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#1 Poster presentation preferred

Towards a better understanding of cell wall dynamics in water-stressed flax with the help of mixOmics

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As part of the "NoStressWall" project (NOvel information on the effects of drought STRESS on the plant cell WALL) financed by the French government agency ANR, we generated a large quantity of data from high-throughput phenotyping techniques (metabolomics, transcriptomics, proteomics…) in flax, a plant of economic interest (e.g. textiles, bio-composite materials, insulation) with sequenced genome information [1]. In order to achieve a detailed understanding of how flax plants are able to cope with water deficit, we decided to consider the different datasets altogether and not separately as has been most commonly done so far [2; 3]. Hopefully, this type of 'integrative biology' approach, will allow us to get a better understanding of the interplay of genes, proteins and other metabolites. As a preliminary piece of work, we present here how our metabolomic, transcriptomic and proteomic data can be related to eight morpho-physiological traits (shoot height; root length; shoot water content; root water content; leaf area; PSII efficiency; bast fiber lumen area; bast fiber cell wall thickness) by using the R package 'mixOmics' (http://mixomics.org). Ultimately, the identification of metabolite(s)/gene(s)/protein(s) whose expression is robustly affected by water deprivation in either short-, middle or long-term scenarios will allow us to better understanding flax biology under water stress conditions, and more especially how the flax cell wall network is modified.

[1] Wang *et al.* (2012) The Genome of Flax (*Linum Usitatissimum*) Assembled *De Novo* from Short Shotgun Sequence Reads. *Plant Journal.* 72(3):461-73

[2] Pont-Lezica *et al.* (2010) Plant cell wall functional genomics : Novelties from proteomics. *Advances in Genetics Research*. 1: 1-24

[3] Ding *et al.* (2015) Network Analysis of Postharvest Senescence Process in Citrus Fruits Revealed by Transcriptomic and Metabolomic Profiling. *Plant Physiology*. 168: 114.255711

#2

Deciphering the microbial mechanisms of biochar involved in suppression of fusarium crown and root rot in tomato

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Biochar, in addition to carbon sequestration, soil amelioration and improvement of plant performance, can significantly reduce plant diseases. Nevertheless, the mechanisms associated with soilborne-disease suppression are not fully understood. This study tested the effects of two biochars at concentration of 0-3% (w:w) on fusarium crown and root rot (FCRR) of tomato caused by *Fusarium oxysporum* f. sp. radices-lycopersici (FORL), with an emphasis on mechanisms of disease suppression. Biochar at higher concentrations suppressed FCRR of tomato by up to 79%. Furthermore, biochar significantly reduced the Fusarium root colonization and survival in soil. Yet, direct toxicity of biochar to FORL was not observed in *in vitro* assay. Biochar amendment significantly increased the culturable counts of general bacteria, fluorescent *Pseudomonas* spp., *Trichoderma* spp. (well-known biocontrol and plant growth promoting agents) and other microorganisms. Indeed, biochar-stimulated fluorescent Pseudomonas have antagonistic activity towards FORL. Illumina sequencing analyses of 16S rRNA gene showed substantial differences in bulk soil, rhizosphere and rhizoplane bacterial taxonomical composition between biochar-amended and control soils. Nevertheless, biochar amendment caused a significant increase in microbial diversity (Shannon's diversity, phylotype richness), microbial activities (respiration rates, dehydrogenase and other enzymes activities) and an overall shift in carbon-source utilization by microbial communities (Biolog Microplates), concurrent with increased plant growth and disease suppression. High microbial diversity and activity in the rhizosphere has been previously associated with soilborne diseases suppression and growth promotion, and this may collectively explain the significant reduction of disease and increase in plant growth observed in the presence of biochar.

#3

Changes in the proteome of pea (*Pisum sativum* L.) seeds germinating under optimal and osmotic stress conditions and subjected to post-stress recovery

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Plants growing under natural conditions are exposed to a variety of stresses, which can lead to undesirable changes in the physiological processes and yielding. These changes can be regulated on different levels, resulting in a synthesis of specific proteins which participate in the plant's response to stress. The purpose of this study has been to determine changes in the accumulation of proteins in germinating pea (*Pisum sativum* L.) seeds under optimal and osmotic (short- and long-term) stress conditions as well as recovery following a short-term stress. For identification of the proteins, two dimension electrophoresis (2DE) and mass spectrometry (MALDI-TOF) were employed. Germination in optimal conditions, increased the accumulation of several proteins involved in glycolysis, Krebs cycle, synthesis of fatty acids, cell growth, cellular transport and detoxification. Osmotic stress, in turn, depressed the accumulation of proteins involved in glycolysis, synthesis of fatty acids, detoxication, methionine conversions, cellular transport, translation, growth control and of cytoskeletal proteins, but raised the accumulation of enzymes of the tricarboxylic acids cycle, proteins participating in signal transduction and protection (chaperones). One protein, 6a-hydroxymaackiain, which is involved in the synthesis of pisatin, was present only under osmotic stress conditions and recovery. Pisatin is synthesized mainly in response to microbiological infections and under stress conditions, what indicate that it play a key role on the acquisition of stress tolerance by plants.

#4

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

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

1) D. Lyon, M.A. Castillejo, C. Staudinger, W. Weckwerth, S. Wienkoop and V. Egelhofer (2014). Automated protein turnover calculations from 15N partial metabolic labeling LC/MS shotgun proteomics data. Plos One15; 9(4)

#5

Two decade evaluating storage proteins of wheat. What, Why, When, Where, How, Who…?

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Wheat has been one of the most important food grain sources for humans throughout history. Over the last two decade, we have conducted several studies on the genetic diversity of endosperm storage proteins in old wheat populations, tetraploid and hexaploid wheat varieties. Proteomic tools have been used to describe the genetic diversity of wheat germplasms from different origins at the level of polymorphisms in alleles encoding glutenin and gliadin, the two main proteins of gluten. Considering that proteomics is a powerful tool to elucidate gluten protein expression, diversity and interactions, different work was carried out since 1994 through these omics technologies. Analysis of Portuguese wheat (*Triticum aestivum* L.) landrace Barbela by 2-DE revealed the existence of high a new x-type high molecular weight-glutenin subunit (HMW-GS) encoded at the Glu-A1 locus, which was named 1Ax1.1 (Mr = 93.648 Da; pI = 5.7; NCBI 1453293 1Ax1.1 JN172932). The different alleles encoded at the six glutenin loci and three w-gliadin loci have been also identified from a set which includes more than 300 hexaploid and tetraploid varieties. Wheat breeding process have gradually increased the frequency of specific alleles coding for storage proteins that have a positive effect on gluten quality, resulting in a reduction in wheat genetic variability. HMW-GS are the major genotypic determinants of dough strength and determine the suitability of the wheat for bread-making by conferring dough viscoelasticity. The knowledge will contribute to the strategic conservation of wheat genetic resources and improve wheat breeding to meet the challenges of the 21st century.

#6

Semantic web for data integration and data exchange among plant phenomics relational databases

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Automation processes in plant research provide a large amount of plant phenotyping data. Relational databases are mostly used to store these data. The goal is to have data exchange and data integration among independently developed databases. Generally, these are heterogeneous and distributed. Heterogeneous databases might use different terminologies, different data types or different measurement units. We propose to use semantic web to achieve interoperability among heterogeneous databases. In semantic web, ontologies are used for describing the domain knowledge. We propose a semi-automated approach using ontologies for supporting data integration and data exchange. According to this approach, we determine the semantic equivalences of database terms in the publicly available ontologies. The proposed approach for solving data exchange and data integration problems is composed of two processes namely, transformation and mapping. Let us assume a database DB and a global ontology GO are given. The input to the transformation process is a relational database DB. This process creates an ontology LO from DB. The ontology LO describes the semantics of DB. The inputs to the mapping process are the ontologies LO and GO. The mapping process returns a set of semantic correspondences which relate constructs of LO to those of GO. Since the approach is semi-automated, the involvement of a human expert is required to curate the mapping results from the semantic perspective and eliminate incorrect results.

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#7

Shoot and nodule development in *Pisum sativum* after infection with *Didymella pinodes*

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Due to its association with root-symbionts, field pea is especially meaningful in crop rotation and winter greening. Above ground infestation by the necrotrophic fungi such as *Didymella pinodes* reduces yields globally. On the plants level, the impact of infection reaches down to the interaction with the symbionts. The development of nodules is affected because of the different needs of above ground parts. However, cultivars exhibit a variety of nodule development, which is, among other things, based on the shoots morphology as well as on the onset of blooming. Here, we examined the nodule development of two field pea cultivars with different resistance levels to *D. pinodes* at 24 h, 48 h and 2 weeks post infection by counting and weighing nodules with subsequent extraction of primary Metabolites (GC-MS) and label free quantification of proteins (Orbitrap Elite). The different nodule development is consistent with the distinguishable shoot morphology and the onset of blooming of each cultivar. Additionally, the formation of shoot nodes post infection appears to depend on the microbial association of the roots. Moreover, we found that the weight of nodules after infection is maintained when co-inoculation with arbuscular mycorrhizal fungi is given. Thus, we presume that nodule development post infection is interrelated with mycorrhizal linkage of the roots. Whether cultivar related nodule development after pathogenic attack shows an impact on yield remains to be elucidated.

#8

Multiple MS methodologies for the identification of proteolysis resistant proteins.

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

#9

Efficiency of the antioxidant defense system in wheat breeding program: An assessment of candidate salt tolerant and salt sensitive doubled haploid mutant lines

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Salinity is one of the most brutal environmental stresses that hampers crop productivity worldwide due to interruption of cell membranes, nutrient imbalance, impairing the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreasing photosynthetic and stomatal activities. Salinity-induced ROS formation can lead to oxidative damages in various cellular components resulting an interruption of vital cellular functions in plants. Therefore, plants possess complex anti-oxidative defense system comprising of non-enzymatic and enzymatic components to scavenge ROS. In recent years, one of the prominent hypotheses is that maintenance of a high antioxidant capacity to scavenge the toxic ROS has been linked to increased tolerance of plants to environmental stresses. Assessment of salt-sensitive and salt-tolerant mutants using antioxidant defense parameters is a useful and low-cost method before identifying genes associated with salt tolerance by high throughput molecular analysis. In this regard, forward genetic screening with sodium azide-mutated doubled haploid wheat (*Triticum aestivum* L. cv. Pehlivan) was used to identify mutants showing tolerance and hypersensitivity to salt stress. A new mutant line exhibiting a severe salt-sensitivity obtained under 100 mM NaCl concentration (as a threshold concentration for commercial cultivar), whereas three mutant lines were detected as healthier than commercial cultivars at the same NaCl concentration. For further comparative investigation of these lines, two-week-old seedlings were exposed to various NaCl concentrations (0, 100, 150, 200, and 250 mM) for 3 days. The activity of SOD, POD, CAT, APX and GR, and the content of H_2O_2, OH, GSH, GSSG, Proline and chlorophylls were measured with UV/Visible spectrophotometer and results were evaluated with different biostatistical methods.

#10

Proteome analysis of crop response to drought stress

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

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#11

Determining phenological patterns associated with the onset of senescence in a wheat MAGIC mapping population

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Appropriate timing of developmental transitions is critical for adapting most temperate crops to their local climatic conditions. Therefore, understanding the genetic basis of different aspects of phenology could be useful in highlighting mechanisms underpinning adaptation, with breeding implications for climate change. For bread wheat, the transition from vegetative to reproductive growth, the start and rate of leaf senescence and the relative timing of different stages of flowering and grain filling could all contribute to plant performance. To evaluate the genetic elements that influence the timing of these stages in European elite varieties, we exploited the genetic variation released in a large, multi-founder wheat population that was derived from eight parental lines that are or recently have been used commercially in the UK and Northern Europe. We undertook a detailed temporal analysis of a core set of recombinant inbred lines and all eight parents that included both traditional manual scoring of growth stage, senescence and grain traits as well as automatically generated high-throughput image data. Quantitative trait loci (QTL) mapping identified markers associated to variation in the timing of key phenological stages associated with senescence such as GS55 as well as derived traits based on the time differences between sequential growth stages. In addition, strong correlations between plant traits and the onset of senescence at the flag leaf were identified.

#12

Role of reactive oxygen species (ROS) in elongation dynamics of the first internode of deep-sown wheat

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Plants develop mechanisms to cope with drought conditions. Deep sowing tolerance is the one of the most efficient way to avoid drought, which can be described as the ability of the elevation of the shoot apical meristem above the soil surface by elongation of first internode. Reactive oxygen species (ROS) play roles on plant growth and development. However, there is no study about role of ROS in elongation dynamics of the first internode of deep-sown wheat. The aim of the study is to characterize the interaction between GA3, ROS and antioxidant machinery in the internode elongation of a deep sowing tolerant wheat variety cultivated in Turkey, *Triticum aestivum* cv. Tir. Wheat seeds were sown depths to 2 cm and 10 cm of soil. For GA3 and unicanazole treatment 2.89 × 10-6 M GA and 2 × 10-7 M unicanazole added to the 1 % agar medium. To determine effect of H_2O_2 on the first internode elongation, seeds were soaked in H_2O_2 solutions (0.05, 1, 10 µM, and 80 mM) for 24 hours. At 10 days, both first internode and coleoptile length of Tir wheats were measured. H_2O_2 content, activities of antioxidant enzymes were determined. Expression levels of GA3 biosynthesis genes (Ta20ox1 and Ta30ox2), semidwarfing genes (RHT-A1, RHT-B1 and RHT-D1) and GAMyB, transcription factor were determined. Epidermal and cortical cell lengths were also measured. To our knowledge, this is the first study that concerns the relationship between GA3, H_2O_2 and antioxidant defence system in the first internode elongation under deep sowing.

#13

Systems biology approach to identify novel biomarkers of wood quality in Mediterranean pine (*Pinus pinaster* Aiton)

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Natural variation of the metabolome and proteome of *Pinus pinaster* was studied to improve our understanding of phenotypic diversity, and wood quality. The metabolome and proteome of needles and the apical and basal section of buds were analyzed in three provenances of *P. pinaster* with contrasting growth capacity selected from mountain in the northwest (CDVO) to the coastal region of southeast Spain (ORIA) also considering a provenance from a sandy Moroccan area (TAMR). The three provenances were grown in a common garden for five years and metabolite and protein extraction were performed from the same sample. For metabolite detection two complementary mass spectrometry techniques: GC-MS and LC-Orbitrap-MS were used, while for protein identification GeLC-Orbitrap/MS combined with the development of custom peptide databases was used. Metabolome, proteome and environmental and growth data were integrated employing modelling and statistical tools to provide a comprehensive picture of phenotypic diversity. A total of 1576 metabolites and 1447 proteins were identified. The metabolites characteristic of each tissue are related to primary metabolism, while provenances were distinguishable when tissues were analysed independently. Integrative studies showed three population clusters, being secondary metabolites, and in particular flavonoid and terpenoid pathways, essential to reach this differential clustering. Additionally, some key enzymes were linked by sPLS networks to wood quality. Altogether these results provide a new perspective of how tree metabolism adapt to different environment, and how these adaptations are also reflected in wood quality, providing these results a new set of biomarkers for breeding programs and forest management practices.

#14

Integrated physiological, proteomic and metabolomic analysis of UV stress responses and adaptation mechanisms in *Pinus radiata*

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Globally expected changes in environmental conditions, especially the increase of UV irradiation, necessitate extending our knowledge of the mechanisms mediating tree species adaptation to this stress. This is crucial for designing new strategies to maintain future forest productivity. Studies focused on environmentally realistic dosages of UV irradiation in forest species are scarce. Pinus sp. are commercially relevant trees and not much is known about their adaptation to UV. UV treatment and recovery of Pinus radiata plants with dosages mimicking future scenarios based on current models of UV radiation were performed in a time-dependent manner. The combined metabolome and proteome analysis was complemented with measurements of physiological parameters and gene expression. Sparse PLS analysis revealed complex molecular interaction networks of molecular and physiological data. Early responses prevented photoxicity by reducing photosystem activity and the electron transfer chain together with the accumulation of photoprotectors and photorespiration. Apart from the reduction in photosynthesis as consequence of the direct UV damage on the photosystems, the whole primary metabolism was rearranged to deal with the oxidative stress while minimizing ROS production. New protein kinases and proteases related to signalling, coordination, and regulation of UV stress responses were revealed. All these processes demonstrate a complex molecular interaction network extending the current knowledge of UV-stress adaptation in pine.

#15

New functional feedback phenotyping platform for controlling soil conditions and evaluating root-plant-atmosphere dynamic response

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Many of the early components of a plant's response to biotic and abiotic stress are related to modifications in plant water relations and, therefore, can be used as characteristic markers at a very early stage of the stress. Here, we reveal a simple, yet effective experimental platform that is based on a gravimetric system combined with a unique irrigation-drainage system and soil and atmospheric probes. This system enables tight control on multiple soil water/salinity scenarios while continuously monitoring in a quantitative manner the soil–plant–atmosphere water relations. The system monitors simultaneously numerous plants for a variety of biotic and abiotic stresses at high resolution. Five Quantitative Physiological Traits (QPT) are determined concurrently: 1) whole-plant transpiration rate; 2) daily/periodically increase in plant biomass; 3) canopy conductance; 4) whole-plant water-use efficiency (WUE); and 5) Root influx. These QPTs are measured for single plants in an array, over time periods ranging from minutes to the entire growing season, under normal, stress and recovery conditions at different phonological stages. A supplemental algorithm that integrates these traits enables to calculate additional important QPTs; the root-to-shoot water flux ratios and whole-plant relative water content (RWC). Use of this experimental platform for the comparative physiological characterization of several crop cultivars has revealed several plant stress-response strategies that we have classified according to their relative "conservative" (isohydric-like) or "risk-taking" (anisohydric-like) character. We describe a "calculated risk-taking" trait that can be used as a marker for the selection of abiotic stress tolerance and resilient plants.

#16

Yield and nutritional assessment of irradiation-developed amaranth mutants

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Plant mutation breeding has gained more recently popularity providing a successful and relatively fast breeding method. The use of mutation induction for creating new germplasm and developing new cultivars is a profitable approach to bred varieties with higher and more stable yield potential, higher adaptability to climate changes and for food security. The main objective of our research is improvement of the quality and quantity of amaranth production through irradiation-induced mutagenesis. We have evaluated obtained grain amaranth (*Amaranthus cruentus* L.) mutants for the phenotypic traits, for important yield parameter and food quality and identified several advanced mutant lines with predictable performance of 1000-seed weight (demonstrated by bigger seeds) across two tested environments during multiyear evaluation. Biochemical analyses of proteins and amino acids, starch, fatty acids and squalene in mutant lines showed that these nutritional components fulfil the WHO/FAO standards. These findings led us to apply for registration of mutagenesis-most influenced mutant line (with respect to evaluated traits) as a new variety. This line was after successful DUS trial registered as first Slovak amaranth variety named "Pribina".

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#17

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

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Plant seeds are important renewable sources of protein, oil and starch, which are produced during the maturation or seed-filling phase of embryo development. Although metabolic networks for storage reserve synthesis have been largely characterized in diverse plant seed we are only beginning to understand the complex regulatory processes involved in carbon partitioning. Protein phosphorylation is a major form of post-translational regulation in eukaryotes as evidenced by over 1000 protein kinases in the Arabidopsis proteome. To begin studying protein phosphorylation in developing seed we performed large-scale, mass spectrometry-based phosphoproteomic studies on seeds at five stages of development in soybean (*Glycine max*), rapeseed (*Brassica napus)*, and Arabidopsis (*Arabidopsis thaliana*). Phosphopeptides were enriched using a combination of immobilized metal affinity and metal oxide affinity chromatography and analyzed by high-resolution tandem mass spectrometry. We identified a total of 2001 phosphopeptides containing 1026 unambiguous phosphorylation sites from 956 proteins with an average FDR of 0.78% for the entire study (Meyer et al., 2012). The dataset was uploaded into the Plant Protein Phosphorylation Database (P^3DB, www.p3db.org), including annotated spectra, for public accession. P^3DB is a public repository for all plant phosphorylation data and allows for homology-based querying of experimentally-determined phosphosites (Gao et al., 2009). Using this database of high quality phosphopeptide assignments as a training set we developed a phosphorylation prediction tool called MUSite (http://musite.sourceforge.net/) that incorporates protein disorder as one of three metrics for prediction (Gao et al., 2010). The sensitivity and reliability of this prediction algorithm is unmatched, and application to whole plant proteomes such as Arabidopsis TAIR10 indicates greater than 17,000 phosphorylation sites at the 99% confidence interval. Clearly, experimental and bioinformatic prediction of phosphorylation sites is rapidly becoming a facile task. However, confirmation and identification of cognate protein kinases responsible for these events remains challenging. To address this problem we developed an assay called the Kinase Client or KiC Assay (Huang et al., 2010). After validating this assay using the pyruvate dehydrogenase kinase (Ahsan et al., 2012) we applied it to identify kinase(s) responsible for phosphorylating a type one protein phosphatase inhibitor (PPI-2, Ahsan et al., 2013) and recently to compare substrate specificities for two families of protein kinases involved in metabolic regulation, the SnRK2 and CDPK superfamilies. Screening of a library of 2100 peptides comprising over 3500 *in vivo* phosphorylation sites in Arabidopsis revealed high specificity for these kinases and a surprisingly low level of overlap among family members.

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#18

Multivariate statistical methods for data exploration, integration and molecular biomarker discovery

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Measuring the activity of thousands of genes at once through gene profiling has dramatically accelerated our understanding of molecular processes leading to break-throughs in diverse areas of biology. Even greater potential now exists, with the recent advent of relatively inexpensive high-throughput next-generation sequencing techniques. These techniques have ushered in the era of big 'omics data (transcriptomics, proteomics, metabolomics) measuring massive quantities of biological features, such as transcripts, proteins and metabolites. With the appropriate statistical and computational tools, these masses of data can be transformed into valuable information in the form of 'molecular signatures' that are involved in biological processes of interest. Further, integrating different 'omics offers an unprecedented opportunity to probe complex molecular interactions of a biological system at multiple functional levels. However, one of the biggest analytical challenges to face is small number of samples (< 50) compared to the extremely large number of variables (> 10,000). Current statistical method can lead to incorrect molecular signatures being identified. We have developed several multivariate statistical methods that shift the current paradigm of assessing the impact of one biomarker at a time to a 'multivariate signature', where several molecular biomarkers are identified and assessed in combination to explain the phenotype. Those methods are available through our extensive mixOmics R toolkit. During this presentation I will introduce some multivariate integrative methods we have specifically developed for biomarker discovery and discuss the benefits of using such methodologies to address complex questions arising from high-throughput molecular biology, with application to plant studies.

#19

The use of Ontologies to describe Plant Experimental Assays

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Ontologies originated from the need to formally specify a controlled set of terms and their relationships in the context of a knowledge domain. The advantages of this type of approach include the ability to share structured information between different users and software tools, to reuse the established vocabulary, and, not less importantly, to make domain assumptions explicit. The Plant domain has been the subject of several attempts to structure and formally define terms and corresponding relations, such as their anatomical features, developmental stages, and the application of particular experimental procedures to a biological problem. Several ontologies describing developmental and anatomical characteristics of plants, their environment and even the types of stress plants can be subject to have already been proposed, e.g. Plant Ontology (PO), Crop Ontology (CO), Plant Infectious Diseases (IDOPlant). Although ontologies specifically dedicated to the description of experimental procedures in general do exist, their foremost concern is the description of experimental design, hypothesis testing and the ultimate goal of the experiments. The ontology proposed here, on the other hand, is mainly dedicated to the description of the pipeline of manipulations performed from specimens to data. In this study, we focus on the development and proposal of an ontology dedicated to the description of these experimental procedures, regardless of the scientific questions that prompted the assays. This ontology includes entities from three distinct realms (biological, physical and computer data), which include both experimental products, their relations and the protocols describing their manipulation. The final outcome is a useful and comprehensive ontology in the plant domain, to be used as a log book by experimentalists, providing a formal relation between entities and easily extensible to incorporate new types of data and experimental manipulations.

#20

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

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Plants have the ability to protect themselves from the threats of pathogens. A good strategy in plant protection is to take advantage of the plant immune system by eliciting the plant's constitutive defenses. Based on this concept resistance inducers have been developed and are commercially available, such as Bion®. An alternative formulation Greenforce CuCa was developed by UFLA partners in Brazil which showed promising results for the control of coffee rust (Hemileia vastatrix) in Coffea arabica. We established as working hypothesis that resistance inducers impose metabolic adjustments at cellular level, mainly on photosynthesis and its regulation. A physiological (leaf gas-exchange) and proteomic (2DE-MALDI/TOF/TOF MS) analysis was performed in coffee leaves sprayed with GreenForce CuCa, Bion® or water (control). Three days after treatment leaves were inoculated with H. vastatrix. Samples were collected at 3, 5 and 7 days after treatments. Our results show large alterations at physiological and proteomic level at 5 days after treatment. GreenForce CuCa and Bion® triggered opposite responses in leaf stomatal conductance and instantaneous photosynthetic rate. While application with GreenForce CuCa increased leaf-gas exchange, application with Bion® caused a decrease in photosynthesis and stomatal conductance. The proteomic data obtained revealed changes at photosynthetic and respiratory metabolism. Additionally, proteins involved in hormonal signaling were also observed as a late response (7 days). Taken together, our data support a role for the primary metabolism in defense responses, but the two resistance inducers seem to operate in different ways. This opens new perspectives for the research of plant induced resistance.

#21

Prioritizing QTLs based DNA molecular markers for heat stress tolerance using cell membrane stability in durum wheat

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Heat stress due to increased temperature is an agricultural problem in many areas of the world. In this study, the association mapping (AM) strategy based on a panel of 183 elite of durum wheat accessions was deployed in order to dissect the genetic control and identify QTLs for heat stress tolerance. Cell membrane stability (CMS) was recorded as a proxy index to evaluate the response to heat stress in a three-step experiment: constitutive heat stress response, acquired heat stress response and constitutive-acquired heat stress response. Significant differences among genotypes were observed for all measured CMS traits. The panel was profiled with simple sequence repeat, Diversity Arrays Technology and sequence-tagged site markers (957 markers in total). Thirty four single marker/QTL regions were located in all chromosomes; four major QTLs (LOD ≥ 3) for constitutive heat stress response were detected on chromosome 5A, 6A, 7B, while one QTL for constitutive-acquired heat stress response was detected on chromosome 6B. It is interesting that a higher number (nearly double) of QTLs were detected for constitutive heat response trait (heat shock applied to detached leaves) as compared to the two traits involving acquired responses, measured on living plants pre-adapted to high temperatures. The reason for this result could be that the constitutive response on detached leaves has a less complex genetic basis and higher heritability than acquired resistance observed on intact plants. The wide range of genetic variation and the limited influence of population structure support the reliability of our results and prompt for additional finer investigations of the physiological bases underlying these QTLs, towards their exploitation in breeding.

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#22

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

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

Sultan A, Andersen B, Svensson B, Finnie C. Exploring the plant-microbe interface by profiling the surface-associated proteins of barley grains. J Proteome Research (2016) 15: 1151–1167

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#23

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

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Abiotic and biotic stresses induce an active plant stress response resulting in stress acclimation and enhanced stress tolerance. Proteins play a crucial role in plant stress response since they are directly involved in an establishment of novel homeostasis leading to stress-acclimated phenotype. Gel-based proteomics using two-dimensional differential gel electrophoresis (2D-DIGE) approach represents a complementary method to gel-free approaches and provides a visual representation of plant proteome based on pI and MW values. 2D-DIGE analysis also enables protein relative quantification leading to an identification of the protein spots revealing an enhanced abundance in stress-treated or stress-tolerant varieties which could be further tested as potential markers of stress tolerance. In the presentation, summary of the major results of our team aimed at 2D-DIGE analyses of proteomes in stress-treated wheat and barley varieties is provided. Proteomic studies aimed at wheat or barley proteome response to cold, drought, salinity and Fusarium head blight disease are briefly summarised. Special attention is paid to the proteins revealing differential abundance between different stress treatments or genotypes revealing differential stress response such as spring versus winter genotypes or winter genotypes revealing differential levels of frost tolerance determined as lethal temperature for 50% of the samples, LT50, subjected to cold. The results of proteomic analyses are interpreted with respect to other physiological data such as parameters related to stress tolerance (LT50), phytohormone levels, water regime-related characteristics (water saturation deficit, osmotic potential), and others. The role of gel-based proteomic analysis in understanding plant stress response and acquisition of plant stress tolerance is discussed.

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#24

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

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Barley (*Hordeum vulgare* L.) belongs to the most important cereal grains, however in Polish climatic conditions its poor winter hardiness results in significant yield losses from cold injury every year. The aim of presented study was the identification of factors that determine freeze tolerance and could improve selection efficiency of genotypes of potential value for future breeding programmes. The plant material consisted of 8 doubled haploid (DH) lines produced by anther culture method from Polish breeding materials (F2 generation). Selected DH lines have been characterized as significantly different in freezing tolerance estimated according to Rapacz et al. (2011). The changes in abundance in protein species were analysed after cold treatment (3 weeks at 4°C) in barley leaves using gel-based proteomics. The proteins were isolated according to the phenol-based procedure (Hajduch et al. 2005) and examined by 2-D electrophoresis and PDQuest Software. Quantitative and qualitative differences in protein expression have been detected. Chosen proteins highly differentiated across examined DH lines were identified by MALDI TOF/TOF MS/MS analysis.

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#25

Proteome analysis of early stages of somatic embryogenesis in *Pinus nigra* Arn.

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Somatic embryogenesis (SE) is of great value because it is the only effective *in vitro* regeneration system that produces large quantity of plants in relatively short period of time. However, the long-term cultivation of Pinus embryogenic tissues on culture media usually results in the loss of maturation capacity and inability to regenerate whole plantlets from somatic embryos. Therefore our attention was paid to the proteins which can play role in early stages of SE in *P. nigra*. Based on our previous studies showing that some physiological or biochemical parameters in *P. nigra* embryogenic tissues with similar embryogenic capacity were cell line dependent, we decided to investigate two *P. nigra* cell lines (E362, E366). Using proteomic methods, we analysed embryogenic tissue with high embryogenic capacity, non-embryogenic callus and embryogenic tissue after loss of embryogenic capacity of each cell line. Total proteins were extracted using phenol-based extraction method, separated by 2-D electrophoresis using IPG strips pH 5-8 and differentially expressed proteins were identified by MALDI. We focused on proteins with altered abundance common for both cell lines. Software analysis revealed differences at protein level between the two cell lines analyzed, despite their similar maturation capacity.

#26

Proteomic analysis to understand pea resistance to *Fusarium oxysporum*

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Fusarium oxysporum f. sp. pisi (Fop) is an important and destructive pathogen affecting pea crop throughout the world. The constant evolution of the pathogen makes difficult the control of this disease necessitating broadening the molecular basis of resistance to Fop. We used a proteomic approach (2-DE coupled to MALDI-TOF/TOF analysis) to study the root proteome of three pea genotypes showing different resistance response to Fop race 2. Multivariate statistical analysis identified 132 differential protein spots under the experimental conditions with a total of 53 proteins identified using a combination of peptide mass fingerprinting (PMF) and MS/MS fragmentation. A functional classification was performed for all of proteins identified. The following main functional categories were assigned to the identified proteins: carbohydrate and energy metabolism, nucleotides and amino acid metabolism, signal transduction and cellular process, folding and degradation, redox and homeostasis, defense, biosynthetic process and transcription/translation. Results obtained in this work suggest that the most susceptible genotypes have increased levels of enzymes involved in the production of reducing power which could then be used as cofactor for enzymes of the redox reactions. This is congruent with the fact that a ROS burst occurred in the same genotypes, as well as an increase of PR proteins. On the other hand, proteins responsible to induce changes in the membrane and cell wall composition related to reinforcement were identified in the resistant genotype.

#27

Chitinase activities indicate pathogen resistance and tolerance to drought stress in wheat with different ploidy

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Chitinases are glycanhydrolases important in defense against pathogens and recently some chitinase isoforms and genes were shown to play role in adaptation to drought in a Slovak wheat breeding line. Here the impact of water shortage on 14 wheat cultivars with different ploidy was screened through selected morpho-physiological parameters, and correlated with total activity of chitinases. Wheat plants at early developmental stage (hexaploids *Triticum aestivum* and *Triticum spelta* and tetraploid *Triticum dicoccum*) were studied in pot experiments. Drought applied for 14 days affected leaf area and water content and several stomatal parameters including gas conductance, CO_2 assimilation rate, water use efficiency and humidity-induced stomatal closure. Induced activity of total chitinase activity was also detected in leaves. Multiple correlation analysis showed low correlation between stomatal characteristics and chitinase content during water deficit (control: R = 0.602/drought: R = 0.568) and also stomatal characteristics and drought tolerance (R values), probably due to variable defense strategies of wheat varieties. However, correlation between the chitinase activity and pathogen resistance characteristics of wheat varieties (R = 0.522) appeared as stronger in drought-stressed plants (R = 0.764). Our results indicate that chitinase activities might be indicative for not only pathogen resistance but also to abiotic stress tolerance level.

#28

A Review of current work on plant phenotyping in Turkey

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Recent developments in plant phenotyping tools and techniques have created opportunities in the genetic improvement of crop plants in order to meet steadily rising demand for food crops, biofuels and feedstock worldwide. Turkey with variable climatic and geographic conditions, rich plant genetic diversity and important agricultural production potential can make important gains by utilising high throughput precision phenotyping techniques. A study was undertaken to highlight the potential of the country in terms of achievements hitherto and institutional and infrastructural capacity. An analysis has been made using the data available on plant phenotyping and plant phenomics by considering temporal variation, sources of the studies, relevant national affiliations and institutions and topics by their importance and frequency. A SWOT (Strength, Weakness, Opportunity, Threats) analysis has been made for Turkey in order to outline its current and future potential for plant phenotyping. Future expectations were highlighted vis a vis national research and development priorities.

#29

Evaluation of the hybrid donor NOSH-aspirin as a priming agent against drought stress in *Medicago sativa* plants

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Terrestrial plants are constantly exposed to multiple abiotic stress factors such as drought, salinity and heat. Melatonin (Mel; N-acetyl-5-methoxytryptamine) is a naturally occurring metabolite, which is involved in multiple physiological processes in plants. The present study attempts to investigate the effect of this molecule in drought-stressed *Medicago sativa* L. plants. Plants were initially pre-treated with Mel by soil watering and were then subsequently exposed to severe water deficit. Preliminary experiments examined the extent of cellular damage in leaves by determining lipid peroxidation (MDA), hydrogen peroxide (H_2O_2) and nitric oxide (NO) content. Interestingly, drought-stressed plants pre-treated with Mel demonstrated significantly lower cellular damage levels compared with non-primed stressed plants, while the primed plants also showed lower reactive oxygen and nitrogen species content. In addition, primed and subsequently stressed plants displayed improved physiological performance in terms of increased chlorophyll fluorescence (indicative of photochemical efficiency of PSII) compared with non-primed, stressed plants. Real-time RT-PCR analysis and enzymatic activity assays are currently underway, examining the expression and activity levels of key defense-related antioxidant enzymes. Our results propose an important role for Mel as a systemic plant priming agent against drought stress conditions, while further experiments are planned which will attempt to delve deeper in the modus operandi of this compound.

#30

Influence of heavy metal stress on the antioxidant response and allergen production in the aromatic plant *Ocimum basilicu*

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Heavy metals constitute one of the most important biosphere contamination problems in many countries. Depending on their oxidation levels, they may be highly reactive and, therefore, toxic to most organisms. They are produced by various anthropogenic activities, which make them an extremely dangerous form of pollution. The toxic effects of heavy metals appear to be related to the increased production of reactive oxygen species (ROS) and consequently cause cell damage in plants. The present study focuses on the effects of three heavy metals (Ni^{+2}, Cu^{+2}, Zn^{+2}) on the antioxidant capacity and allergenic biosynthesis in the aromatic plant *Ocimum basilicum* using physiological, biochemical and analytical methods. The concentrations used for the three heavy metals were based on the limits set by the Government of Poland (i. rural areas: 100 ppm Ni^{+2}, 210 ppm Ni^{+2}, 200 ppm Cu^{+2}, 500 ppm Cu^{+2}, 720 ppm Zn^{+2} and ii. industrial areas: 500 ppm Ni^{+2}, 1000 ppm Cu^{+2}, 1500 ppm Zn^{+2}, 3000 ppm Zn^{+2}). Phenotypic damage was observed on the plant *Ocimum basilicum* after treatment with the three heavy metals, which was more pronounced at high concentrations (500 ppm Ni^{+2}, 1000 ppm Cu^{+2}, 3000 ppm Zn^{+2}). For detecting the concentration of heavy metals, the ICP-MS method was performed, which yielded increasing rates for all three concentrations of heavy metals (Ni^{+2}, Cu^{+2} and Zn^{+2}) compared with control plants. Specifically, increasing concentrations of Cu^{+2} resulted in increased damage to both physiological and cellular level, which was determined by measuring the concentration of MDA, hydrogen peroxide (H_2O_2), photosynthetic pigments and chlorophyll fluorescence. The levels of proline as an additional stress marker were higher at 1500 ppm Zn^{+2} compared with controls. The Elisa method showed higher concentrations of protein allergens with respect to the control plants for all three concentrations of Cu^{+2}. Increasing concentrations of Cu^{+2} and Zn^{+2} led to a decrease in the concentration of total proteins and antioxidant capacity, which was measured by two different methods (FRAP and Phosphomolybdate). Specific proteins that were differentially regulated due to heavy metal stress are currently being identified by LC-MS-MS/MS.

#31

The role of proteomics towards the study of PTMs in plant priming phenomena against abiotic stress factors

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Priming is the cellular state in which the harmful effects of abiotic stress factors in plants are hindered by pre-exposure to a stimulus, thus resulting in greater survival. It is becoming increasingly evident that priming techniques (e.g. external application of natural or synthetic compounds in plants) can enhance the tolerance of crops to environmental stresses. Thanks to the development of cutting-edge omics´; technologies such as proteomic analysis, scientists now have powerful tools to understand the molecular mechanisms underlying plant responses to environmental stimuli and priming phenomena. The few published proteomic studies on priming in the context of environmental stress identify key protein targets and signaling pathways which are being involved in the alleviation of negative effects of stress factors. Such tools can also focus on the study of post-translational modifications which appear to act as key regulators in these plant responses. Among others, our work has led to the identification of a number of carbonylated, nitrated and S-nitrosylated proteins with distinct or overlapping signatures as a result of priming against salt and drought stress with a series of chemical agents including hydrogen peroxide, nitric oxide, hydrogen sulfide and polyamines. Since priming is a very promising strategy in modern crop production management, further research is needed in order to establish the global picture of priming phenomena against environmental challenges as well as to characterize specific priming-related protein indicators in plants.

#32

Functional roles of HT1 and MPK12 kinases in CO_2-induced stomatal responses

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Plants are immobile organisms and to survive they need to respond to the constantly changing environment. The communication between plants and surroundings is mediated by guard cells, highly specialized cells that form stomatal pores. Guard cells sense various signals from the environment and adjust the stomatal pore size to maximize CO_2 uptake for photosynthesis and to minimize the water loss by transpiration. The opening and closure of stomatal pores are regulated by dynamic changes of protein phosphorylation in guard cells. Plants possessing several kinase mutants have been shown to be defective in regulating guard cells movement but the direct substrates and roles of these kinases are often unknown. We have been studying two protein kinases, HT1 and MPK12, as key regulators in CO_2-induced stomatal responses. Using in vitro kinase assays we show that protein kinase HT1 phosphorylates well-known proteins associated with stomatal pore movement. Additionally, we revealed that HT1 kinase activity is inhibited by MAP kinase MPK12. In the light of these findings we provide a new, more detailed model for CO_2 signalling and guard cells movement.

#33

Quantification and identification of allele specific proteins for polyploid non-model crops: proof of principle for 3 banana genotypes/phenotypes

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Polyploid crops are governed by various allelic copies of genes arising from their chromosome redundancies. We search for causal relationships between observed phenotypes and allele specific genes. The detection and quantification of allelic variants can be determined by RNA sequencing, but most read mapping software is not designed for polyploid genomes and quantitative allele specific information is lost. In LC-MS/MS proteomics the allele specific product - its unicity revealed by its tryptic specific peptides - is as such separated and quantified. In this study, with three contrasting phenotypes under osmotic stress, we develop a new workflow by integrating transcriptomics and proteomics to pick up, identify, and validate allele specific protein isoforms. 234,000 spectra were aligned and quantified in LC-MSMS and using uni- and multivariate statistics on the quantitative data 2,342 allele specific peptides were picked up. To identify these allele specific peptides we link the MS2 spectrum to its corresponding protein / gene in (1) a species specific database, (2) a database developed from mRNAseq, (3) a cross-species database. As a last identification effort we use the spectral properties for spectral library clustering and de novo sequence derivation. Finally, using read alignment software, we verify if the allele specific protein abundance is supported by RNA reads. Identification of specific alleles and coupling this to their phenotypes provide valuable insight for breeding of polyploid non-model crops and for characterization of valuable biodiversity.

#34

Phenotyping *Phaseolus* sp. germplasm collected from high altitudes for cold tolerance at generative stage

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Late season chilling stress (temperatures below 10ºC) is primary stress factor limiting the production of green beans in the autumn period[1]. Germplasm adapted to high altitudes may include tolerant genotypes that can be utilised for breeding tolerant cultivars for autumn sowings to extended period of bean production especially in relatively moderate climate coastal areas. An experiment was set up in order to screen 59 genotypes previously selected for their chilling tolerance at seedling stage at +5ºC for 24 hours at 700 µmol $m^{-2}s^{-1}$ light intensity in controlled climatic conditions. Chilling damage was assessed on the bases of visual scales, cell membrane leakage, chlorophyll measurements, leaf colour parameters in comparison with control plants. Considerable variation existed within the germplasm screened for chilling tolerance at generative stage. Seven genotypes showed no visual chilling damage, 9 negligible damage and 11 slight damage (scale of 1, 2, and 3 respectively) while the remaining genotypes ranged between 4 to 9 (dead plants). Phenotyping parameters were assessed in relation to breeding mild chilling tolerant green bean cultivars for late autumn sowings.

References

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#35 Poster presentation preferred

Nutrition conditions affect glucanase enzymes and arsenic uptake in wheat

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Nutrition strongly affects plant growth and provides resources for activation of defence. The molecular background of plant defence against extreme nitrogen conditions is, however, rarely studied within a single experiment. In this work, wheat plants (*Triticum aestivum*) were cultivated in hydroponic media with different N content (0 mM as starvation to 35 mM as excess). Afterwards, plant responses to presence/absence of 5 $mg.l^{-1}$ As^{3+} were studied. We quantified the activities of glucanases (EC 3.2.1.39) as typical defence enzymes and evaluated the impact of N conditions, arsenic and their combination. Several isoforms responded to the concentration of nitrogen in the medium, and many of these were induced by arsenic, too. The data indicate that nutrition affects the inducible defence as well as metal uptake. Responses of several isoforms to arsenic correlated positively or negatively with concentration of nitrogen in the medium. Others were not affected by nutritional conditions. We assume that this is related to the specific roles of individual enzymes under given conditions. The identified enzymes might be evaluated as indicators for nutritional conditions or presence of arsenic.

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#36

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

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Response to water deficit is a complex character, and its genetic variability is likely to involve a large number of mechanisms. In this context, a greater knowledge of these mechanisms and of the involved proteins can be useful to accelerate the genetic improvement of drought tolerance in maize. With various examples of proteomics analyses based on the separation of proteins by two dimensional gels and on gel-free proteome and phosphoproteome analyses, we will show that quantitative proteomics makes possible the identification of processes involved in the response to water deficit in maize, and to seek candidate proteins involved in the genetic variability of plant responses to this constraint. While the "candidate protein" strategy classically involve the analysis of proteome variations in segregating populations obtained from a cross between two inbred lines, we will describe a new strategy based on genome-wide association study (GWAS), where the variations of protein abundances is analyzed in hundreds of unrelated genotypes. We analyzed the proteome of 251 genotypes grown in a high throughput phenotyping platform (PhenoArch platform in Montpellier) under normal irrigation or moderate water deficit. At the pre-flowering stage, 1008 samples (251 genotypes x 2 watering conditions x 2 replicates) were collected on the last ligulated leaf and analyzed by shotgun label-free proteomics. Proteins were identified by using X!Tandem the X!TandemPipeline (http://pappso.inra.fr/bioinfo/xtandempipeline/), and quantification was performed by using MassChroQ [1]. Genome wide association studies were performed with 700.000 SNP for about 2000 protein x treatment combinations. Our results evidenced general proteome responses to water deficit which were shared by all genotypes, but they also showed the existence of genetic variations, that may be related to the variation of physiologic or agronomic traits. The GWAS allowed the identification of cis and trans protein quantity loci (PQLs). Their distribution on chromosomes and the use of this strategy to identify candidate proteins will be discussed.

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