrane-associated protein complexes from aleurone representing oil body-associated proteins, mitochondrial respiratory chain components, vacuolar and ER complexes were characterized.

L 16

Keywords:

MULTIPLEXED PROTEIN QUANTIFICATION DEPLOYING HIGH RESOLUTION, ACCURATE MASS STRATEGIES

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Proteomics relies on the confident identification, characterization and correct quantification of protein components of a sample set. In this presentation, several strategies for protein quantification will be reviewed and examples given to highlight their respective benefits and strengths.

Tandem Mass Tag™ (TMT™) Reagents allow researchers to derive results from multiplexed separate samples in a single LC-MS analysis, boosting lab productivity up to 10X and allowing proteomics results to play a critical role in large scale biological studies. Highly optimized and accurate TMT workflows have been developed departing from MS2- to MS3-based quantification. Examples will be shown from algae and human protein quantification.

Data-Independent Analysis (DIA) on Thermo Scientific™ Orbitrap™-based systems offers the ability to detect and quantify large numbers of proteins or small molecules in a sample by systematically collecting multiplexed MS and MS/MS data for all ionizable compounds in a sample. DIA workflow options will be discussed along with the critical benefits of data quality enabled by Orbitrap high resolution and mass accuracy.

When deploying targeted quantification strategies in combination with high resolution, accurate mass approaches, high sensitivity as well as selectivity are benefitting the analytical outcomes. Parallel Reaction Monitoring will be reviewed with application examples from recently published literature.

L 17

Keywords: Glandular trichomes, Mentha x spicata, MudPIT, Nicotiana tabacum, Terpenoids

PROTEOMICS AS A TOOL FOR MONITORING PLANT SECONDARY METABOLISM AND IDENTIFYING CANDIDATE PROTEINS FOR UNATTRIBUTED FUNCTIONS

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Large scale proteomics is today becoming a powerful tool, giving the opportunity to investigate low abundant proteins and to unveil metabolic pathways. We are investigating plant metabolic pathways using proteomics to unravel new putative functions of cell types and identify candidates for unclear biosynthetic steps and transport activity. Proteomic analysis of *Mentha spicata* glandular trichomes resulted in the identification of more than 1.600 proteins, including 57 enzymes potentially involved in secondary metabolism, often considered as low-abundant proteins. In *M. spicata*, proteins identified included 21 involved in the synthesis of phenylpropanoids and phenolics, 32 involved in terpenoid synthesis, and 14 membrane transporters, among which 11 ATP-binding cassette transporters that are predicted transporters putatively involved in their transport. Using quantitative proteomic analysis of the tall and short glandular trichomes from *Nicotiana tabacum,* we were able to identify 97 and 95 proteins, respectively, that are significantly more abundant in one trichome type. These results shed light on *N. tabacum* trichome functions and open the way to in-depth functional studies.

L 18

Keywords: Cabernet Sauvignon, temperature stress, label-free, filter aided sample preparation (FASP)

QUANTITATIVE LABEL-FREE SHOTGUN PROTEOMIC ANALYSIS OF A RED GRAPEVINE VARIETY EXPOSED TO HOT AND COLD TEMPERATURE STRESSES.

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Abiotic stresses trigger complex responses in grapevine.This project investigates quantitative shotgun proteomic changes induced by temperature stress on Cabernet Sauvignon to provide insights into metabolic pathways related to temperature stress in grapevines.Grape cells were grown at 26°C, exposed to 34°C, 42°C (high) and 18°C, 10°C (low) temperatures, harvested in biological triplicates, proteins extracted with methanol-chloroform, digested by FASP[1] and trypsin.Proteins were identified with LC-MS/MS spectra by gas phase fractionation (Velos-Pro linear ion-trap mass spectrometer), searched against UniProtKB *Vitis vinifera* genome and quantified by spectral counting.Proteins identified in biological triplicates ranged from 1056 to 1167 with a protein false discovery rate of <1%.Gene Ontology annotations of the up- and down-regulated proteins revealed the crucial role of proteins metabolic processes and carbohydrate metabolism.This is the first label-free shotgun proteomic study on grapes exposed to hot and cold temperature stress.

Reference

[1] Wisniewski, J. R., Zougman, A., Nagaraj, N., Mann, M., 2009 *Nature methods 6*, 359-362

L 19

Keywords: Brassica juncea, seedlings, s-nitrosylation, NO signaling, cold stress

BIRD'S EYE VIEW OF NITRICOXIDE SIGNALING IN COLD STRESS VIA NITROSYLATION IN BRASSICA JUNCEA SEEDLINGS

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Cold stress affects growth as well as crop yield in the oilseed Brassica. Nitric oxide (NO) is an important signaling molecule which manifests its effect by post translational modification (PTM) of proteins. S-nitrosylation is one such modification which nitrosylatesRuBisCo leading to a 40% decrease in the photosynthetic efficiency in cold stress in Brassica seedlings. To gain insight in NO mediated cold stress signaling, subcellular s-nitrosoproteome analysis in apoplast, the communication channel and nucleus, the regulatory hub was performed. Biotin switch technique iwithneutravidin affinity chromatography and mass spectrometry showed distinct spatial regulation as nitrosylation of ascorbate-glutathione cycle, cell wall modifying and proteolytic enzymes was observed in apoplast along with nitrosylation of the brassicaceae specific glucosinolate pathway. Out of 103 identified nitrosylated target proteins almost 50% were novel and hence provide a strong evidence for cross talk in cold stress and NO signaling. Positive regulation of dehydroascorbate reductase, superoxide dismutase and glutathione s-transferase activities provides clues on fortification of redox defence in cold.

L 20

Keywords: grain germination, secretome, electrochemistry, glycosylation sites, ER stress

SECRETORY PROTEINS AND PROGRAMMED CELL DEATH: INSIGHTS FROM THE BARLEY GRAIN ALEURONE LAYER

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The cereal grain aleurone layer is a hormone-reponsive tissue with a specialized role in protein secretion. In response to the phytohormone gibberellic acid (GA), the aleurone layer synthesises an array of enzymes that are secreted to the endosperm for the degradation of storage products. Subsequently, the aleurone cells undergo programmed cell death (PCD). Another phytohormone, abscisic acid (ABA) can counteract GA. The barley aleurone layer can be separated from the other grain tissues and retains its specific responses to GA and ABA in culture, allowing the study of the effect of added signalling molecules in an isolated system. Using aleurone layers incubated *in vitro,* application of hormones, agents disrupting protein secretion, and measurements of redox activity and cell death are combined with proteome and glycoproteome analysis to provide new insight into the plant protein secretory machinery and its potential influence on PCD. Immobilisation of the tissue on a polymer support allows parallel analyses of cell death, redox state and transient gene expression in single aleurone layers.

L 21

Keywords: lysine acetylation

EXPLORING THE ROLE OF LYSINE ACETYLATION IN THE REGULATION OF PLANT METABOLISM

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Acetylation of the ε-amino group of lysine is a reversible post-translational modification recently discovered to be widespread, occurring on proteins outside the nucleus, in most sub-cellular locations in mammalian cells. Until recently, almost nothing was known about this modification in plants beyond the well-studied acetylation of histone proteins in the nucleus. In a shot-gun proteomics approach we identified several hundredsorganellar and cytosolic Arabidopsis proteins to be lysine-acetylated. One of the main questions of our research is to identify the acetylation sites which are improtant for the regulation of enzyme functions and to quantify changes in lysine aceytlation under different environmental conditions using LC-MS/MS-based proteomics.

L 22

Keywords: leaf tissues, proteasome, ubiquitylation, Arabidosis

PROTEASOME TARGETING OF PROTEINS IN ARABIDOPSIS LEAF MESOPHYLL, EPIDERMAL AND VASCULAR TISSUES

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The Arabidopsis leaf is composed of different tissues that maintain specialized biological functions. We developed a method to separate leaf epidermis, vasculature and mesophyll and demonstrated an enrichment of individual tissue types which was basically free of contamination with other tissues. Employing that, we investigated tissue type specific protein composition, analyzed changes in protein accumulation after inhibition of the proteasome and identified affinity-enriched ubiquitylated proteins using high-throughput proteomics. In the tissue specific total protein extracts we identified 1114 proteins. Several proteins were exclusively identified in only one tissue type indicating the importance and specificity of cellular processes for the function of the respective tissue. After inhibition of the proteasome we identified 226 accumulating and 519 ubiquitylated proteins in a tissue specific manner. In the mesophyll the plastid import machinery was targeted by the proteasome, while in the epidermis proteins involved in TCA cycle and cell wall biosynthesis increased after proteasome inhibition. In vasculature many proteins involved in glucosinolate biosynthesis were ubiquitylated.

L 23

Keywords: aquaporin, phosphorylation, quantitative proteomics

QUANTITATIVE PROTEOMICS UNRAVELS NOVEL POST-TRANSLATIONAL REGULATORY MECHANISMS OF PLANT AQUAPORINS

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In plants, aquaporins play a crucial role in regulating water transport in response to environmental cues [1]. By using Absolute QUAntification method [2], Multiple Reaction Monitoring [3] and label-free quantitative proteomics [4] our group demonstrated that changes in phosphorylation status of plasma membrane aquaporins (PIPs) are positively correlated to changes in tissue hydraulic conductivity. An unexpected role for the calcium- dependent protein kinase 7 (CPK7) in contributing to the control of PIP1 cellular abundance was also revealed. The overall work provides deep insights into post-translational events triggered by environmental constraints and their role in regulating the plant water status.

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L 24

Keywords: SILAC, Phosphoproteomics, Signaling, Gravitation

SILAC FOR THE ANALYSIS OF PHOSPHORYLATION EVENTS UNDER MICRO-GRAVITATION IN ARABIDOPSIS THALIANA CELL CULTURES

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Callus cells of *Arabidopsis thaliana* are able to respond immediately to gravitational changes with adaption in their physiology. In Order to assess the effect of micro-gravitation (<0,02*g*; weightlessness) we exposed suspension cultures of *A.th.*to parabolic flights on board of an Airbus A300 (Novespace). Because of the short exposure time (about 20 s of e.g. microgravity) we focused on changes in the degree of protein phosphorylation to investigate the initial steps of signalling cascades after perception. We show that modulation of proteins is a very fast means of metabolic control

The extended SILAC (stable isotope labelling by amino acids in cell culture) method can overcome the so far restricted analysis of protein phosphorylation in plant cells. For the flight experiment *A. th*.cells could be successfully labelled by $^{13}C/^{15}N$-lysine for up to 90% for further MS-Analysis. The data show that the phospho-proteome reacts within seconds to transduce the signalling events after altered gravitation. Mainly calcium related proteins and different kinases/phosphatases were altered in degree of phosphorylation. In addition proteins of detoxification and primary metabolism are affected.

L 25

Keywords: Arabidopsis thaliana, formative cell division, protein-protein interactions, phosphoproteomics, ACR4

SYSTEMS APPROACHES TO IDENTIFY ACR4 INTERACTORS AND SUBSTRATES IN ARABIDOPSIS

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In plants, coordinated formative cell divisions enable the formation of new cell types and tissues**.** The membrane-localised receptor-like kinase ACR4 plays a central role in controlling formative cell divisions in the *Arabidopsis* root. To unravel the molecular mechanism underlying ACR4-mediated signalling, various complementary proteome-wide approaches were used to study the protein-protein interaction network of this key regulator. As a result, PP2A-3 - a catalytic component of a PP2A phosphatase complex - was identified as an ACR4-interacting protein. Biochemical studies demonstrated that ACR4 phosphorylates PP2A-3 *in vitro*, pinpointing PP2A-3 as the first direct substrate for ACR4 kinase activity. Phenotypic analysis of *pp2a-3* and *pp2a-3 pp2a-4* double mutants further corroborates the role of PP2A phosphatases downstream of ACR4. In addition, I will present our progress on decoding the ACR4−PP2A-3 signalling network through a large-scale quantitative mass spectrometric analysis of the phosphoproteome in the relevant loss-of-function backgrounds

L 26

Keywords: phospho-proteins, membrane proteins, soluble proteins, in-gel activity, native 2D

NATIVE 2D-PAGE FOR COMPREHENSIVE ANALYSIS OF NATIVE PHOSPHO-PROTEINS

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Post-translational modifications are common ways to alter protein activities, subcellular localizations, degradation and protein-protein interactions. Among post-translational modifications protein phosphorylation is one of the most common. Many cellular processes in all examined organisms are regulated via reversible protein phosphorylation. Methods that can be used for monitoring the phosphorylation status of proteins are very important with respect to the evaluation of diverse biological processes. Phos-tag technology was combined with 2D native PAGE, consisting of native IEF PAGE or NEPHGE in the first dimension and high resolution clear native PAGE in the second dimension. The here presented native 2D method can be used to study phosphorylation depended changes in protein activity and changes in protein complex composition. Furthermore it is combinable with further downstream analysis like Western blot, phospho-peptide enrichment after digestion and mass spectrometry. Protocols were developed for application on soluble and membrane proteins.

L 27

Keywords: cell wall, Golgi, plasma membrane, free-flow electrophoresis

EXPLORING THE PLANT ENDOMEMBRANE BY FREE-FLOW ELECTROPHORESIS

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In recent years there has been interest in the development of a cellulosic biofuels industry resulting in the establishment of multiple research facilities with a focus on plant cell walls. We have been utilizing free-flow electrophoresis (FFE) to characterize the plant endomembrane, with a focus on compartments involved in cell wall biosynthesis. While the approach resulted in high purity fractions of Golgi membranes from Arabidopsis cell cultures, its application on enriched microsomal fractions from developing xylem tissue from pine was less successful. The success of plant cell cultures led us to apply the approach to rice cultures. In contrast to the Arabidopsis results, the ER and Golgi were tightly interconnected. Although we disrupted this interaction, the resultant post FFE fractions were enriched for ER. Utilizing computational approaches, we were able to obtain a rice Golgi proteome. While the Golgi is a major source of cell wall polysaccharides, cellulose is synthesized on the plasma membrane. We applied FFE to enriched membranes fractions from Arabidopsis and in contrast to the rest of the endomembrane, the PM migrated to the cathode and was present as a distinct peak.

L 28

Keywords: Plant, chloroplast, subcellular localization

SUBCELLULAR AND SUBPLASTIDIAL PROTEOMICS

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Many high throughput proteomic experiments were conducted on purified cell organelles. We recently went a step further into the definition of the accurate localization of proteins within the chloroplast. We first performed a comprehensive analysis of the chloroplast proteome starting from the whole chloroplast and its purified three subcompartments (envelope, stroma and thylakoids) and assessed the partitioning of each protein in the 3 above-cited compartments (Ferro et al., *Mol. Cell. Proteomics* 2010). Using these data, we created the AT_CHLORO database (Bruley et al., *Front. Plant Sci*. 2012). In depth investigation of the subplastidial localization of identified proteins revealed new insights over chloroplast metabolism (Joyard et al., *Mol. Plant* 2009 & *Prog. Lipid Res.* 2010; Rolland et al., *Annu. Rev. Genet.* 2012). More recently, we performed new studies targeting the thylakoid subcompartments (Tomizioli et al., *Mol. Cell. Proteomics* 2014). These recent findings corroborate previous observations obtained for photosynthetic proteins that used nonproteomic approaches, and identify about a hundred minor thylakoid proteins being potential regulators of the chloroplast physiology.

L 29

Keywords: Absolute quantification, MS(E), Chloroplast stroma proteome, Chloroplast development.

FROM VALIDATION TO APPLICATION: QUANTITATIVE MULTIPLEX MS ANALYSIS (MSE) OF CHLOROPLAST DEVELOPMENT

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LC-MS^E–based absolute and relative protein quantification is a new strategy for label-free quantification [1]. New MS^E approaches use ion mobility separation of peptides as additional dimension to reduce sample complexity, resulting in higher sensitivity and higher proteome coverage. In a recent study, we validated HD-MS^E-based protein quantification using spiked proteins as quantitative reference. We applied this method to the absolute quantification of proteins in the chloroplast stroma, and performed a meta-analysis with previously published quantitative chloroplast proteomics data. We find that the quantitative distribution of enzymes within a pathway is robust under different experimental conditions and correctly reflected by different MS-based quantification strategies [2]. Here, we report on the application of HD-MS^E-based absolute protein quantification to the quantitative analysis of chloroplast development.

Refereces

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2. Helm, S., et al., 2014, JoP, **98**(0): p. 79-89.

L 30

Keywords: MRM, organelle, development, quantitative proteomics

USING MULTIPLE REACTION MONITORING (MRM) TO DETERMINE ORGANELLE ABUNDANCE DURING PLANT DEVELOPMENT AND ORGANELLE ISOLATION PROCEDURES

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Using Multiple Reaction Monitoring, we have described the relative proportions of the major subcellular compartments in a range of Arabidopsis tissues and developmental stages. Peptides from at least two marker proteins per organelle have been quantified and an organelle profile built for each tissue. These data are being combined into a free online tool which, together with data from MASCP Gator (http://gator.masc-proteomics.org/), can be used to determine the organellar composition/purity of a sample. Results from samples analyzed by shotgun proteomics are inputted as lists of AGIs. By comparing inputs with extensive and robust organelle marker lists, an organelle profile of the sample based on protein numbers is generated. Estimates of protein abundance can be optionally included using protein abundance scores from MASCP Gator or a scoring system based on MRM analysis. The scarcity of commercially available antibodies and limited scope of enzyme assays means estimation of a sample's organelle composition is problematic for the plant science community, despite this being a fundamental requirement for a broad range of studies. The tool aims to directly address this limitation.

L 31

Keywords: seagrasses, chloroplasts proteomics, marine proteomics

PURIFICATION OF INTACT CHLOROPLASTS FROM MARINE PLANT POSIDONIA OCEANICA SUITABLE FOR ORGANELLE PROTEOMIC.

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Posidonia oceanica belongs to the polyphyletic group of marine angiosperm called seagrasses adapted to grow to the underwater life in shallow waters as n depth waters. This pose questions on how their photosynthesis adapted to the attenuation of light through the water column and leads to the assumption that biochemistry and metabolisms of the chloroplast are the bases of adaptive capacity. We start the construction of a catalog of proteins expressed and/or localized in the organelle of the marine plants. The intact chloroplasts were extracted from leaves growing at shallow depth and performing an isopynic separation and purification from the other cell components. After protein extraction from intact chloroplasts and separation by 1DE SDS-PAGE, we performed the mass spectrometry analyses of polypeptide bands by nLC-ESI-IT-MS/MS. Database searching for protein identification was performed against NCBInr green plant databases, seagrasses database and a local customized dataset. The assignments of chloroplast proteins and their localization in the sub-compartment envelope, stroma and thylakoids were validated by peptide searching against the AT_Chloro database

L 32

Keywords: MIAPE, scientific standards

DO PLANT PROTEOMICS CURRENT PUBLICATIONS FIT IN THE MIAPES AND SCIENTIFIC STANDARDS REQUIREMENTS?

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In the light of the plant proteomics papers so far published we can state that not all of them fit in the standards required for a scientific publication or follow the HUPO-MIAPE recommendations. Both, standards and MIAPEs, are supposed to be known as clearly presented in the major proteomics publications instruction to authors, MIAPE [1], and some other reviews [2], but even so, total or partially ignored. This could be the main reason of rejection for our submitted manuscripts, according to the author´s editing or reviewing experience. Several key issues related to a proteomics publication will be discussed, including: the employed terminology, experimental design,statistical analysis. and a blind acceptance of the matches for protein identification provided by MASCOT and other search algorithms. It is quite common to convert our contribution, supposed to be an original one, in a review of the biological objective and identified proteins. In the best of the cases, and with no data validation, plant proteomics can be defined as mostly descriptive and speculative.

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L 33

Keywords: Protamine sulfate precipitation, Oil seed, Seed storage proteins

PROTAMINE SULFATE PRECIPITATION: A RELIABLE, SIMPLE AND EFFICIENT DEPLETION METHOD FOR HIGH ABUNDANT STORAGE PROTEINS IN OILSEEDS

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We describe a protamine sulfate (PS)-based differential precipitation method to increase detection of both high abundant (HAPs) and low abundant proteins (LAPs) in oilseeds of soybean and peanut. The protocol was developed using proteins extracted from dried soybean seeds with Tris-Mg/NP-40 buffer, followed by precipitation by different concentrations of PS (0.01–0.1%). Results from SDS-PAGE, 2-DE and Western blotting showed that 0.05% PS efficiently and differentially precipitated the HAPs. It was observed that HAPs, such as glycinin and β-conglycinins, were fractionated into PS-pellet while LAPs were enriched in PS-supernatant. Moreover, application of PS fractionated LAPs to MALDI-TOF/TOF analysis led to their successful identification, suggesting that PS precipitation method is MS compatible. Furthermore, efficacy of PS precipitation method was also tested in broad bean, pea, wild soybean, and peanut, which showed that this method is suitable in fractionation of HAPs in all these plants. We therefore conclude that PS precipitation approach presents: (1) a simple method for fractionation of HAPs and LAPs, (2) is suitable for downstream proteomic analysis.

L 34

Keywords: Drought, barley, rapeseed, vegetables, DIGE

QUANTITATIVE ANALYSIS OF PROTEOME EXTRACTED FROM CROPS UNDER DROUGHT STRESS

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Different genotypes of crops (barley, rapeseed, melon, tomato) were used to study the quantitative changes in proteome during drought by 2D-DIGE (two-dimensional difference gel electrophoresis). The dry tissue powder prepared from crowns or leaves by TCA/SDS-Phenol protein extraction. Protein extracts were labelled prior to electrophoresis (IPG strips, pH 4-7, 24 cm; 12.5% SDS-PAGE) with the CyDyes (GE Healthcare) according to the manufacturer's instruction. The differentially expressed polypeptides (absolute abundance variation of at least 2-fold, *p*, 0.05) were analyzed by Applied Biosystems 4800 Proteomics Analyzer (Applied Biosystems). Proteins were identified by searching against the *Viridiplantae* protein database downloaded from the NCBI database using a MASCOT server (www.matrixscience.com). A lot of identified proteins belong to known stress-associated proteins as HSPs, ascorbate peroxidase and enolase. However, some of identified proteins were not well known as stress-regulated proteins.

Acknowledgements: This work was supported by the Ministry of Agriculture of the Czech Republic (QI 111A075, QJ1310055) and by the Ministry of Education, Youth and Sports (LD14064).

L 35

Keywords: 2D-DIGE analysis; cold stress; spring and winter growth habit; wheat crown; cold acclimation; development response

PROTEOME ANALYSIS OF COLD RESPONSE IN SPRING AND WINTER WHEAT (TRITICUM AESTIVUM) CROWNS REVEALS SIMILARITIES IN STRESS ADAPTATION AND DIFFERENCES IN REGULATORY PROCESSES BETWEEN THE GROWTH HABITS

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A proteomic response to cold (4 °C) has been studied in crowns of frost-tolerant winter wheat cultivar Samanta and frost-sensitive spring wheat cultivar Sandra after short-term (3 days) and long-term (21 days) treatments. Densitometric analysis of 2D-DIGE gels led to detection of 386 differentially abundant protein spots revealing at least 2-fold change between experimental variants. 58 representative protein spots have been selected for MALDI-TOF/TOF identification and 36 proteins have been identified. Identified proteins revealing a cold-increased relative abundance in both growth habits are involved in carbohydrate catabolism (glycolysis enzymes), redox metabolism (thioredoxin-dependent peroxidase), chaperones as well as defence-related proteins (protein revealing similarity to thaumatin). Proteins exhibiting a cold-induced increase in winter cultivar only are involved in stress response and development (germin E, lectin VER2) while proteins showing a cold-induced increase in spring cultivar only are involved in restoration of cell division and growth (eIF5A2, glycine-rich RNA-binding protein, adenine phosphoribosyltransferase).

L 36

Keywords: soybena, flax, seed development

SEVEN YEARS OF PLANT PROTEOMICS IN RADIO-CONTAMINATED CHERNOBYL AREA

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Although there has been more than 80 years of research addressing the effects of ionizing radiation on plants, the ongoing success of plants in radio-contaminated Chernobyl area was not anticipated. In order to characterize proteome of plants adapting to radio-contaminated Chernobyl area we established non-radioactive and radio-contaminated experimental fields in 2007. Using two-dimensional electrophoresis and tandem mass spectrometry, we comparatively analyze on yearly basis proteomes of developing and mature soybean and flax seeds harvested from both experimental fields. Initial analyses suggested limited effect of radio-contaminated environment on mature seed proteomes. More detailed analyses revealed alteration in carbon assimilation during seed development that resulted into modification of fatty acid composition and total oil content. The results of this study are being continuously deposited into online database http://www.chernobylproteomics.sav.sk.

Acknowledgment

This research was supported by Slovak Research and Development Agency (APVV-0740-11) and European Community: project 26220220180: Building Research Centre „AgroBioTech".

L 37

Keywords: Prototheca, microalgae, Bovine Mastitis, DIGE, MALDI TOF MS

PROTEOMICS ANALYSIS OF 'ORPHAN SPECIES' - THE CASE OF PROTOTHECA ZOPFII (FAMILY CHLORELLACEAE), THE ONLY KNOWN PLANT-LIKE INFECTIOUS ORGANISM

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Proteomics analysis of unsequenced organisms (orphan species) is rare. The mass spectrometry-based protein identification of these species results in matching with protein sequences of other species and lower identification scores. One such species is *Prototheca zopfii,* an achlorophyllous alga associated with therapy-resistant bovine mastitis and rare human infection. Two genotypes of *Prototheca zopfii* have been described: non-pathogenic genotype 1 and pathogenic genotype 2. Recently, proteomic differences between these two genotypes were investigated using 2D-DIGE MALDI-TOF MS analysis (1) and NCBI database resulted in identification of 110 out of 209 proteins spots. Despite 280 *Prototheca* proteins entries (*P. wickerhamii* -272, *P. zopfii* -2, *P. blaschkeae*-1 and deduced from microbes-3) in the database, the rate of identification was low (52%) and the matching occurred with proteins from *Prototheca* (4%), other algae (36%), bacteria (39%), plants (10%), fungi (4%), and others (7%). Expressed sequence tag (EST)-based protein identification might enhance identification rate due to the larger number of EST entries (5906) in the database.

1. Murugaiyan et al, 2013, Proteomics. 13, 2664-9

L 38

Keywords: MYB transcription factor, Co-immunoprecipitation, LC-MS/MS, Protein-protein interaction, muti-protein complex

REGULATION OF ISOFLAVONOID BIOSYNTHESIS AND GMMYB176 INTERACTOME IN SOYBEAN

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An R1MYB transcription factor, GmMYB176 regulates *chalcone synthase8 (CHS8)* gene expression and isoflavonoid biosynthesis in soybean. We have demonstrated that GmMYB176 alone is not sufficient for *CHS8* gene regulation and hypothesized that GmMYB176 acts cooperatively with other factor (s). The goal of this study is to elucidate the GmMYB176 interactome for *CHS8* gene regulation and isoflavonoid biosynthesis in soybean. Two translational fusions of GmMYB176 (GmMYB176-YFP and YFP-GmMYB176) were created with yellow fluorescent protein (YFP). The fusion proteins were used as baits in separate experiments to pull down their interacting proteins by co-immunoprecipitation and identified by liquid chromatography tandem mass spectrometry. Proteins such as 14-3-3, WD40, bHLH, kinases and uncharacterized proteins were identified. The interaction of each of the candidate with GmMYB176 was validated *inplanta* by bimolecular fluorescence complementation assay. The results will be discussed. Understanding the role of these candidates on *CHS8* expression and isoflavonoid biosynthesis will allow genetic manipulation of isoflavonoid level in soybean and/or introduce isoflavonoid pathway in non-legumes.

L 39

Keywords: Western-blot, antibody, antigen

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 proteomic research. They can be either custom made or purchased from commercial supplier. In either case their production is a complex process, consisting of three very important components which has to be carefully considered. These are: Antigen-Animal-Testing. Which source of antigen is most optimal for your project: peptide, recombinant protein or a native protein isolated from tissue? Which animal species to chose? 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?
Over 15 years spent in antibody production and validation process will aid to answer such questions during this presentation.

L 40

Keywords: seed proteomics, phylogeny, ancestral plants

PROTEOMICS HIGHLIGHTS SPECIFIC FEATURES OF ANCESTRAL AMBORELLA TRICHOPODA SEEDS

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Amborella trichopoda, an understory shrub endemic to New Caledonia, is the sole surviving sister species of all other living flowering plants (angiosperms). We have performed a proteomic analysis of the seeds of this plant (1).

In particular we characterized the seed storage proteins (11S globulins), which are critical for early seedling development in seed plants. Both structural and phylogenetic analyses support the view that Amborella 11S globulins can be both reminiscent of modern angiosperms, and still exhibit specific features of corresponding seed storage proteins in basal species and gymnosperms.

We will also provide information on other protein families from Amborella seeds as well as on the proteome evolution of the seed during the germination process.

(1) Amborella Genome Project (2013) The Amborella genome and the evolution of flowering plants. Science Dec 20; 342(6165)

L 41

Keywords: early-flowering, plant development, equatorial region, production, subtractive cDNA library, bidimensional gels

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN THE SHOOT APICAL MERISTEM USING CONTRASTING SUGARCANE CULTIVARS FOR FLOWERING

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Sugarcane is a tropical crop used for sugar and ethanol production around the world. Early-flowering is an undesirable characteristic since it reduces sugar accumulation in the stalks. The present work used a proteomic tool to identify differentially expressed proteins in the shoot apical meristem (SAM) using contrasting sugarcane cultivars (late-flowering and early-flowering) grown in fields under equatorial conditions. Results obtained here with bidimensional gels and peptide sequence suggest that polyphenoloxidase (PPO), thaumatin-like proteins, glycosyl transferase, peroxidase ATP6a, and some unknown proteins may play a role as promoter or inhibitors in the SAM during the flowering process. The PPO sequence it was also identified in the cDNA subtractive libraries. These results reinforce a potential role for this sequence in this physiologic process. Thus, proteomic and interactome data demonstrate a potential crosstalk from the stress pathway, signal pathway and flowering pathway. This work is the first evidence that suggests a relationship between these proteins and the flowering process in sugarcane plants.

Supported by CNPq and CAPES

L 42

Keywords: ethephon, B serum, Hevea, shotgun proteomics

ANALYSIS OF ETHEPHON STIMULATION EFFECTS ON B SERUM PROTEINS FROM HEVEA LATEX USING SHOTGUN PROTEOMICS APPROACH

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B serum significantly affects the latex flow by plugging the latex vessel/ laticifers [1]. Application of ethephon helps to prolong the flow. In order to gain insights into the B serum proteins implicated in ethephon stimulation, the proteins of ethephon-treated and untreated samples were identified using shotgun proteomic application. Both control and ethephon-treated B serum were subjected to in-solution tryptic digestion and analysed by nano-LC MS/MS and all MS/MS spectra were searched using the SEQUEST. Results demonstrate the successful application of this shotgun proteomics analysis and a total of 720 proteins were identified from both samples. Analysis and identification of proteins revealed that ethephon-treated B serum contains more proteins involved in ATP synthesis, protein transport and proteolysis. In non-ethephon treated B serum, more proteins responsive to oxidative stress, wounding and defense response were recognised. These early results help to gain better understanding of how ethephon affects B serum proteins and latex in manipulating the latex flow.

1. Yeang, H. Y. (1989a). *J. Nat. Rubb. Research.* 4 (1): 47-55.

L 43

Keywords: Germination, halophyte, photosynthesis, proteomics, salt-tolerance

PROTEOMIC APPROACH PROVIDES NEW INSIGHTS INTO SALT-TOLERANCE OF THE OILSEED HALOPHYTE CAKILE MARITIMA

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Proteomics is gaining interest for addressing the molecular responses of plants when challenged with salinity. Yet, data on naturally salt-tolerant species (halophytes) are scarce. Here, we focused on salt-induced changes of the proteome profile of the oilseed halophyte *Cakile maritima* at two developmental stages (germination and vegetative) under moderate to high salinities. Increasing salinity (up to 200 mM NaCl) significantly inhibited seed germination, together with a slower degradation of seed storage proteins. Increased abundance of proteins involved in several metabolic pathways (glycolysis, amino acid metabolism, photosynthesis, and protein folding) during germination was less pronounced under salinity. At the vegetative stage, moderate salinity (100 mM NaCl) was optimal for plant growth. A specific set of leaf proteins involved in photosynthesis, cell expansion and respiration showed significantly higher abundance in salt-treated plants. Altogether, integrating data gained using the proteomic approach with eco-physiological traits provided valuable information which improved our understanding of the salt-response strategy adopted by this halophyte in its natural habitat.

L 44

Keywords: Barley aleurone layer, redox activity, programmed cell death, plant tissue culture

PROGRAMMED CELL DEATH: THE LIFE AMBITION OF THE BARLEY ALEURONE LAYER

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We have developed a 24-well multiplate tissue culture system with electrochemical and optical detection techniques for tissue culture of immobilised barley aleurone layers. We have applied the system for the purpose of studying the underlying mechanisms of programmed cell death (PCD) in plants.

We have optimised an electrochemical, intracellular, whole-cell redox activity assay [1] that probes the NAD(P):NAD(P)H ratio via a double-mediator system. Experiments show that redox activity changes depend on phytohormone activation or inactivation of aleurone layer metabolism and subsequent PCD.

We have successfully applied a fluorescent double-probe system [2] to detect PCD to ensure that our redox activity data match with known responses of barley aleurone layers to phytohormones.

We have also used the system for transformation of barley aleurone cells with α-amylase-GFP constructs for the purpose of studying the timing of α-amylase production in relation to PCD. These studies will be combined with activity assays and quantitative proteomics studies of α-amylase and other target enzymes.

References

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[2] Fath et al., 2001, Plant Physiol, 126, 156-166

L 45

Keywords: Ribo-seq, iTRAQ, iron deficiency, post-transcriptional regulation

TRANSLATIONAL FITNESS OF MESSENGER RNAS: IMPACT OF ENVIRONMENTAL SIGNALS

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Several comparative studies in mammals, yeast and plants showed that transcript levels are not a good proxy for protein abundance, indicative of regulatory processes that disengage changes in protein abundance from transcriptional activity. In contrast to animals, plant r-proteins are encoded by paralogous families consisting of several members, allowing for adjusting the composition of r-proteins in a condition-sensitive manner. Re-organization of the translational machinery may lead to specialized ribosomes that preferentially translate subsets of mRNAs. In addition, stress conditions triggers the production of non-functional transcripts by differential alternative splicing to fine-tune the abundance of proteins that are critical for acclimation. Here, we address the impact of environmental factors on the translational efficiency of mRNAs by using a combination of GWIPS (genome-wide information on protein synthesis *in vivo* by ribosome sequencing) and quantitative iTRAQ proteomics of Arabidopsis leaves and roots subjected to iron deficiency. This analysis revealed a highly dynamic composition and density of ribosomes that prioritizes vital transcripts for translation.

L 46

Keywords: Vitis; drought; root proteome; 1D-PAGE-MS/MS

DROUGHT EFFECTS ON ROOT PROTEOME IN TWO GRAPEVINE ROOTSTOCKS WITH DIFFERENT SUSCEPTIBILITY

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This work focused the attention on the changes induced by water stress (WS) in the root proteome, comparing the susceptible commercial rootstock 101.14 with the new tolerant genotype M4. The biological samples, consisting in lignified root organ of one-year-old plants grown in soil, posed technical challenges related to the paucity of biochemical active tissues as well as to the high content of phenolic substances. In order to overcome these aspects, we used SDS-PAGE combined with nLC-nESI-Q-TOF analyses. The MS/MS experiments were optimized to quantify and to compare the identified proteins and showed both high reproducibility and dynamic range, whit no limits for protein hydrophobicity and *pI*.

The study allowed to quantify one-thousand proteins in each genotype, providing the first proteomic characterization of root organ in *Vitis* and showing as WS significantly affected the levels of about the 15% of the proteome. The main changes were especially linked to energy and redox metabolism, protein turn-over and (a)biotic stress responses, highlighting interesting differences between the two rootstocks.

Acknowledgments: Work was supported by SERRES-AGER project (n°2010-2105).

L 47

Keywords: quantitative genetics, drought, Zea mais, integrative biology

PROTEOMICS ANALYSIS OF THE GENETIC DIVERSITY OF DROUGHT TOLERANCE IN MAIZE

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Maize is one of the main crop worldwide and drought can severely affect its yield. Its responses to drought involve numerous physiologic and molecular functions (e.g. reduction of stomatal conductance, changes in the primary metabolism) that could be involved in the variations of drought tolerance between genotypes. Knowing which of these responses are genotype-dependent can help to identify QTLs for drought tolerance. To address this issue, we analyzed the proteome of 24 genotypes grown under normal irrigation or water deficit. A total of 96 leaf samples (24 genotypes x 2 treatements x 2 replicates) were analyzed by shotgun proteomics. Of 1 125 reproducibly quantified proteins, 552 and 647 varied significantly according to the treatment and to the genotype, respectively. We found evidence for genetic variability of the responses of proteins to water deficit. This opens up new perspectives of breeding based on the combination of complementary responses to drought. This work is a first evaluation of a large-scale analysis (252 genotypes) aiming at performing association genetics on protein abundances, to map their PQLs and analyze their relationships with QTLs of drought tolerance.

L 48

Keywords: Poplar, Gravitropic stimulus, Tension wood, Quantitative phosphoproteomics

A QUANTITATIVE PHOSPHOPROTEOMIC APPROACH TO DECIPHER THE SIGNALING PATHWAY OF TENSION WOOD FORMATION IN POPLAR

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A tree exposed to gravitropic stimulus produces tension wood (TW) allowing it to recover a favorable position. Signaling cascades involving phosphorylation and dephosphorylation of proteins are crucial in this mechanism. To identify members of these cascades, a quantitative proteomic and phosphoproteomic analysis of poplar in control condition or under gravitropic stimulus was achieved. Peptides of straight wood (SW), TW and opposite wood (OW) were labeled with stable isotopes; the fractions obtained after enrichment in phosphopeptides with TiO_2 column were run on a LC/MS-MS spectrometer. !TANDEM pipeline and MassChroq were used to identify and quantify 1,155 proteins and 468 phosphoproteins. The phosphorylation patterns differed between SW, TW and OW, suggesting a specificity of kinases for each wood type. An analysis of the annotations showed a differential abundance of Cellulose synthases: those involved in primary cell wall being over-abundant in TW, while those involved in secondary cell wall being over-abundant in OW. This work provides insights into the gravity response leading to TW formation, a desirable wood for biofuel production due to its higher cellulose content.

L 49

Keywords: Powdery mildews, barley, haustoria proteome

PROTEO(GENOMICS) TO UNDERSTAND MOLECULAR PLANT-PATHOGEN INTERACTIONS BETWEEN BARLEY AND ITS POWDERY MILDEW

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Powdery mildews (PMs) are obligate biotrophic fungal pathogens affecting many crops. Blumeria graminis f.sp. hordei (Bgh) is the causal agent of barley PM. A large scale proteogenomics approach was undertaken to facilitate the genome annotation and the validation of ORF models during the assembly of the sequenced genome of Bgh (Spanu et al, 2010, Bindschedler et al, 2009 & 2011). To compromise host immunity, pathogenic microbes secrete an arsenal of effector proteins into their hosts for successful infection. PM effectors are secreted by haustoria, the feeding structure of biotrophic fungi. 50+ Blumeria Effector Candidate proteins (BECs) were identified exclusively in the haustoria proteome and predicted to be secreted into barley apoplast or epidermal cells. Effector function was validated by RNAi for 8 BECs, including BEC1011 and BEC1054 RNAse-Like proteins, BEC1005 glucosidase and BEC1019 metallo-protease like protein (Pliego et al, 2013). We used a pull-down approach to identify barley proteins interacting with BEC1054 and validated some by Y2H. This included an elongation factor, pathogen related protein PR5 and a GST. The BEC1019 metallo-protease like protein is a universal protein found in more than half of the sequenced fungi including the human pathogen Candida albicans. It might have a role to play in zinc transport or in targeting barley proteins to compromise the immune response.In conclusion, the proteomics analysis of Bgh has been successful to help the annotation of the genome and the discovery of novel effectors with a virulence function. By identifying the plant proteins targeted by these effectors, it will be possible to identify key components plant immunity. Moreover, by adapting a new RNAi methodology to Silence Targeted effector in planta (STEP) and Hydroponic isotope labelling in entire plants (HILEP) of a wheat dwarf variety, we will set up a crop model system to quantitatively study proteomes of plant-pathogen interactions.

L 50

Keywords: DIGE, microarray, stilbenoids, cell culture, grapevine

AN OMICS EXPLORATION OF ELICITOR-MEDIATED STILBENOIDS ACCUMULATION IN GRAPEVINE (VITIS VINIFERA L.) CELL CULTURES

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Grapevine cell suspension cultures accumulate large extracellular amounts of the stilbenoid resveratrol in response to elicitation with methylated cyclodextrins (MBCD) and methyl jasmonate (MeJA) [1]. Here we investigate changes in both the transcriptome and cellular proteome potentially related to stilbenoids production in response to elicitor treatments. Both, the GrapeGenAffymetrixGeneChip® [2] and DIGE-MS/MS-database search techniques were used to detect statistically significant changes and qPCR for profile validation. While CD or CD+MeJA induced accumulation at 24h of transcripts from shikimate, general pheylpropanoid, stilbenoid and malonylCoA pathways; MeJA induced only general phenylpropanoid and stilbenoid pathways. In the proteome kinetics experiment (6-120h), 212 de-regulated unique spots were selected. Protein identification revealed a strong correlation with transcriptome but also a large multiplicity of spots containing proteins relevant to resveratrol synthesis and accumulation. Major findings will be presented and discussed in relation to the effect of elicitors.

References

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2) Grimplet et al. (2012) BMC Res Notes 5:213.

L 51

Keywords: 2-DE, mass spectrometry, MALDI-Profiling, MALDI-Imaging

BRASSICA OLERACEA-XANTHOMONAS CAMPESTRIS INTERACTION: CHALLENGES AND CONTRIBUTIONS OF PROTEOMIC METHODS

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Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Xcc), is one of the main diseases that affects all cruciferous plants. In this study we have identified proteins modulated by the presence of the bacterium Xcc in two genotypes of *B. oleracea*. Plants from the resistant (União) and susceptible (Kenzan) cabbage genotypes were inoculated with Xcc and leaves were collected at 5, 10 and 15 days after inoculation. Leaf samples were analyzed by 2-DE and several differential proteins were identified by mass spectrometry, including proteins involved in defense, metabolism and photosynthesis. Interestingly, proteins associated to photosynthesis were increased in the resistant genotype. RT-qPCR was also performed to further analyze the expression of some genes corresponding to the proteins identified. We have also explored methods such as MALDI profiling using the Biotypersoftware(Bruker Daltonics) and MALDI Imaging to search for differential proteins during *B. oleracea*-Xcc interaction. The results obtained show the potential use of approaches such as MALDI Profiling and Imaging to determine the protein profile during plant-pathogen interaction.

L 52

Keywords: leaf proteomics, ectomycorrhizal fungi, poplars

LEAF PROTEOMICS OF ECTOMYCORRHIZAL POPLARS

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Ectomycorrhizal (ECM) fungi, important partners of plants, most often found in symbiosis with vascular plants, increase the growth parameters of the host as well its resistance to stress conditions i.a. by improving the uptake of nutrients and water by the plant in exchange for its carbohydrates. Despite the relatively large amount of information gathered on ectomycorrhizas, still little is known about the molecular background of the observed changes. In this work, we present the results of proteomic analyzes performed for poplar, *Populus x canescens*, inoculated *in vitro* with two strains of *Paxillus involutus*. The strains, collected in the field, showed significantly different levels of root mycorrhization. Six weeks after inoculation, poplar root tips mycorrhized with the first strain accounted for over 20% of the root system, while mycorrhization with the second strain was recorded in less than 3% of poplar roots. These differences were not only clearly reflected in the biometric features of roots, but also visible in the leaf biometrics. In this poster, we show how the observed phenotypic changes are linked to leaf proteomes of the inoculated poplar seedlings.

P 1

Keywords: Cuminum cyminum, In-silico, Seed Spices, RZ-19, Protein, Structure, Function

INSILCO APPROACH TO DECODE SEQUENTIAL LANGUAGE FOR STRUCTURAL SCRUTINY OF CUMIN (CUMINUM CYMINUM) PROTEIN

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Emergence of in-silico techniques have revolutionariese the traditional methods and opened up new vistas for protein structure and function prediction of proteins in Cumin (*Cuminum cyminum*). In the present study, high quality 3D structure and function of cumin RZ-19 protein has been predicted for hypothetical amino acid sequence and showed homology with the protein domain of humans and E-coli illustrating that database available on Apicaeae family is very low. The estimated molecular weight of identified cumin RZ-19 protein was 55028.6 and was predicted as an acidic protein with pI 5.14. Results of functional scrutiny were broadly classified into 2 categories: 1) Biological Process: oxidation-reduction and ion transport with a probability of 98.9% and 97.8% respectively and 2) Molecular function: structural constituent of ribosome with 99.0% probability. We have also identified a channel in cumin transmembrane protein, through which a ligand might pass. The present finding may be a valuable addition to the proteomic information available on cumin. Further validation can be performed using wet lab experiments.

P 2

Keywords: fatty acid desaturase, restriction analysis, radioactivity

CHARACTERIZATION OF FATTY ACID DESATURASE GENES IN PLANTS GROWN IN RADIO-CONTAMINATED CHERNOBYL AREA

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The aim of our study was to follow previously acquired proteomics data that suggested involvements of fatty acid biosynthesis during plant adaptation in radio-contaminated Chernobyl area[1]. Herein we characterized key enzyme of fatty acid biosynthesis, fatty acid desaturase (FAD) by restriction fragment length polymorphism mapping. Isolated FAD genes were digested using multiple restriction endonucleases. After the comparison of DNA samples from radio-contaminated and non-radioactive fields, restriction profiles of FAD genes were evaluated as monomorphic in the case of all cleavage sites analyzed in this study. Our present experiments are focused on the analyses of DNA methylation and single nucleotide polymorphisms of FAD genes of plants grown in radio-contaminated Chernobyl area.

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P 3

Keywords: pea, cultivar, mutation, identification

THE BATTLE WITH INCOMPLETE GENOME SEQUENCE INFORMATION IN COMPARATIVE CROP SCIENCE – HIGH THROUGHPUT DENOVO SEQUENCING FOR IMPROVED CULTIVAR-SPECIFIC PROTEIN IDENTIFICATION OF PISUM SATIVUM

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Research on fully sequenced model plants applies gained knowledge to ecological or agricultural interesting species. *P. sativum* consists of a large and complex genome and accordingly few sequence information is available. Dealing with different cultivars complicates protein identification due to sequence mutations. We aimed to match the proteome of two cultivars from *P. sativum* (Messire and Model) by comparing the outputs of three commercially available programs (Proteom Discoverer - SEQUEST, MaxQuant - Andromeda, Peaks - deNovo) and found that protein identifications vary notably among these. Adding deNovo sequencing before a database search crucially increases the number of identifications. With Peaks´ homology search tool we found 38 novel commonly mutated proteotypic peptides in both cultivars. Messire and Model showed 69 and 87 cultivar dependent mutations of proteotypic peptides, respectively. The initially used database is going to be complemented with mutated protein sequences for improved identification with any preferred search algorithm. We here present a workflow to be included in proteomic comparison of crop cultivars to enhance protein identification and quantitation.

P 4

Keywords: protein methylation, chloroplast, methyltransferase, Arabidopsis, AT_Chloro

UNCOVERING THE PROTEIN K/R METHYLATION NETWORK IN ARABIDOPSIS CHLOROPLASTS

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The present work aimed at establishing the first repertoire of chloroplastic **methylproteins** in the **chloroplast** of *Arabidopsis thaliana*. Using a dedicated pipeline [1], we datamined the large pool of MS/MS data previously collated for the chloroplast protein database AT-CHLORO [2].

We identified 24 methyl-Lys and 7 methyl-Arg sites from **23 chloroplastic proteins** which belong to essential processes including photosynthesis or chloroplast biogenesis/maintenance. Also, using an *in silico* approach we produced a list of **9 methyltransferases** that are known or predicted to be targeted to plastids.

Biochemical assays with either chloroplast stroma or individual recombinant methyltransferases validated the fructose-bisphosphate aldolase isoforms [3], the plastid ribosomal protein L11 and the β-subunit of ATP synthase [1] as methylated targets and allowed the identification of the involved enzymes.

Future investigation could provide important clues on the biological role of protein methylation in the chloroplasts.

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P 5

Keywords: B. napus, endosperm, embryo, mass spectrometry, 2D IEF/SDS PAGE

BRASSICA NAPUS SEED ENDOSPERM – METABOLISM AND SIGNALING IN A DEAD END TISSUE

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In dicotyledons, such as *B. napus*, the endosperm is of transient nature and only the aleurone layer persists until seed maturity whereas the bulk of the endosperm is replaced by the growing embryo. The interplay of both tissues on various levels is crucial for coordinated seed development. To give insights into the physiological role *B. napus* endosperm a proteomic study including (1) endosperm proteome map, (2) endosperm development and (3) embryo development was performed. Proteins were separated by IEF/SDS PAGE, identified by tandem MS and quantified by Delta2D. This study represents the characteristics of the *B. napus* endosperm proteome, revealing dynamic changes in its metabolic and cellular processes[1]. The metabolic architecture of endosperm comprises the entire set of central metabolic pathways. Its enzymatic machinery turns it into a self-sustaining, metabolically competent tissue. Endosperm development is a unique and complex process which is reflected in the proteome by sequential appearance of proteins involved in energy, carbohydrate, amino acid and lipid metabolism.

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P 6

Keywords: allergens, gliadins, glutenins, proteomics, MSE quantification

THE MSE-PROTEOMIC ANALYSIS OF GLIADINS AND GLUTENINS IN WHEAT GRAIN IDENTIFIED AND QUANTIFIED ALLERGEN PROTEINS

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Precise content of gliadin (Glia) and glutenin (Glu) proteins in wheat grain are largely unknown despite their association with celiac disease, various allergies, and physical processing properties of wheat. In this work, alcohol soluble extracts of Glia and Glu proteins were analyzed using a data-independent mass spectrometry (MS^E) approach in combination with 76 wheat allergenic sequences downloaded from the AllergenOnline database (www.allergenonline.org). This approach quantified 15 allergenic protein isoforms that belong to amylase/trypsin inhibitors, γ-gliadins, and high/low molecular weight glutenins. The identified allergens are associated with Baker's asthma, food allergy, atopic dermatitis, and celiac disease. Also, several peptides carrying four previously discovered epitopes of γ-gliadin B precursor have been detected (QPQQPFP, QTQQPQQPFP, LALQTLPAMC, YIPPHCSTTI). These results showed that the MS^E is suitable approach for quantitative analysis and profiling of clinically relevant proteins in wheat and other food matrices.

Acknowledgment

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Keywords: wheat, Coeliac disease

PROTEOMIC ANALYSIS OF ALLERGENIC PROTEINS IN WHEAT

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Wheat (*Triticum aestivum* L.) is major agriculture crop for countries with moderate climate. Wheat grains are used for human consumption and as animal feed. The quality of baked goods depends mainly on protein composition of wheat grains. However, many wheat grain proteins are clinically relevant for various food intolerances and allergies. For instance, gluten proteins are associated with Baker's asthma and Coeliac disease.Nowadays proteomic approaches are being investigated as promising method for detection and quantification of wheat grain allergens. Herein we used wheat variety Viginta to establish quantitative protein map of wheat grain allergens. The approach based on phenol-based extraction method and protein two-dimensional electrophoresis (2-DE) in combination with MALDI-TOF/TOF resulted into the quantification of 200 2-DE protein spots, out of which 90 were identified.

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P 8

Keywords: sugarcane, quantitative proteomics, lignin biosynthesis

STALK PROTEOME OF SUGARCANE ASSOCIATED TO THE CONTROL OF LIGNIN BIOSYNTHESIS

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While Brazil is the largest producer sugar in the world, most of its sugarcane is actually used to produce ethanol. Surveys show the exponential increase in bioethanol production just exploring the residual biomass of the plants. Improving the availability of the carbon chains for yeasts to produce the cellulosic-ethanol is the challenge now. So, understanding how the lignin biosynthesis occurs in the cell wall of sugarcane stalks, will help to understand the modulation of these genes and contribute for future manipulation of monolignol levels deposited.Therefore, the aim of this work is on the perspective of the proteome investigate the control of lignin formation in stalks of sugarcane from contrasting cultivars using specific environments(drought and nitrogen fertilization) as the main modulators of lignin formation. The GeLC-MS/MS proteomic approach is being performed to characterize the proteomes in the related experiments. Peptides are being analyzed in the Q-Exactive to acquire the peptide MS spectra that will be used in a quantitative strategy to evaluate differential proteins. Differentially expressed proteins will provide new insights about lignin biosynthesis regulation.

P 9

Keywords: analysis of proteins, HTPLC, HPTLC-MALDI-MS, HPTLC-ESI-MS

CHARACTERIZATION OF PROTEINS BY HPTLC-MS – PROTEOMICS REVISITED ?

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Due to the wide range of functionality and reactivity, proteins form a significant focus of research in food and nutritional science. In addition to the protein identification are changes in the protein (post-translational modifications - PTMs) of particular interest. The analysis has always been based on classical chromatographic and electrophoretic separation methods. Studies of such protein derivatives led with the previous methods often only to unsatisfactory results.

In this work, a new form of methodology will be developed, combining traditional mass spectrometry based protein analysis with thin-layer chromatography. The first step is an optimized separation of proteins by means of the multidimensional *high performance thin layer chromatography* (HPTLC). The identification and characterization of proteins and protein species will be carried out by mass spectrometry, which requires the establishment of innovative coupling techniques such as HPTLC-MALDI, HPTLC-ESI, or HPTLC-LESA-ESI-MS. The implementation and development of these methods are initially based on purified proteins. In the further course the developed procedures will be extended to food- and feed-related questions.

P 10

Keywords: Eragrostis tef, drought, iTRAQ, protein-bioinformatics, stress-response

A PROTEOMIC APPROACH TO INVESTIGATE THE RESPONSE OF ERAGROSTIS TEF TO DROUGHT

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Tef (*Eragrostis tef*) is an important staple cereal crop grown for both human consumption and as a forage crop [1]. Tef is of great nutritional value, is highly adaptable to environmental conditions and is grown as an insurance crop when drought conditions arise [1, 2, 3]. In order to observe the proteomic profile of Tef under drought conditions, iTRAQ mass spectrometry data was searched against the *Liliopsida* database in UniProt and the Tef transcriptome using PEAKS software, to identify and quantify proteins changing in response to drought. A total of 205 proteins showed statistically significant changes in expression between the Tef hydrated vs. dehydrated leaf tissue. Validation studies of selected proteins were performed which included physiological enzyme assays and western blot analyses.

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P 11

Keywords: HPTLC, LESA, nanoESI-MS, PTM

A FURTHER LOOK AT PROTEIN ANALYSIS – USEFUL APPLICATIONS OF NANOESI-MS TO IDENTIFY PROTEINS AND POSSIBLE MODIFICATIONS

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The last decade proteomics devoted to map the entirety of proteins. By now the focus places on detailed analysis of individual proteins.

Of special interest is the dissection of so called posttranslational modified (PTM) proteins. To explore the diversity of different possible modifications there are only a few methods the research could avail. By using an off-line nanoESI-MS (e.g. TriVersa Nanomate® Advion, USA) it is possible to identify purified proteins respectively modifications.

Furthermore, the possibility to link a MS with the mentioned Nanomate-source leads to an innovative extraction technique: with a so called *liquid extraction surface analysis (*LESA*)* the substance of interest is leached out of a matrix e.g. a *high performance thin layer chromatographie* (HPTLC) plate. Besides the analysis of intact proteins also a dissection of tryptic peptides could lead to the purposed protein identification. Due to a higher resolution of single peptides by MS-analysis, a previous separation is beneficial. By using a HPTLC separation the gain of advantage is a selective excitation of interesting spots (peptides) which where reckoned to bear a modification.

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Keywords: acetylation, acetyltransferase, Arabidopsis

CHARACTERIZATION OF LYSINE ACETYLTRANSFERASES IN ARABIDOPSIS

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Protein lysine acetylation is one of the most important protein post-translational modification in eukaryotes. It plays an important role in protein synthesis, stability and localization. In plants, the function of acetylated proteins and their catalyst acetyltransferases is not yet well studied. Lysine acetylation is catalyzed by acetyltransferases (KAT) resulting neutralized ε-*N*-acetyllysine. In histones, this conversion results a relaxed structure of chromatin to increase the level of gene transcription. Recently, lysine acetylation of proteins outside of the nucleus was experimentally proven (Finkemeier et al., 2011; Wu et al., 2011). However, the function of those acetylated proteins and especially their catalyst acetyltransferases remains unknown. Therefore, it becomes a new research frontier to reveal the biological function of lysine acetyltransferases in plants. To enrich and identify acetyltransferases, an artificial synthesized peptide probe was utilized, which can mimic the substrate of acetyltransferases in vitro. In this study, we aim to identify lysine acetyltransferases in Arabidopsis by using subcellular localisation analysis and co-immunoprecipitation approaches.

P 13

Keywords: Drought,proteome,root,LC-MS/MS,Medicago

SOLUBLE AND MICROSOMAL ROOT PROTEOME CHANGES OF MEDICAGO TRUNCATULA IN RESPONSE TO TWO LEVELS OF DROUGHT STRESS

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Changes in soluble and microsomal proteins in response to drought stress were analyzed via shotgun LC-MS/MS in roots of *Medicago truncatula* plants. Comparative proteome analysis revealed that cellular membranes are highly sensitive to drought, with a significant microsomal protein breakdown concomitant with the level of stress. The down-regulation of protein synthesis and nitrogen assimilation-related proteins with the inducement of protein targeting and degradation indicate a protein recycling and re-location process. Membrane transport was also affected, activating some drought tolerance mechanisms such as the down-regulation of aquaporins and inducement of vacuolar H^+-ATPases, to minimize water flow through the cell and sequester toxic cytoplasmic Na^+ into the vacuoles, respectively. Other drought tolerance mechanisms were also induced, such as the accumulation of sucrose or the removal of ROS and lipid peroxides. This study provides new insights that may lead to a better understanding of the molecular basis of the drought stress responses.

P 14

Keywords: chloroplast, modelling, metabolic pathways

TOWARDS THE VIRTUAL CHLOROPLAST

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The chloroplast is a complex and integrated metabolic network. One way to improve our knowledge of such a “metabolic factory” is to automatically build metabolic pathways with well-curated and integrated knowledge. Unfortunately, current knowledge of the plastidial metabolism is still dispersed in the scientific literature.

Thus we decided to create a virtual chloroplast, by manually and automatically integrating all the qualitative and quantitative data currently available, especially proteomic data. It will contain a user-friendly interface allowing rapid visualization and virtual modulation of metabolic fluxes, for research or for teaching purposes. First, we built a series of metabolic maps of the Arabidopsis thaliana chloroplast using the CellDesigner software. These maps have been integrated into a web interface and provide direct link with biological and bibliographical databanks (e.g AT_CHLORO database). Each component of a given map has a dedicated description page. Furthermore, every map is connected with each-other allowing to follow a metabolite from one metabolism to another. A first release of this knowledge base is expected by Q1 2015.

P 15

Keywords: Peptide, bioactive, nut

PROTEOMIC PROFILE OF THE SEED OF CARYA ILLINOINENSIS (FAGALES: JUGLANDACEAE): SEARCH FOR PEPTIDES WITH POTENTIAL BENEFITS FOR HUMAN HEALTH.

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Bioactive peptides are small peptide chains composed between 2 and 9 amino acid residues derived from foods [1]. In the last decade it has been shown that these have different biological activities, with benefit effects on the consumer. An alternative source is the seed of pecan tree (*Carya illinoinensis*). It has a protein content of 9%, and is considered a crop with a great potential neutraceutical as it provides a balanced supply of fatty acids [2]. The aim of this work was to obtain proteomic profile of *C. illinoinensis* by two-dimensional 2D-PAGE. It was quantified over 226 spots. 78 peptides were identified by MASCOT, and bioinformatics prediction indicated that the peptides have different biological activities highlighting antimicrobial, antioxidants, immunomodulators, anticancer,and antithrombotics activities. This is the first work that gives a real approximation of the proteomic profile of *C. illioinensis*; and support the isolation and testing of individual biological activity of each peptide identified in this study.1. Maldonado, et al 2010.Peptides.3 1: 1635–1642. 2. Oro, et al 2008. Grasas y Aceites.59(2):132-138. The Research thanks to Conacyt Ciencia Basica No. 182549.

P 16

Keywords: Quantitative proteomics, rice, germination, cold stress, shotgun

QUANTITATIVE SHOTGUN PROTEOMIC ANALYSIS OF RICE GERMINATION UNDER THE COLD CONDITION

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We performed comparative shotgun proteomic analysis of germination of rice grain under in cold water. Tong-887(T887) is resistant to cold treatment and Milyang23(M23) is susceptible. Brown rice were germinated in 13℃. Brown rice geminated in 28℃ were used as control. When the hypocotyl emerged, proteins were extracted from the whole rice grain including embryo, endosperm, and hypocotyl. To construct an in-depth proteome reference map, monitoring the expression patterns of the identified proteins, and to detect proteins that are expressed differentially in the cold treatment in Tong-887, the protein expression patterns were revealed by a quantitative shotgun proteomic analysis. Extracted proteins were separated in 1D-SDS-PAGE then the gel was sliced into seven. Chopped gels were in-gel digested. M23 was lower than that of T-887 until 7 DAI. In 8DAI, the germination rates of both varieties were ~80%. In cold condition, the length of hypocotyl of T-887 was longer than that of M23. By merging all of identified proteins, ~2,200 non-redundant proteins were detected. The glycolysis associated proteins were highly abundant in the germinated rice grain.

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Keywords: cryptophytes, ocean, Guillardia theta, light harvesting, iTRAQ

ALL 21 ALPHA-PHYCOERYTHRINS OF THE CRYPTOPHYTE GUILLARDIA THETA ARE LIGHT-DEPENDENTLY EXPRESSED

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Cryptophytes are a group of flagellated algae living in marine or freshwater environments where light is limiting. As a typical adaptation to this condition, cryptophytes harvest light using two different antenna systems; the membrane embedded chl *a*/c_2 binding proteins as well as the lumenal localized phycoerythrins. In *Guillardia theta*, a representative of the cryptophytes, the unique and evolutionary conserved β-phycoerythrin is encoded in the chloroplast genome, while the divergent α-phycoerythrins are encoded by 21 nuclear genes. The different α-phycoerythrins are considered to form distinct holoproteins, which alter the properties of the light harvesting unit. Using iTRAQ we quantified expression of the α- and β-phycoerythrins under different light fluxes that are relevant in the natural habitat of *G. theta*. Our preliminary data show that β-phycoerythrin and all 21 α-subunits are expressed in a light dependent manner. Additionally to these light harvesting proteins, we detected 90 other proteins with significantly altered expression levels.

P 18

Keywords: Activity Based Protein Profiling, Herbicides

ELUCIDATING THE MODE OF ACTION OF K3 HERBICIDES USING ACTIVITY BASED PROTEIN PROFILING

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K3 herbicides are believed to work by disrupting VLCFA synthesis through inhibition of a family of enzymes called KCSs. Differential inhibition by K3 herbicides has been established in the literature but exact targets are unknown. We intend to use Activity Based Protein Profiling, a form of chemical proteomics, to find the target profiles of a variety of K3 herbicides and thereby identify the key targets crucial to the mode of weed lethality. We have successfully developed a proteomics workflow to identify the protein targets of one such K3 herbicide.

P 19

Keywords: SpikeTides, Peptide Reference Standards, Proteotypic Peptides

SPIKEMIX PEPTIDES – A NOVEL APPROACH FOR SYNTHETIC PREPARATION OF LOW COST & SMALL SCALE PEPTIDE POOLS FOR LARGE SCALE PROTEOMICS

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Recently, SpikeTides™ were reported as small-scale, inexpensive, heavy labeled or non-labeled and/or absolutely quantified peptides for SRM/MRM assay development and absolute/relative protein quantification [1].

The synthesis of non-quantified SpikeTides™ is performed via economical SPOT™ synthesis on cellulose membranes [2], yielding individual peptides. While these peptides sufficiently support discovery and targeted proteomics studies, for large scale approaches including thousands of peptides, handling efforts (pooling) and costs remain significant.

Here we present an advanced procedure for the preparation of synthetic peptide pools with increased efficacy and throughput.

Synthesized peptides were used to support weak peptide identifications in ProteomicsDB, a new database containing high-quality proteomics data for the whole human proteome. [3]

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P 20

Keywords: abscisic acid, ubiquitylation, PTMs

WHICH PTMS ARE IMPLICATED IN ATHB6 REGULATION?

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Abscisic acid (ABA) biosynthesis is directly induced by drought. The presence of this hormone initiates a signaling cascade that leads to the expression of stress-related genes and stomata closure. In the model plant Arabidopsis only few transcription factors have been described to be involved in stomatal movements. The class I HD ZIP transcription factor, ATHB6 is among them. ATHB6 act as a negative regulator of ABA and its expression is induced by ABA, salt and drought. ATHB6 is also tightly controlled at protein level by post-translational modifications (PTMs), in particular ubiquitylation by a specific class of CRL3 E3 ligase [1]. Our work aims to identify and characterize all PTMs on ATHB6 using proteomic approaches in order to better understand the regulation and function of ATHB6 in drought stress responses.

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Keywords: SUMOylation, carbohydrate metabolism

SUMOYLATION OF ARABIDOPSIS PROTEINS AND ITS INVOLVEMENT IN CARBOHYDRATE METABOLISM

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SUMOylation is a post-translational modification involved in a variety of cellular functions by affecting protein localization, stability and activity [1]. In animals, SUMOylation shows to play a role in sugar metabolism [2]. In plants, enzymes of the central carbohydrate metabolism have been shown to be SUMOylated [3]. However, its relevance in the regulation of the respective pathways has not been established.

We follow two proteome wide approaches to identify SUMOylated proteins and study the link between SUMO and plant carbohydrate metabolism. This poster presents preliminary results obtained using a gel-shift assay to uncover SUMOylated proteins. The second approach aims to characterize putative chloroplast localized SUMO proteases to uncover their role in the regulation of carbohydrate metabolism related enzymes.

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P 22

Keywords: Cell Wall, Protein Interactions, Golgi, Covalent bond formation

COVALENT BOND FORMATION AMONG GOLGI-LOCALIZED GLYCOSYLTRANSFERASES

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Plant cell walls represent one of the most abundant resources present on the planet. Influenced by environmental changes they are constantly rebuilding themselves and the extensive biosynthetic machinery required for its control and regulation remains unclear. Large parts of the plant cell wall polymers are synthesized by groups of glycosyltransferases within the Golgi apparatus. Recent observations and studies indicate that enzymes involved in plant cell wall biosynthesis indeed acts in complexes via both covalent and non-covalent bonds [1,2]. To study covalent interactions of plant cell wall related enzymes in the Golgi apparatus, isolated Golgi fractions from plants cell wall cultures and plant seedlings were treated with or without protein denaturation, reduction and alkylation agents followed by protease digestion prior to LC-MS analysis. Initial findings highlighting Golgi enrichment approaches, the ability to identify cross-linked residues using the outlined approach and the extent to which cell wall related enzymes appear to be forming complexes will be presented.

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P 23

Keywords: vesicular trafficking, proteomics, Arabidopsis, brefeldin A, wortmannin, LY294002

PROTEOMIC STUDIES ON ARABIDOPSIS USING INHIBITORS OF VESICULAR TRAFFICKING – A COMPARATIVE SURVEY

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Brefeldin A (BFA), wortmannin and LY294002 (LY) are widely used in the investigation of vesicular trafficking (VT), mainly because of known subcellular effects as well as molecular targets. Nevertheless, the proteome-wide changes caused by these inhibitors are not well known. In this contribution we summarize and cross-compare the proteomic changes occurring after treatments of Arabidopsis roots with BFA, wortmannin and LY. The proteomic data are accomplished by detailed cell biological studies.

Among proteins involved inVT, we have found for example varying abundance of small RabGTPase RabA1d in response to BFA (upregulated) versus wortmannin and LY (downregulated). Additionally, we obtained proteome differences between wortmannin and LY, likely related to their PI3K specificity. Both substances consistently altered VT-related proteins, but they had diverse effects on proteins involved in stress response. In general, proteomics revealed that these inhibitors alter apart from VT-related events also other cellular and metabolic processes.

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P 24

Keywords: Plant cell wall, Plant proteomics, CWPs, Extraction, Alfalfa

COMPARISON OF DIFFERENT METHODS FOR THE EXTRACTION OF CELL WALL PROTEINS FROM ALFALFA STEMS

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The plant cell wall (CW) is a complex and highly organized entity, modified throughout the plant's life, containing economically highly valuable compounds. Although mainly composed of polysaccharides, plant cell walls also contain proteins (CWPs) assuring the *in situ* deposition of the CW but also stress-protective functions. Numerous proteomic studies, using specific extraction protocols, have been performed on the plant CW, but a comparison of the different protocols on the same sample is currently not published. As one of the most used forage plants the study and improvement of alfalfa (*Medicago sativa*) knows an economic interest. This work consists of comparing previously used extraction protocols and the establishment of a hybrid protocol on alfalfa stems improving CWPs enrichment. CWPs were identified by two complementary techniques, nanoLC-MS/MS and 2DE-MS/MS, to assess the efficiency of the different protocols. Preliminary data wherein the optimized protocol was used to study the influence of Cu nutrition on the CW proteome of alfalfa will likewise be presented.

*equal contribution

P 25

Keywords: gel-based proteomics; methods comparison

GOING DEEPER IN COFFEA ARABICA LEAF PROTEOME

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Three extraction and cleaning methods were tested to select the most effective protocol for *C. arabica* leaf protein analysis by 2D-electrophoresis. Trichloroacetic acid precipitation followed by resuspension in 2% SDS sample buffer was selected as the best method when combined with the 2D clean-up kit®. Protein (300 µg) was loaded to 18 cm IPGstrips pH 3-10, and the second dimension was performed in 12.5% T gel. The analysis of colloidal Coomassie Blue stained gels revealed 376 spots. By MALDI-TOF/TOF MS, followed by homology search in NCBI and ESTs Coffee databases, 185 proteins were identified. Using the Blast2GO functional annotation it was found that the most represented proteins are involved in photosynthesis, respiration and amino acid metabolism. This proteome contrasts with that of the apoplast [1] which shows the predominance of proteins of cell wall metabolism, phenylpropanoid biosynthesis and proteolysis. The information obtained from the two studies allows a deeper understanding of the coffee leaf metabolism.

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This work was supported by FCT (PTDC/AGR-GPL/109990/2009, PEst-OE/EQB/LA0004/2011) Portugal,CNPq and INCT-Café from Brazil

P 26

Keywords: plasma membrane, sugar beet, iron deficiency, shotgun proteomics, detergent resistant microdomain

CHANGES IN THE PROTEIN PROFILES OF PLASMA MEMBRANE AND DETERGENT RESISTANT MICRODOMAIN PREPARATIONS FROM BETA VULGARIS ROOTS AS AFFECTED BY FE DEFICIENCY

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The aim of this work was to characterize the changes induced by Fe deficiency on the protein profiles of plasma membrane (PM) and detergent resistant PM microdomain (DRMs) preparations from *B. vulgaris* roots. Highly pure PM and DRMs were prepared by aqueous two phase partitioning and Triton X-100 treatment followed by ultracentrifugation in a sucrose gradient, respectively. Samples were analyzed by shotgun proteomics (nanoLC-MS/MS), spectra processed with Progenesis software and identification carried out using MASCOT and a custom made database containing *Beta ssp.* UniProtKB and published genome entries. A total of 278 proteins were identified and quantified with 2 or more peptides in all samples, and 66 and 187 of them showed significant changes in abundance (ANOVA $p<0.05$, $n>4$, fold change>1.5) in the PM and DRM preparations, respectively, when Fe-deficient samples were compared to Fe-sufficient ones. A preliminary analysis revealed that membrane trafficking and protein phosphorylation and signaling were the functional categories containing a larger number of protein species showing decreases in abundance, whereas increases were found in proteins related to carbon metabolism.

P 27

Keywords: proteins, carotenoids, membrane

INFLUENCE OF PROTEINS AND THEIR COMPLEXES WITH CAROTENOID PIGMENTS ON MODEL LIPID MEMBRANES STUDIED WITH APPLICATION OF THE BLM – BLACK LIPID MEMBRANE-METHOD

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Black Lipid Membrane (BLM) technique was used to examine effects of BSA (Bovine serum albumin) on properties of the model membrane. BLMs were formed with EYPC

(L-α-Phospatydylocholine from eggs yolk) deposited in n-dekane. BLM studies were performed in phosphate buffer (pH 7.4).

Various concentrations of BSA (between 0,01 mg/ml and $2{,}5 \times 10^{-3}$mg/ml) were used in order to optimize experimental conditions. BSA concentrations above 0,01 mg/ml proved too high as they caused collapse of the lipid membrane. Supplementation of the protein and protein - pigment complexes with carotenoids resulted in change of resistance of the lipid membrane.

The results of the experiments indicated that the pure protein and protein complex with carotenoids interact with the lipid membrane.

P 28

Keywords: Allium sativum, development, low-temperature, 2-DE, LC-MS/MS

LOW-TEMPERATURE CONDITIONING OF "SEED" CLOVES INDUCES DIFFERENTIAL RESPONSE IN 'COREANO' GARLIC (ALLIUM SATIVUM) PROTEOME DURING PLANT DEVELOPMENT

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Low-temperature conditioning of garlic seed cloves accelerated the development of the crop cycle and increased the synthesis of phenolic compounds and anthocyanins in the bulbs at harvest time [1]. The aim of this work was to apply a 2-DE gel coupled with LC-MS/MS approach to study the changes in the protein profiles during the plant development of garlic subjected to cold conditioning. Garlic seed cloves were conditioned at 5°C for 5 weeks and were sown in a field. Proteins were extracted from three biological replicates of samples collected during plant development and at harvest time with an adapted method [2] and separated by IEF using 24 cm, pH 4-7 strips. 2-DE gels were stained with Coomassie blue and analyzed using Melanie v7.0. So far, 62 protein spots showing statistically significant changes in abundance have been analyzed by LC–MS/MS and identified by database analysis using the Mascot search tool. This is the first report analyzing the garlic proteome modifications induced by the cold conditioning of the seed cloves during plant development.

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P 29

Keywords: radioactivity, seed, development, abiotic stress, fatty acids

THE ANALYSIS OF FLAX SEEDS HARVESTED FROM CHERNOBYL AREA WITH INCREASED OIL CONTENT

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We investigated seed development of flax (*Linum usitatissimum*, L. variety Kyivskyi) grown in radio-contaminated and non-radioactive experimental fields located in Chernobyl area since 2007. Surprisingly, Chernobyl environment resulted into 12% increased oil content in mature seeds. In order to elucidate molecular bases for such increase, flax seeds of second generation were harvested at 2, 4, 6 weeks after flowering and maturity stage and analyzed using 2-D gel electrophoresis (pH 5-8 strips) followed by LC-MS/MS. Our results indicate significantly increased abundance of proteins associated with carbon assimilation and fatty acid metabolism in early stages of seed development what is in concordance with increased level of seed oil. Increased abundance of seed storage proteins confirms data obtained from previous analyses. The updated model for plant adaptation toward a radioactive contamination based on these and previous results was suggested.

Acknowledgements

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P 30

Keywords: androgenesis, triticale, 2D-electrophoresis

PROTEOMIC ANALYSIS OF WINTER TRITICALE (×TRITICOSECALE WITTM.) ANTHERS AFTER ANDROGENESIS-INDUCING TREATMENT

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Microspore embryogenesis (androgenesis) is regarded as one of the most striking examples of cellular totipotency in the plant kingdom. This phenomenon is expressed only under certain circumstances different for each species. It has been proved that one of the main factor responsible for the change of microspore developmental pathway is stress treatment. Since cold treatment (2-3 weeks at 4°C) occurred to be the most efficient in triticale androgenesis induction, proteomic study can provide new information about molecular mechanisms involved in this remarkable process.

We investigated changes in abundance of protein species after cold treatment in triticale anthers using gel-based proteomics. The proteins were isolated according to the phenol-based procedure (Hajduch et al. 2005). The protein expression patterns were examined by using 2-D electrophoresis and chosen proteins were identified by MALDI TOF/TOF MS/MS analysis. It was shown that androgenesis-inducing treatment changes the anthers protein profile. The identified proteins were mainly associated with metabolism or cell structure.

Acknowledgement

The research is supported by the National Project 2011/01/N/NZ9/02541

P 31

Keywords: Chernobyl, flax, two-dimensional electrophoresis, ionizing radiation

PROTEOMIC ANALYSIS OF SEED DEVELOPMENT OF FLAX GROWN IN RADIO-CONTAMINATED CHERNOBYL AREA FOR THREE GENERATIONS

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We follow flax during adaptation in radio-contaminated Chernobyl area since 2007. Herein we present the analysis of third flax generation. In order to reveal differences between proteomes of developing seeds grown in non-radioactive and radio-contaminated Chernobyl area, we employed quantitative proteomics approach based on protein two-dimensional gel electrophoresis and tandem mass spectrometry (MS/MS). This approaches resulted into the establishment of joint abundance profiles for 130 protein spots out of which 75 were found differentially abundant. All differentially abundant spots were reliably identified by comparison to previously established reference map or by MS/MS. The identity of differentially abundant proteins confirmed association of carbon metabolism with flax adaptation toward radio-contaminated Chernobyl environment that was detected in first two flax generations. The data also showed differential abundance of unknown proteins that constitute targets for further investigations.

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P 32

Keywords: Proteomics Bioinformatics Software Gel Electrophoresis

A TOOL FOR THE ASSESSMENT OF 2D GEL ELECTROPHORESIS ANALYSIS PROCEDURES, FROM MISSING VALUES TO GENE ONTOLOGY

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The analysis of 2D gels for proteomics requires the choice of methods to deal with missing values, data normalization and statistical tests; we developed a tool, available in the R software environment, to aid in this decision, which can significantly change the biological interpretation based on the relevant results. The set of available commands allows assessing data quality in terms of missing values distribution. Then the data can be processed by combining several methods for missing values imputation, normalization and statistical evaluation. The results of each different procedure are analysed according to the gene ontology classes evoked by the resulting proteins sets. All the ontological profiles are finally compared. In this way the user can select a procedure for the data processing which, while methodologically sound, provides the results best matching a coherent and plausible data interpretation.

P 33

Keywords: populus, 2D DiGE, climate change, isoprene emission, VOC

EFFECTS OF FUTURE CLIMATE SCENARIO'S ON ISOPRENE EMITTING AND NON-EMITTING POPLARS

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An experiment was run with poplar lines modified in isoprene emission potential, in which isoprene emission was genetically repressed or strongly down-regulated. Isoprene emitting and non-emitting lines were grown in four controlled growth chambers, each accommodating four different treatments, focused on the effect of climate change on isoprenoid metabolism, and the consequent response to integrated stress events. The different treatments included a) control plants grown under unstressed conditions and ambient CO2 concentration; b) plants grown at high CO_2 concentration, and plants grown as in b) but exposed to c) intermittent or d) chronic drought stress. Samples were taken at the maximum of stress intensity and after a week of recovery. Non-invasive measurements, biochemical and proteomic analyses were conducted. Proteomics were run as 2D-DIGE, and results indicate that primary effects on protein abundance variations were due to (i) stress, then (ii) recovery period, (iii) to isoprene emission and (iv) elevation of CO2, respectively.

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P 34

Keywords: Ginseng roots, Protamine Sulfate, Low abundant proteins, 2D-PAGE

DEVELOPMENT OF A SIMPLE AND RAPID PROCEDURE FOR EXTRACTION OF PROTEINS FROM PLANT TISSUES: A CASE STUDY OF GINSENG

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In this study, a highly efficient method for extraction of ginseng root proteins and depletion of high abundant protein (HAPs) is reported. Total proteins from ginseng roots were isolated using Mg/NP40 buffer and samples were clarified using an in-house developed procedure to get rid-off from secondary metabolites and other contaminants. Purified proteins were then precipitated using 0.5% protamine sulfate (PS) to deplete HAPs like ribonucleases, leading to the enrichment of low abundant proteins (LAPs). Comparative SDS-PAGE and 2D-PAGE analysis revealed that our method is rapid and highly efficient in removal of contaminants in comparison with other previously published methods. A combination of in-house developed procedure with PS precipitation resulted in fractionation of highly abundant ribonucleases into pellet (P) fraction, while LAPs were preferentially enriched in the supernatant (S) compared to the total extract (T). Totally, 760.5±119.5, 935.5±78.5, and 563.5±23.5 spots were detected in high resolution 2-D maps of T, S, and P samples, respectively. Thus, this method can be used for in-depth proteomic analysis in ginseng and other plants, rich in secondary metabolites.

P 35

Keywords: Large pore BN-PAGE, thylakoids, Photosystem II super- and mega-complexes

LARGE PORE BLUE NATIVE GELS FOR SEPARATION OF THYLAKOID PROTEIN COMPLEXES AND IDENTIFICATION OF PHOTOSYSTEM II SUPER- AND MEGA-COMPLEXES ISOLATED FROM HIGHER PLANTS

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Gel-based analysis of thylakoid membrane protein complexes represents a valuable tool to monitor the dynamics of the photosynthetic machinery, of which Photosystem II (PSII) is a key component. In plants PSII has associated with it an antenna system, the light harvesting complex II (LHCII), responsible for increasing its light harvesting efficiency, forming PSII-LHCII super-complexes and mega-complexes (PSII-LHCII-sc and -mc).

Pea thylakoid membranes solubilised with the detergent n-dodecyl-α-D-maltoside according to [1] were separated either by sucrose density gradient or native PAGE.

Large pore blue native electrophoresis [2] was applied for: 1) the separation of pea thylakoid membrane protein complexes, searching for high-molecular-mass thylakoid membrane complexes; 2) the identification of the typology of PSII-LHCII-sc and -mc isolated by sucrose density gradient.

Further, transmission electron microscopic investigations were performed on the isolated PSII-LHCII-sc and -mc to correlate their protein composition to the supramolecular organization.

References

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P 36

Keywords: abiotic stress, drought, proteomics, 2D-DIGE, hop

PROTEOMIC AND PHYSIOLOGICAL RESPONSE OF HOP (HUMULUS LUPULUS L.) TO DROUGHT STRESS

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Drought is one of the most important constraints on the growth and productivity of many crops, including hops (*Humulus lupulus* L.). To improve our understanding of the complex mechanisms involved in the response of hop to drought stress, a proteomic approach was used to identify proteins in the leaves of two cultivars (Aurora and Savinjski golding) differing in their response to drought. A growth experiment was carried out in 2013 and 2014. Measurements of physiological parameters (gas exchange techniques, fluorescence, water potential measurements) gave a better insight into the drought response of plants and enabled the selection of plants for proteomic analysis. Proteins in leaves were extracted from four biological replicates, the concentration of proteins was measured with a 2D Quant kit, while 2D-DIGE technology was used to compare differences in protein abundance between control and stressed plants. Physiological measurements and preliminary proteomic results of the experiment will be presented.

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Keywords: apoplastic, iron deficiency, two-dimensional gel electrophoresis, sugar beet.

CHANGES IN THE PROTEIN PROFILES OF BETA VULGARIS LEAF APOPLASTIC FLUID WITH IRON DEFICIENCY AND IRON RESUPPLY

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The aim of this work was to study the effects of Fe deficiency and Fe resupply on the protein profile of *Beta vulgaris* leaf apoplastic fluid. Plants were grown in Fe-sufficient and deficient conditions, and Fe resupply was carried out with 45 µM Fe(III)-EDTA for 24 h. Apoplastic fluid was obtained by leaf centrifugation, proteins were precipitated with 10% TCA and analyzed by 2-DE IEF SDS-PAGE. Eighty µg of protein were loaded in IPG strips (pI 3-10) and 12% gels were used for the second dimension. Gels were analyzed using PDQuest and 203 spots were consistently detected in 100% of the four biological replicates. Sixteen proteins changed in relative abundance in the different treatments (increases >2-fold or decreases <2-fold; $p<0.05$). When compared to controls, 4 spots increased in Fe-deficient and Fe-resupplied samples, whereas 1 and 4 spots decreased, respectively. In the comparison of Fe-resupplied *vs.*-Fe, 2 and 4 spots increased and decreased, respectively. Fourteen (88%) of the 16 protein species were identified using nanoLC-MS/MS, with polysaccharide metabolism being the most affected metabolic pathway. Some changes in the protein profile were checked for gene expression.

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Keywords: Xyrophyta viscosa, desiccation tolerance, post translational modification, phosphoproteins

PHOSPHO-PROTEOMIC ANALYSIS OF XEROPHYTA VISCOSA UNDER DEHYDRATION STRESS

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Xerophyta viscosa (X. viscosa) is an ideal genetic model to study plant abiotic stress and serves as a novel source of stress tolerance genes [1].Isolation of phosphorylated proteins from tissue samples requires removal of non-phosphorylated proteins followed by enrichment steps selective for phosphoproteins.Enrichment strategies such as immobilized metal affinity chromatography and the use of an Agilent phosphochip will be employed. All LC−MS/MS data will be analyzed using both Mascot and PEAKS against all plant proteins in the Swissprot database. Our motivation for this study is twofold; firstly, to identify important and specific phosphoproteins involved in desiccation tolerance. Secondly, to relate these *X.viscosa* phosphoproteins to known plant signalling mechanisms in order to elucidate unique and common mechanisms in response to abiotic stress.

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P 39

Keywords: Coffee, Coffee leaf rust, Apoplastic proteins, 2-DE, MALDI-TOF/TOF MS

HIGHLY SPECIFIC ANTIBODIES AGAINST DIFFERENTIALLY EXPRESSED PROTEINS OF COFFEA ARABICA-HEMILEIA VASTATRIX INTERACTIONS

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Comparative proteomic analysis of *C. arabica* leaves healthy and infected with *H. vastatrix* (resistant and susceptible samples) allowed the identification of differentially expressed proteins, namely, chitinases, pectin methylesterase, serine carboxypeptidase, reticuline oxidase-like protein and subtilisin-like protease. By bioinformatic means, peptide candidates with 11-12 amino acids were selected, on the basis of hydrophobic status, accessibility at the surface and structure prediction. These peptides were synthesised and conjugated with bovine serum albumin and ovalbumine (OVA) by the glutaraldehyde one-step method and used in the immunization protocol. The highly specific antibodies (Anb) were produced and their titer against each peptide was evaluated by indirect-ELISA with the OVA-peptide conjugate. These Anb showed higher level of detection in the resistant than in the healthy or susceptible samples. The Anb will be used in an ELISA kit assay as resistant markers to assist in the selection of appropriate coffee genotypes with relevant traits for breeding programs.

Acknowledgement

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P 40

Keywords: cell wall, proteomics, UVB

SUGARCANE CELL WALL PROTEOME RESPONSE TO HIGH ULTRAVIOLET-B RADIATION STRESS

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Development of new economically feasible usage of ligno-cellulosic component from sugarcane biomass has been attempted. Plant cell wall polymers structural networks compose the extracellular matrix, whose dynamics is mostly coordinating by its proteome (about 1% of total cell proteome). Despite of its relevance, cell wall proteome studies are relatively very few. Here, the usually considered drought-tolerant and high fiber content sugarcane commercial hybrid (RB867515) was exposed to high or low intensity ultraviolet-B (UVB) radiation stress during 7 days. Cell wall-enriched protein extracts were obtained by using CaCl2 and LiCl buffers, and submitted to 2D-PAGE profiling. Obtained digital 2D profiles were analyzed in LabScan and Image Master 2D Platinum v.7.05 (GE Life Sciences) programs, where the selected differential protein spots (DEPs) could be selected based on significant ANOVA and normalized frequency ratio. DEPs were excised from gels, digested by trypsin and analyzed in AutoFlex III MALDI-ToF/ToF (Bruker Daltonics) mass spectrometer. Among the selected 18 DEPs, about 29% could be putatively assigned to extracellular matrix and/or functionally involved with it. Also, only four DEPs could be identified as present in both conditions but significantly variable in the accumulation level between them; all these common DEPs presented reduced accumulation under higher UVB stress. Meanwhile, six DEPs were detected exclusively in the lower UVB intensity treatment, and eight 8 DEPs were found as exclusive from higher UVB condition. The identified proteins were putatively assigned to signaling, membrane transport and cell wall enzymes associated to glycans biosynthesis, and starts to point an initial picture on sugarcane cell wall proteome dynamics in response to UVB radiation stress. The results may be helpful on future research on sugarcane and other plant species cell wall proteome regulation associated to abiotic factors, as well as the cell wall structural features that may be affected by such molecular regulation with consequences in produced biomass.

Acknowledgement

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Keywords: Allergen, Foodomics, Citrus, SDS-PAGE, Mass spectrometry

ALLERGOMICS IN CLEMENTINE JUICE IN A TRANSLATIONAL PROTEOMICS PERSPECTIVE

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Nowadays the allergens in foods is of big importance for allergic consumers. The cutting edge omics technologies permits a deep knowledge in features of these molecules referring to foodomics that can be considered a new area of translational proteomics. In particular clementine fruits represent one of the most appreciated crop of south Italy and widely used in industrial preparation. In this study the clementine pulp was investigated for the presence of putative allergens that were detected previously in *Citrus clementina* fruit peel. Extracted proteins from juice were subjected to SDS-PAGE and LC-MS/MS analyses taking account in gel slices at molecular mass referred to allergen detected in our previous study in clementine and limon and in independent studies in orange. Differential expression in gel slices were detected about the three citrus allergens Cit c1, Cit c2, Cit c3 between juice and peel by means MS method. The methodology applied is more sensitive among a broad range of masses and it was possible to detect only the Cit c1 allergen hundreds time less concentrated than in peel. This suggest to remove the peel prior to crush the fruits to get an hypoallergenic juice

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Keywords: oil palm, proteomics

THE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS/MS)-BASED PROTEOME PROFILING OF OIL PALM (ELAEIS GUINEENSIS JACQ.) FRUIT

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Palm oil is an edible vegetable oil derived from the lipid-rich fleshy mesocarp tissue of the notable economic and nutritional relevance oil palm (*Elaeis guineensis* Jacq.) fruit. To facilitate the understanding of the oil palm development, proteomics offers the possibility to dig deeper into major changes during ripening and to classify the patterns of protein accumulation during these complex physiological processes. In this work, we have developed a shotgun approach using LC-MS/MS as a complement to the gel-based method. Total protein was extracted from ripening stage of oil palm fruit (20 week after anthesis) and was then subjected to one-dimensional (1D) and two-dimensional-liquid chromatography-mass spectrometry (2D-LC-MS/MS) technique. The identification of proteins using the 2D-LC-MS/MS increased five-fold compared to the 1D-LC-MS/MS strategy using both internal (MPOB Transcripts) and public databases (NCBInr, SwissProt). By using this comprehensive and species-specific internal database, identification of the proteins improved by 39%. This study provides insights into oil palm fruit metabolism and oil accumulation during the oil palm fruit development.

P 43

Keywords: alfalfa, stem, cellulose, lignin, 2D-DiGE

MULTIPLE APPROACHES TO PROFILE THE GROWING STEM OF ALFALFA

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The regain of interest for plant by-products in industrial fields urges the scientific community to study molecular mechanisms of production of lignocellulosic biomass. Here we depict the differences in the molecular machinery active in young stem tissues of alfalfa *vs* adult woody tissues.

A proteome analysis reveals that the stems' upper regions display higher abundance of proteins involved in chloroplast establishment and activity. Enzymes of the production of early precursors of cellulose biosynthesis or in the carbon flux to the Shikimate pathway are also more abundant. This accumulation is accompanied by a higher expression of cellulose synthase genes (qPCR). These observations coincide with an increased proportion of cellulose and lignin from the apical to the medial region of the stem.

In the lower stem redox- and stress-related proteins accumulate together with those of mitochondrial activity. At the structural level, this shift results in the stabilization of the stem's molecular composition (NIR spectroscopy).

Altogether these observations reflect metabolic change from the production of fibres in the apex of the stem to the acquisition of defence functions in its basal part.

P 44

Keywords: membrane proteomics, Norway spruce, lignification, xylem

MEMBRANE PROTEOMICS OF NORWAY SPRUCE DEVELOPING XYLEM

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The vessels and tracheids of water-transporting xylem tissue of vascular plants have strong secondary cell walls. Their strength is greatly affected by the macromolecule lignin that is a phenolic polymer of monolignols. The biosynthesis of lignin includes monolignol biosynthesis from phenylalanine via the phenylpropanoid pathway, transport of these precursors to the cell wall, monolignol oxidation, and finally polymerization into lignin. The final steps of lignification, the transport, oxidization, and polymerization of monolignols, occur at the plasma membrane or in the cell wall. Therefore, understanding the roles of plasma membrane-localized proteins involved in this process is of vital importance [1, 2, 3]. We are currently studying the total membrane proteome of developing xylem, phloem, and a lignin-forming tissue culture of Norway spruce (*Picea abies*) in order to identify candidates for lignification-related membrane proteins such as monolignol transporters.

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P 45

Keywords: mitogen activated protein kinase, proteomics, plant, YODA, SIMKK

FUNCTIONAL PROTEOMICS ON ARABIDOPSIS MAP3K MUTANTS AND MAP2K OVEREXPRESSOR LINE

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Along with transcriptomic studies, proteomic and phosphoproteomic approaches provide significant functional information about mitogen-activated protein kinases (MAPKs) in plants. We provide a shot-gun proteomic analyses of Arabidopsis plants overexpressing *Medicago sativa* stress-induced MAPKK (SIMKK) and of loss- and gain-of-function Arabidopsis *yda1* and *ΔNyda1* mutants in *MAPKKK4* gene called *YODA*.

Comparative proteomic analyses of SIMKK-YFP overexpressor line versus wild type showed decreased abundance of proteins involved in salt-induced oxidative stress, which may determine the lower tolerance of SIMKK-YFP line to salt stress. Similarly, the auxin-related phenotypes of *yoda* and *ΔNyda1* mutants were accompanied by changes in abundances of auxin biosynthesis proteins. When supported by appropriate biochemical and cell biological validation we consider this proteomic approach feasible for functional characterization of respective MAPKs including molecular interpretation of phenotypes and stress responses of such transgenic and mutant plants.

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P 46

Keywords: Physcomitrella patens, sORF, endogenous peptides, transcriptome profiling

IDENTIFICATION OF SMALL OPEN READING FRAMES (SORFS) IN GENOME OF THE MOSS PHYSCOMITRELLA PATENS

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Small open reading frames (sORFs, up to 100 codons) have the potential to encode biologically active peptides that have regulatory roles in eukaryotic cells [1]. The aim of our work was to identify of the sORFs in genome of model plant – *P.patens* moss. To identify sORF that coding peptides, we carried out a high-throughput RNA-seq analysis and identified native peptide pools by tandem mass spectrometry analysis in gametophores, protonemata and protoplast cells of *P. patens.*

Using sORFfinder [2] , we distinguished 241,228 sORFs within intergenic region with high coding potential. RNA-Seq confirmed transcription of 8,450 sORFs from intergenic region and 16,928 previously known genes of *P. patens*. Tandem mass-spectrometry analysis resulted in identification of 44 peptides derived from 27 sORFs within intergenic region, 52 peptides derived from 42 sORFs that were previously thought to be untranslated region of mRNAs and more than 100 peptides from about 100 alternative sORFs within previously known ORFs.

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P 47

Keywords: Somatic embryogenesis, translucent and opaque somatic embryos types

COMPARATIVE PROTEOMIC ANALYSIS OF THEOBROMA CACAO L. TRANSLUCENT AND OPAQUE SOMATIC EMBRYOS TYPES.

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Somatic embryogenesishas been routinely used as high performance micropropagation system as well as a model system to investigate structural, physiological and molecular events that occur during embryonic development. Proteomic approaches may provide a better understanding of this morphogenetic route at a molecular level. The present study aimed at to establish a protein profile as well as identify differentially expressed proteins in translucent and opaque cotyledonary-staged somatic embryos of *T. cacao* by means of 2D electrophoresis. Differentially expressed proteins identified in two types of embryos showed the presence of 3 exclusive spots associated to opaque type, 4 exclusive spots to translucent type and 17 spots were differentially expressed in two types embryos. Analyses of these protein spots were identified by the mass spectrometry. Many of the identified proteins are involved in genetic information processing and stress response. Many stress related proteins were similar with zygotic embryos proteins. These proteins have a prominent function in storage compound metabolism in cacao seeds. These findings shed light to improvement of somatic embryogenesis protocol in cacao.

P 48

Keywords: action potentials, hydroxyl radical, ion channels

THE IMPACT OF HYDROXYL RADICAL TO THE BIOELECTRIC SIGNALS IN THE LIVERWORT CONOCEPHALUM CONICUM

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Plants are able to generate electrical responses to various mechanical, electrical, chemical, or light stimuli causing the disturbance of the balance between cation and anion fluxes through the plasma membrane. The aim of the research was to determine the impact of hydroxyl radical (1-10 mM) on activation of ion channels in the plasma membrane of the liverwort *Conocephalum conicum*. The study was conducted using intracellular microelectrodes. Application of hydroxyl radical in concentrations higher than 1 mM resulted in vanishing series of action potentials (APs), leading to permanent depolarization of the membrane potential. Potassium channel inhibitor (TEA, 10 mM) and anion channel inhibitor (A9C, 2 mM) caused the suppression of AP series evoked by hydroxyl radical.

P 49

Keywords: drought stress, shotgun proteomics, metabolomics, legume biology

LEGUME-RHIZOBIA INTERACTION PLAYS A CRUCIAL ROLE IN PLANT ACCLIMATION TO DROUGHT

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Drought stress hampers plant energy and biomass production; however it is still unknown how internal C/N balance impacts on plant response to water limitation. Here, we tested the effect of differential optimal nitrogen nutrition on drought stress and recovery responses of Medicago truncatula.
One group of plants was nodulated with Sinorhizobium medicae; the second group of plants was grown in a rhizobia-free medium and watered with mineral nitrogen fertilizer.
We found different patterns of protein abundance fluctuations between N-fed and nodulated plants linked to chloroplast metabolism and redox homeostasis. Few of the quantified proteins showed similar changes in both experimental systems. N-fed plants showed accelerated leaf senescence compared to nodulated plants. The remobilized reserves were preferentially invested in enhanced root biomass production for better water uptake. Nodulated plants invested to a greater extent in reprogramming of the leaf metabolism yielding osmoprotectants.

We conclude that symbiotic interaction with rhizobacteria facilitates shoot biomass maintenance and protective compound synthesis in vegetative Medicago truncatula under water limited conditions.

P 50

Keywords: non-glandular, olive, physiology, trichome

A COMPARATIVE PROTEOMIC ANALYSIS TO VERIFY THE PHYSIOLOGICAL AND PRODUCTION STATUS OF OLIVE (OLEA EUROPAEA L.) ABAXIAL TRICHOME

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Olive is one of the most important fruit crop trees in the history of Mediterranean because of the high quality oil. Olive oil has a well-balanced fatty acid composition along with biophenols, which makes it exceptional in human diet and provide an exceptional value to the olive oil. Accumulated data have correlated the olive oil consumption with the reduction of cardiovascular diseases and the breast cancer. Leaf non-glandular peltate trichomes are specialized cell types known to have a number of secondary metabolites. In order to establish a holistic approach to verify the proteome of this highly differentiated cell type, we performed a comparative proteomic analysis among isolated trichomes and leaves without trichomes. Proteins were identified using the 2-D MALDI-TOF MS method. A number of enzymes involved in biochemical networks producing secondary metabolites are present. Different transcriptional factors were also detected. The results show that this highly differentiated cell type is physiologically active fulfilling the demands of the trichomes in furnishing the leaf with a highly protective mechanism. This work was funded by ARISTEIA/GSRT 1200 and SYNERGASIA to PH.

P 51

Keywords: differential display; ESI-MS/MS; flavonoids; protein identification; proteomics;
Robinia pseudoacacia; sapwood; transition zone.

IDENTIFICATION OF BIOCHEMICAL DIFFERENCES BETWEEN THE SAPWOOD AND TRANSITION ZONE IN ROBINIA PSEUDOACACIA L. BY DIFFERENTIAL DISPLAY OF PROTEINS

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The predominant proteins and enzymes in the sapwood and transition zones of *Robinia pseudoacacia* L. were identifyed and expressed by two methods: 2D SDS-PAGE and electrospray ionisation tandem mass spectrometry (ESI-MS/MS). Large differences in the amount of proteins extracted were observed between the bark, sapwood and transition zones. Soluble proteins strongly expressed in sapwood are responsible for carbohydrate metabolism and flavonoid turnover. By contrast, proteins strongly expressed in the transition zone are mainly responsible for flavonoid biosynthesis. Lectins were found in protein fractions of both wood zones, and heat-stress proteins were detected only in the transition zone. The results are a further proof that flavonoids are synthesised directly at the transition zone between sapwood and heartwood, and that materials deposited in the sapwood are the source for synthesis of metabolites in heartwood, such as flavonoids and tannins.

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P 52

Keywords: Quercus ilex, drought, root proteome

2-DE PROTEOMIC RESPONSE OF QUERCUS ILEX ROOTS TO DROUGHT STRESS AT EARLY DEVELOPMENTAL STAGE

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Holm oak is dominant tree in western Mediterranean region. Despite being well adapted to dry hot climate, drought is the main cause of mortality post-transplanting in reforestation programs. An active response to drought is critical for tree establishment and survival [1]. Most of the drought responses studies in plants have been performed on leaves, thought roots are directly exposed to the stress.

Water stress was applied on 20 days-old seedlings by water shortage for 10 and 20 days, with recovery. Root proteins were extracted using TCA/acetone/phenol [2] and subjected to 2-DE (pI 5-8 and 12% SDS PAGE). After multivariate statistical analysis [3], proteins of interest were identified using MALDI TOF/TOF. Reliable score was achieved in 82 out of 90 spots. Several enzymes of the carbohydrate metabolism were down accumulated while some related to ATP synthesis and secondary metabolism - up accumulated. Results indicate complex changes in root proteome in response to drought.

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P 53

Keywords: potato-pvy interaction, shotgun proteomics

EXPLORING POTATO-PVY INTERACTION USING LABEL-FREE SHOTGUN PROTEOMICS

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We are studding potato-PVY (potato virus Y) interaction using systems biology approaches and one of them is label-free shotgun proteomics.

Proteins were extracted with TriZol and digested with Lys-C and Trypsin. Peptides were subjected to LC MS/MS analysis using OrbiTrap mass spectrometer (University of Vienna). Relative quantification of peptides was determined by spectral counting. Proteins were identified by searching the database combining all known potato genes (prepared on National Institute of Biology (Ljubljana) and Jožef Stefan Institute (Ljubljana)).

We investigated differences in potato proteome 4 days after infection with virus PVY on cultivar Desiree using non-transformed Desiree and two different transformants (defective in salicylic acid and jasmonic acid signaling pathway). Approx. 300 proteins were identified. Differentially expressed proteins were mostly included in photosynthesis.

Some of proteins were found interesting on transcriptional level but not identified with shot gun proteomics. To observe some of those proteins we have in plan to use another approach like MRM (multiple reaction monitoring) technique and detection with antibodies.

P 54

Keywords: cyclophilin, phloem, Brassica napus

CYCLOPHILINS IN THE PHLOEM OF BRASSICA NAPUS

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Since the first mammalian cyclophilin (CyP) has been identified in 1984 [1] many other CyPs could be identified in various organisms like yeast, invertebrates and plants [2, 3]. Besides its manifold distribution, also functions of CyPs are widely varied. They include the involvement in protein trafficking and maturation, RNA processing and most likely the modulation of viral replication [4].

CyPs occurring in the phloem of higher plants [5] might play an important role in stress response and virus defense as the phloem serves as a long-distance transport system for assimilates and signals. Thus the identification and characterization of all CyPs in the phloem are of particular interest. Here we show identification strategies and first results.

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P 55

Keywords: glycoproteins/drought stress/common bean

ANALYSIS OF GLYCOPROTEINS IN LEAVES OF COMMON BEAN (PHASEOLUS VULGARIS L.) UNDER DROUGHT

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Glycosylation is one of the most important and common forms of protein post-translational modification that is involved in many physiological functions and biological pathways. To identify glycoproteins changes of common bean under drought, the extracted proteins from leaves were subjected to lectin affinity chromatography and glycoproteins were separated by SDS-PAGE. The gel lanes of control and stressed plants were divided into ten slices, proteins were digested with trypsin and analyzed by LC-MS/MS. Mass spectrometry data were quantified using MaxQuant software. Thirty-five proteins with a predicted N-terminal ER targeting signal peptide and N-glycosylation sites changed in abundance between control and stressed samples. Proteins were classified into several functional groups, mainly into cell wall metabolism, stress and defence related proteins, proteolysis and energy metabolism. Structures of high mannose, complex and hybrid types of N-glycans were found by manual inspection from spectra. The results suggest that drought stress might affect biochemical metabolism in cell wall and provide new insights into understanding of the molecular basis of drought stress in common bean.

P 56

Keywords: Apoplast, Arabidopsis, Pseudomonas syringae, Protease

QUANTITAIVE APOPLASTIC PROTEOMICS REVEALS ARABIDOPSIS SECRETED PROTEASES ATTACKING THE BACTERIAL PATHOGEN PSEUDOMONAS SYRINGAE

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Pseudomonas syringae pv. *tomato* (*Pto*) grows in the intercellular space in plants called apoplast where they acquire necessary nutrients for growth. Thus, the apoplastic space is an actual battle field between plants and bacteria, and it has been shown that plants secret proteins into apoplast during infection to suppress bacterial growth. However, crucial information about how/what plant defense outputs suppress bacterial growth is still missing. Here, we investigated protein profiles in Arabidopsis apoplast upon *Pto* infection by quantitative proteomics approach. Secreted proteins related with cell wall modification, ROSs detoxifying, and proteolysis were highly accumulated. We investigated molecular function of secreted proteolysis proteins. Overexpression of proteases in Arabidopsis significantly reduced *Pto* growth. Moreover, those proteases have ability to directly suppress *Pto* growth *in vitro*. Proteomics based analysis showed that several bacterial secreted or membrane associated proteins were degraded by protease. Our finding for the first time gives the evidence that plant secreted proteases are direct weapons to suppress bacterial growth through attacking bacterial proteins.

P 57

Keywords: waterlogging; peroxidase; native PAGE; shoot; *Zea mays* L.

ALTERATIONS OF PEROXIDASE PROFILES IN MAIZE SHOOTS BY WATERLOGGING

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Due to an increase in flooding events, waterlogged soils and submergence becomes a major problem in crop production. In the present study peroxidase profiles of maize (*Zea mays* L.) shoots have been investigated in dependence on waterlogging. Protein abundance of several isoperoxidases were altered after flooding and increased guaiacol peroxidase activity was detected by proteomic approaches (native IEF-PAGE and high resolution Clear Native Electrophorese) . Two ascorbate peroxidases and 11 class III peroxidases have been identified in the samples by mass spectrometry (Meisrimler et al., 2014). Three of these enzymes have been described earlier to be regulated by oxidative stress. Using qRT-PCR, up-regulation of membrane bound Prx01 and Respiratory burst oxidase homologes (Rboh) have been demonstrated in the samples after flooding stress. Although some peroxidases may be involved in adaptive mechanisms, our results suggest a major function of class III peroxidases in ROS scavenging during flooding stress.

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Keywords: cadmium, peroxidase; 2D-PAGE; native PAGE, root; *Zea mays* L.

SUB-CELLULAR LOCATIONS OF CLASS III PEROXIDASES AND THEIR ALTERATIONS BY CADMIUM EXPOSURE

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Peroxidase activity is used as a general stress marker. At least 143 class III peroxidases have been indicated by the maize genome [1]. About 50 % of these isoenzymes have a membrane-bound prediction by *in silico* analysis. Membrane-bound peroxidases have been detected in chloroplasts, highly enriched plasma membranes and tonoplast. Cadmium is well known to cause an oxidative stress and up-regulation of total peroxidase activity. Isoperoxidases involved in this reaction will need further investigation.

In the present study regulation of isoperoxidases from maize (Zea mays L.) roots were investigated after 18 days of cadmium exposure. Soluble and microsomal proteins were analysed by 2D-PAGE (IEF/SDS-PAGE). Several proteins appear to change in abundance after Cd exposure. In addition alterations in peroxidase profiles of plasma membranes and tonoplast were investigated after fractionation of the microsomal fraction. Highly enriched membrane fractions were prepared by a combination of aqueous polymer two-phase partitioning (APTPP) and a sucrose step gradient [2]. Alterations in peroxidase profiles of plasma membranes and tonoplast were studied by native PAGE (hrCNE) or modified SDS-PAGE and specific in-gel activity staining. In addition, regulation of Prx1, Prx66 and Prx70 transcripts was investigated by quantitative PCR. Localisation of gene products was characterized by GFP-fusion products.

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Methods in Molecular Plant Biology: Plant Proteomics, 687-706

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Keywords: cadmium, peroxidase; 2D-PAGE; root; *Zea mays* L.

THE PEA SECRETOME: ALTERATIONS OF CLASS III PEROXIDASES AND MALATE DEHYDROGENASE ISOFORMES BY WOUNDING

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The pea secretome can be divided in at least three sub-proteomes by cell fractionation: i) cell wall, ii) apoplastic fluid and iii) plasma membrane. The cell wall fraction can be further divided in ionically and covalently bound proteins. Up-regulation of antioxidant systems like peroxidases after wounding is well known. The molecular compounds responsible for this observation will need further elucidation.
In the present study samples of control and wounded pea roots were studied by proteomic approaches. Peroxidase activities of crude extracts from control and wounded samples showed similar peroxidase activity. Cell fractionation, however, revealed significant alterations in peroxidase profiles of cell wall, apopplastic fluid and plasma membranes between control and wounded samples. 2D-PAGE of crude extracts and apoplastic fluids of control and wounded pea roots showed further differences. In total 592 protein spots were detected in the control, whereas 614 protein spots were detected in wounded samples suggesting an induction of several proteins under stress conditions. Among the proteins found in 2D-PAGE, several proteins were unique for the control and others for stressed samples. In contrast to the crude extracts, 2D-PAGE analysis of apoplastic fluid revealed 158 protein spots in the control and 171 protein spots in wounded samples. In accordance to the crude extract several proteins were induced after wounding in apoplastic fluids. In comparison 22 protein spots were induced in crude extracts and 13 protein spots were induced in apoplastic fluids by wound stress. Thus at least 9 of the induced proteins found in crude extracts appeared to have a location different from the apoplast.

P 60

Keywords: cadmium, peroxidase; 2D-PAGE; root; *Zea mays* L.

A SYSTEMS BIOLOGY APPROACH TO UNRAVEL THE EFFECTS OF CADMIUM EXPOSURE ON ARABIDOPSIS THALIANA

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In a growing population, plants will become increasingly vital as a sustainable resource for food, feed, fibre, and fuel. Environmental stresses can limit plant growth and quality, leading to obvious problems in a scenario where plant products are a primary resource.
Cadmium (Cd) pollution, mostly from anthropogenic origin, can have detrimental effects on plants. Exposure to Cd induces oxidative stress, leading to changes in metabolic processes that can lead to reduced yield or death.
The present study made use of a multi-omics approach, combining proteomics (DiGE) in combination with transcriptomics (qPCR) to shed some light on the molecular processes that respond to Cd treatment. Arabidopsis thaliana (Columbia) plants were grown hydroponically and exposed to 5 µM CdSO4. Rosettes from treated plants and non-treated controls were harvested after 0, 24 and 72 hours of exposure.
The multi-omics data was visualized on Arabidopsis thaliana pathways using Pathvisio and network analysis was carried out using Cytoscape. Significantly differentially abundant proteins were involved primarily in carbon metabolism and detoxification, and are often associated with NADPH or NADH, the most important cofactors for redox processes. Gene expression data was mostly confirmative of proteomics data and constituted a strong element of validation.

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Keywords: iTRAQ-OFFGEL; *Arabidopsis thaliana* L., iron, zinc

ITRAQ-OFFGEL ANALYSIS OF ARABIDOPSIS SHOOT MICROSOMAL PROTEINS REVEALS ROLE FOR SUGAR TRANSPORTERS IN A CROSS-TALK BETWEEN EXCESS ZINC AND IRON DEFICIENCY

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Iron (Fe) deficiency has significant effects on plant growth and development such as chlorosis and root growth inhibition. It has been observed that excess zinc (Zn) shows resembling symptoms with Fe deficient plants. To better understand the cross talk between Fe and Zn, we examined protein expression changes in microsomal fraction from Arabidopsis shoots using iTRAQ-OFFGEL method. Fe-deficient conditions were created by basal medium without Fe and addition of excess Zn into basal medium. Basal medium with excess of both Zn and Fe was also prepared, and which lead to recovery of chlorosis. As a result, 909 proteins were commonly identified in three biological replicates. Most of them were either transporters or proteins involved in photosynthesis, and ribosomal proteins. Interestingly, protein expression changes were of similar pattern between the two Fe-deficient conditions. Furthermore, changed protein expressions due to excess Zn were recovered on application of excess Fe that was comparable to those on basal condition. Among Fe-deficiency responsive proteins we identified two highly increased sugar transporters namely, STP 13 and STP 4. Indeed, the concentrations of sugars (i.e., sucrose, fructose and glucose) were significantly increased by Fe deficiencies. Similarly, decreased abundance of photosynthetic proteins was seen, which might be related to high sugar concentrations as sugar-driven signals regulate their expressions and lead to lower photosynthetic activity. We hypothesize that the negative effect on expression levels of proteins involved in photosynthesis may be due higher accumulations of sugars under Fe deficiency conditions.

	Sunday, August 31	Monday, September 1	Tuesday, September 2	Wednesday, September 3	Thursday, September 4
08:00		Registration	INPPO Office open	INPPO Office open	INPPO Office open
9:00		**Bioinformatics & Mass Spec** Chair: Wolfgang Schröder 9:00 Kathryn Lilley 9:25 Sebastien Carpentier 9:45 Christof Ramptisch	**Quantitative Proteomics** Chair: Stefanie Wienkoop 9:00 Sascha Baginsky 9:25 Natalia V. Bykova 9:45 Madalina Oppermann	**Subcellular Proteomics** Chair: Sabine Lüthje 9:00 Joshua Haezlewood 9:25 Norbert Rolland 9:45 Stefan Helm	**Plant Growth Development** Chair: Michel Zivy 9:00 Dominique Job 9:25 Katia Castanho Scortecci 9:45 Norazeen Abd Rahman
10:00		10:05 Isa Catarina Ribeiro 10:25 Stefanie Wienkoop	10:05 Antoine Champagne 10:25 Iniga S. George	10:05 Harriet T. Parsons 10:25 Silvia Mazzuca	10:05 Ahmed Debez 10:25 Christina Mark
11:00		Coffee break (11:00-11:30)	Coffee break (11:00-11:30)	Coffee break (11:00-11:30)	Coffee break (11:00-11:30)
12:00		**Systems Biology / Mass Spec** Chair: Wolfram Weckwerth 11:30 Waltraud Schulze 11:55 Alexander Graf 12:15 Lilian Carmo 12:35 Mathilde Decourcelle	**PTM (Part 1)** Chair: Thomas Kieselbach 11:30 Renu Deswal 11:55 Christine Finnie 12:15 Iris Finkemeier 12:35 Julia Svozil	Poster Viewing (11:30-12:30) Odd numbers	**Interaction Environment I** Chair: Carla Pinheiro 11:30 Wolfgang Schmidt 11:55 Bhakti Prinsi 12:15 B-N. Melisande 12:35 Mélanie Mauriat
13:00		Lunch break (13:00-14:30) COST-Meeting	Lunch break (13:00-14:30) Committee Meetings	Lunch break (13:00-14:30)	Lunch break (13:00-14:30)
14:00		Coffee	Coffee	Coffee	Coffee
15:00	Registration open	**Integrative Proteomics** Chair: Dominique Job 14:30 Kentaro Tamura 14:55 Mark Galland 15:15 Luca Espen 15:35 Pingfang Yang	**PTM II (Part 2)** Chair: Renu Deswal 14:30 Veronique Santoni 14:55 Niklas Hausmann 15:15 Elisabeth Stes 15:35 Claudia N. Meisrimler	**Gel-based Proteomics (Part 1)** Chair: Jenny Renaut 14:30 Jesus Novo-Jorrin 14:55 San Tae Kim 15:15 Pavel Vitamvas 15:35 Klara Kosova	**Interaction Environment II** Chair: Laurence Bindschedler 14:30 Laurence Bindschedler 14:55 Roque Bru-Martinez 15:15 Angela Metha 15:35 Agniszka Szuba
16:00		Coffee break (16:00-16:30)	Coffee break (16:00-16:30)	Coffee break (16:00-16:30)	Coffee break (16:00-16:30)
17:00	Opening (17:15-18:00) Sabine Lüthje Ganesh K. Agrawal Dominique Job	Doctoral´s Office (16:30-17:30) *Mass Spectrometry* Thomas Kieselbach Stefanie Wienkoop Poster Viewing (17:30-19:30) Even numbers	Round Table (16:30-18:30) *Plant proteomics and international organizations/ initiatives* Organized by Jesus Jorrin and Joshua Haezlewood	**Gel-based Proteomics (Part 2)** Chair: San Tae Kim 16:30 Martin Hajduch 16:55 Jayaseelan Murugaiyan 17:15 Arun K. Angural Vadivel 17:35 J. Porankiewicz-Asplund Doctoral´s Office (18:00-19:00) *Gel-based Methods* Christine Finnie Joanna Porankiewicz-Asplund Jenny Renaut Claudia Meisrimler	Round Table (16:30-18:00) *INPPO Perspectives* Ganesh K. Agrawal, Dominique Job Jenny Renaut Closing, Poster dismounting
18:00	Public Evening Lecture Pier G. Righetti *(18:00-19:00)*	Wine & Cheese	Member gathering Ganesh K. Agrawal, Dominique Job		
19:00	Welcome Reception (19:00-21:00)				Farewell (19:00-22:00)
20:00				Congress Dinner (20:00-24:00)	
21:00					
22:00					Departure
23:00					
24:00					