



INSTITUTO  
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ANTÓNIO XAVIER /UNL

Knowledge Creation

# The quest for tolerant and stable varieties I: the role of "omics"

## WG2 Meeting

Hotel Praia Mar

Carcavelos, 26-27 February 2015

### *Scientific and Organizing Committee*

Carla Pinheiro, ITQB & FCT-UNL, Portugal

Estelle Goulas, Université Lille1, France

Sebastien Carpentier, KULeuven, Belgium



**Program (Outline) and time table**

	25 Feb	26 Feb	27 Feb
8h30min		Registration	
9h		Welcome	
9h10min		Invited Talk – Ric de Vos	Invited Talk – Luis Valledor
9h50min		Selected Abstracts Margarida Fortes (page 7) Vasileios Fotopoulos (page 13)	Selected Abstracts Elisabeth Jamet (page 14) Michel Zivy (page 19)
10h30min		Coffee break	Coffee break
11h		Selected Abstracts Estelle Goulas (page 9) M <sup>a</sup> Rosário Domingues (page 10) Aurélio Gomez-Cadenas (page 11) Elena Prats (page 16)	Round table Omic’s contribution to breeding Current and Future needs Technological barriers
12h20min		Lunch	Lunch
14h30min		Invited Talk – Carla António	Invited Talk – Ahmed Jahoor
15h10min		Selected Abstracts Ivan Paponov (page 15) Vesselin Baev (page 18) Shot-gun sessions: R. Turetschek (page 40) Ana Soares (page 31)	Selected Abstracts Alma Balestrazzi (page 12) Sebastien Carpentier (page 20)
16h10min		Coffee break	Coffee break
16h30min		Selected Abstracts Astrid Junker (page 8) Klaus Palme (page 17) Shot-gun sessions: Rita Tenente (page 33) Sandrine Arribat (page 41)	Round table remarks
18h00min	Welcome drink*	Drinks & snacks*	Farewell

Invited talks, 30 min + 10 min; Selected abstracts, 15 min + 5 min; Shot-gun sessions, max 10 min

## Invited speaker

### Metabolomics: a powerful tool for molecular phenotyping

Ric de Vos, Roland Mumm, Robert Hall

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Metabolomics is the latest -omics technology and aims to analyze and (relatively) quantify all low molecular weight compounds present in a biological sample, thereby providing an holistic view of the metabolome of a cell, tissue, organ or complete organism. As changes in the metabolite composition are the ultimate response of a biological system to genetic or environmental variations, metabolomics can be regarded as a large-scale molecular phenotyping tool. In plant biology and crop research, metabolomics techniques are nowadays frequently used to get more insight into the effects of genetic variation and modifications, plant growth and development, biotic and abiotic stress, post-harvest treatments, food processing, etcetera, on the metabolite composition of the plant or its derived products. Especially the so-called untargeted approaches, in which all metabolites detected in the sample extracts, both known and yet unknown, are taken into consideration, have provided novel and detailed insights into metabolites and biochemical pathways that are key to economically important traits of (crop) plants or products derived thereof, into the in vivo functioning of genes within functional genomics studies, and into hidden or unforeseen effects of natural or induced mutations. During the last decade we developed both targeted and untargeted metabolomics platforms for large-scale plant molecular phenotyping, based on mass spectrometry, including dedicated data processing workflows and multivariate analyses techniques. In this presentation a few examples of our research, using metabolomics as plant phenotyping and functional genomics tool, will be highlighted.

## Invited speaker

### Metabolomics challenges in plant abiotic stress: flooding, a case study

Carla António<sup>1,2</sup> and Joost T. van Dongen<sup>2,3</sup>

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Due to their sessile nature, plants cannot escape from regularly changing environmental and seasonal conditions that adversely affect their growth and development. Their survival depends largely on the initiation of highly complex adaptive responses involving stress sensing, signal transduction, and the activation of a number of stress-related genes and metabolites. Central metabolism including carbohydrate, nitrogen and energy metabolism, is essential for plant life, and flexibility to reconfigure these primary metabolic pathways to sustain cellular homeostasis is crucial for plants to develop strategies that allow them to survive. In this presentation, current challenges in the analysis of the complex plant primary metabolome will be presented, focusing on a study of the metabolic adaptations to hypoxia of wild-type roots of the crop legume soybean (*Glycine max*) using GC-TOF-MS metabolite profiling. Furthermore, the use of stable isotope flux analysis will be presented to further infer the relative metabolic activities of the various constituent pathways of central carbon metabolism. <sup>13</sup>C-pyruvate labelling was performed to compare metabolism through the TCA-cycle, fermentation, alanine metabolism and the  $\gamma$ -amino butyric acid (GABA)-shunt, whilst <sup>13</sup>C-glutamate labelling was performed to address the metabolism via glutamate to succinate. Our combined labelling data reveal the inhibition of the TCA-cycle enzyme succinate-dehydrogenase explaining the bifurcation of the cycle and the down regulation of the rate of respiration measured during hypoxic stress. Moreover, up-regulation of the GABA shunt and alanine metabolism explained the accumulation of succinate and alanine during hypoxia.

## Invited speaker

### **Pinus: a case study for a high throughput phenotyping in a non-model species**

Luis Valledor<sup>1</sup>, M<sup>a</sup> Jesús Cañal<sup>1</sup>, Jesús Jorrín-Novo<sup>2</sup>, Meijón Mónica<sup>3</sup>

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Current technology for the high-throughput phenotyping of model species at the different -omic levels (metabolomics, proteomics, transcriptomics) and its integrative analysis following a systems biology approach, promises to revolutionize the way we how we understand plant biology. In agricultural and forestry research these techniques are employed, following a non-targeted approach, to characterize key processes behind plant productivity including (a) biotic stress resistance, growth or fruit production, etc. Independently of the -omic level to be studied, a non-targeted approach aims to analyze and quantify all of the target molecules present in a biological sample for providing a detailed snapshot of cellular biology. The comparison of different snapshots allows a precise definition of the biological processes leading to specific responses, providing potential targets for plant improvement and dense data for modeling metabolic processes. However, this approach cannot be applied straightforward to most of the species since the available molecule extraction methods are not correctly set up and the reduced number of accessions in gene and protein databases dramatically impacts over the final biological meaning of these studies. These limitations are a major drawback for non-sequenced species for which these -omic approaches produce dramatically lower analytical yields compared to model species since the lower number of identification hinders easy pathway mapping and the capabilities to extract hidden biological meanings. In this presentation we will show our efforts for developing new analytical and bioinformatics workflows aimed to overcome the limitations associated to recalcitrant non-model species. We will cover the entire proteomic and metabolomics workflow, from molecule isolation to data processing and statistical analyses, highlighting the applications for the study of stress-responses and the characterization of the natural variation in this tree species.

**Invited speaker****Public private partnership in pre-breeding project: combining knowledge from field and from laboratory for pre-breeding in barley**Ahmed Jahoor

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In total 180 barley lines were collected from the participating breeding companies in Denmark (Sejet Plant breeding and Nordic Seed) Finland (Boreal) Iceland (LBHI), Norway (Graminor) and Sweden (Lantmännen) (30 lines were provided from each participant). These 180 lines were genotyped using the 9K iSELECT SNP chip 42 Simple Sequence Repeats (SSRs) markers. Meanwhile, these 180 lines were under the participants' observation phenotyping 7 major barley diseases and 12 agronomical traits underlying different climatic conditions. Barley diseases were studied under 28 different environments and the agronomical traits were studied under 92 different environments during 2012 and 2013 spring barley growing seasons. Association mapping was conducted using 7000 SNPs and disease resistance as well agronomic traits. A handful of linked markers were identified in this material. Usefulness of these linked markers have been validated at each companies. During the presentation, the population structure of the material as well as the results of association mapping will be presented.

## Oral Presentation

### Transcriptome and metabolome reprogramming in *Vitis vinifera* cv. Trincadeira berries upon infection with *Botrytis cinerea*

Patricia Agudelo-Romero<sup>1</sup>, Alexander Erban<sup>2</sup>, Cecília Rego<sup>3</sup>, Pablo Carbonell-Bejerano<sup>4</sup>, Teresa Nascimento<sup>3</sup>, José M. Martínez-Zapater<sup>4</sup>, Joachim Kopka<sup>2</sup>, Ana Margarida Fortes<sup>1</sup>

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*Vitis vinifera* berries are sensitive towards infection by the necrotrophic pathogen *Botrytis cinerea* leading to important economic losses worldwide. In an attempt to identify the molecular and metabolic mechanisms associated with the infection, pepper-corn size fruits were infected in-field. Green and *véraison* berries were then collected for microarray analysis and metabolic profiling. The results provide evidence of a reprogramming of carbohydrate and lipid metabolisms towards increased synthesis of secondary metabolites involved in plant defense. The response is already activated in infected green berries with the putative involvement of jasmonic acid and ethylene. Genes encoding WRKY transcription factors, pathogenesis-related proteins, stilbene synthase and phenylalanine ammonia-lyase were up-regulated in infected berries. However, salicylic acid signaling is activated in healthy ripening berries along with the expression of proteins of NBS-LRR superfamily suggesting that the pathogen is able to shutdown defenses existing in healthy berries. This study also provided metabolic biomarkers of infection such as azelaic acid, a substance known to prime plant defense responses.

## Oral Presentation

### Integrated and network-based visualization of multi-omics datasets

Astrid Junker<sup>1</sup>, Hendrik Rohn<sup>1</sup>, Anja Hartmann<sup>1</sup>, Eva Grafahrend-Belau<sup>2</sup>, Matthias Klapperstück<sup>3</sup>, Tobias Czauderna<sup>3</sup>, Christian Klukas<sup>1</sup> and Falk Schreiber<sup>3</sup>

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Experimental datasets are becoming larger and increasingly complex, spanning different data domains (metabolomics, transcriptomics, proteomics etc), thereby expanding the requirements for respective tool support for intuitive and compact visualization and enhanced visual analysis of large-scale datasets. The presentation will introduce the VANTED tool, a framework for systems biology applications. It comprises a comprehensive set of tasks ranging from network reconstruction, data visualization, integration of various data types, network simulation to data exploration combined with a manifold support of systems biology standards for visualization and data exchange. The offered set of functionalities enables users to view and explore data from different perspectives in the context of various kinds of biological networks accessible through VANTED, thereby facilitating the systemic analysis of a biological object. The support of various standards enables users to easily exchange files and allow for an accurate exchange of biological information using an unambiguous graphical representation (SBGN). The presentation will highlight VANTED functionalities using a number of -omics use cases.

Rohn H et al 2012. BMC Systems Biology 6:139.

Junker A et al 2012. Nature Protocols 7, 579-593.

Junker et al 2012. Frontiers in Plant Science 3:52.

Junker et al 2012. Trends in Biotechnology 30, 555-557.

Schreiber et al 2012. Nucleic Acids Research 40, D1173-D1177.

## Oral Presentation

### The 'NoStressWall' project: for a better understanding of drought-induced modifications in flax (*Linum usitatissimum* L.)

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Climate changes, fluctuating oil prices and diminishing fossil reserves are driving a worldwide increase in the use of plant biomass as a source for both biofuels and bio-based materials. For many thousands of years, Flax (*Linum usitatissimum* L.) has been cultivated for both its seed oil naturally rich in alpha-linolenic acid, and for its cellulose-rich bast fibers used to make textiles (linen) and to reinforce composite materials. The quality of textiles and composite material is related to fiber architecture and cell wall structure/composition and it is therefore essential to learn more about the different factors and mechanisms impacting cell wall formation during growth. To our knowledge, there is currently little information available concerning the impact of drought stress (and other abiotic stress) on cell wall formation, development and structure. The NoStressWall project intends to produce comprehensive data via multi-scale -omics analyses on the impact of drought stress – with a major focus on the cell wall - in flax. We aim to: i) generate and integrate large amounts of transcriptome, metabolome and proteome data together with comprehensive analyses of cell wall structure and modifications induced by drought stress, ii) use a reverse genetics screen to identify specific mutants in available flax chemical mutant populations, and iii) functionally characterize selected mutants. This work will improve not only our understanding of how flax plants react to water stress, but will also provide new detailed data on how abiotic stress modify cell wall structure and impact on fiber quality.

## Oral Presentation

### **Plant Lipidomics: a valuable tool to profile variation in lipidome towards plant growth and in adaption to stress**

M. Rosário Domingues

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Lipids are major components of plant membranes playing important roles in the regulation of plant metabolism. It is widely recognized that the functionality of lipids are far beyond from pure structural role, and that they are important signaling molecules. Changes in the lipid profile are associated somehow to lipid remodeling, and adjustments in lipid metabolism occur during plant growth and in plant adaptation to seasonality, environmental and stress conditions.

The lipidome of eukaryotes comprises thousands of lipids that are structurally and functionally diverse, and thus the identification of specific lipidomes or their variation is still a challenge. In the last years, the recent advances of mass spectrometry associated with high sensitivity, and capability of high throughput analysis opened new perspectives in the understanding of the role of lipids in plant biochemistry. The information gained with lipidomic analysis allow to infer the understanding of plant lipid function at molecular and cellular levels, and can provide clues concerning the roles of the enzymes and genes involved in lipid metabolism, in homeostasis and during plant adaptation. Most of the work developed until now have mainly been focused on *Arabidopsis* and more recently have been extended to other plants and algae. However, this is still an unexplored field that needs to be developed.

In our presentation, we will briefly present the most common analytical strategies based on mass spectrometry analysis used in plant lipidomics, same examples will be presented to illustrate how variation can be assessed in plant lipidome during growth or plant adaptation to stress.

## Oral Presentation

### **Analysis of secondary metabolite responses in citrus to drought and soil flooding**

Vicent Arbona, Rosa M. Pérez-Clemente and [Aurelio Gómez-Cadenas](#)

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In citrus, comparison of closely-related stress-tolerant and -sensitive species has allowed the identification of several abiotic stress tolerance traits such as the ability to exclude chloride from photosynthetic organs or the relationship between increased gas exchange parameters and soil flooding tolerance. Moreover, plant species experience changes at the metabolic level in response to adverse environmental conditions, and these changes could be used as early markers for tolerant genotype selection. To investigate these metabolic responses, two citrus rootstocks, Carrizo citrange and Cleopatra mandarin, with a different ability to tolerate soil flooding and drought were subjected to these abiotic stress conditions. Both stress conditions, induced alterations in root proline and cinnamic acid concentrations that could not be associated to any particular tolerance behaviour and were likely a common response to stress. In addition, concentration of plant hormones abscisic acid (ABA) and jasmonic acid (JA) decreased in response to soil flooding irrespective of the relative flooding tolerance but increased in response to drought. These contrasting responses in ABA and JA accumulation are probably associated with particular regulatory processes under soil flooding and drought. Non-targeted metabolite profiling of root tissues indicated a high number of genotype- and stress-specific responses with low degree of overlapping, indicating a specific mechanism to cope with stress in plant species. Analysis of the basal metabolic status of plant roots also indicated different metabolite levels under control conditions in the two genotypes which could also contribute to stress tolerance.

## Oral Presentation

### Molecular tools for assessing seed quality in crops: focus on the seed repair mechanisms

Patrizia Vaccino<sup>1</sup>, Stefania Paparella<sup>2</sup>, Davide Gerna<sup>1</sup>, Margherita Limonta<sup>1</sup>, Susana Araújo<sup>3</sup>, Alma Balestrazzi<sup>2</sup>

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Seed vigor positively correlates with enhanced germination, harvest rate and improved stress tolerance (Rajjou et al. 2012, *Annu Rev Plant Biol* 63: 507-533). A critical aspect of seed vigor is the seed ability to repair oxidative injury. DNA repair mechanisms, activated during seed rehydration to preserve genome integrity are still poorly explored (Ventura et al. 2012, *Plant Physiol Biochem* 60: 196-206). A deeper knowledge of the expression profiles of DNA repair genes, used as hallmarks of seed viability, will support the development of phenotyping methodologies to help monitoring the seed response during vigorization treatments performed in the Seed Industry. These aspects are investigated in the PRIMTECH project, funded by the Italian Lombardy Region and Cariplo Foundation. The project started at the beginning of 2014, and scheduled activities are in progress.

## Oral Presentation

### Systems biology approach towards deciphering the response of *Medicago truncatula* plants against salt stress

Panagiota Filippou<sup>1</sup>, Antoniou Chrystalla<sup>1</sup>, Toshihiro Obata<sup>2</sup>, Evangelos Harokopos<sup>3</sup>, Xavier Zarza<sup>4</sup>, Antonio F. Tiburcio<sup>4</sup>, Vassilis Aidinis<sup>3</sup>, Alisdair R. Fernie<sup>2</sup> and Vasileios Fotopoulos<sup>1</sup>

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Salt stress is one of the most important factors limiting plant productivity, with salinity affecting plant physiology and metabolism at multiple levels. The aim of this study was to explore and elucidate the role of antioxidant and salt tolerance mechanisms in the model legume *Medicago truncatula*. For this reason, three ecotypes of *M. truncatula* showing differential response to salinity were used: Jemalong A17 (moderate response), TN6.18 (sensitive to salinity) and TN1.11 (tolerant to salinity). Cellular damage levels were monitored in roots and leaves after 48 h of salt stress application with 200 mM NaCl by measuring lipid peroxidation (MDA) levels, as well as reactive oxygen and nitrogen species (RONS) content, further supported by leaf stomatal conductance and chlorophyll fluorescence readings. The salt-tolerant genotype TN1.11 displayed the lowest MDA and RONS content, while the salt-sensitive TN6.18 was affected the greatest. Transcriptional profiling using Affymetrix microarray analysis of salt-stressed *M. truncatula* plants compared with control samples identified approximately 794 transcripts that are differentially regulated in both a genotype and tissue-dependent manner. Furthermore, metabolite profiling of *M. truncatula* plants was employed to analyze the effect of salt stress in the accumulation of key metabolites (including sugars and amino acids), leading to exclusive insights into the plants' metabolic networks which however appear to be genotype- and not tissue-dependent. This holistic approach will hopefully contribute in gaining new insights into the cellular response to salt stress in *M. truncatula* plants.

## Oral Presentation

### Cell wall plasticity in response to temperature changes: A cell wall proteomic study of two contrasted ecotypes of *Arabidopsis thaliana* (Col0 and Sha)

Vincent Hervé, Cécile Albenne, Josiane Chourré, Vincent Burlat, Harold Duruflé, Thierry Balliau, Michel Zivy, [Elisabeth Jamet](#), Christophe Dunand

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Plant cell walls are critical for plant adaptation to their environment and play numerous roles, such as physical control of growth, establishment of cell shape, structural integrity of the plant body and defense against environmental stresses. Their structure and composition are modified to maximize plant adaptation and it has been shown that cell wall proteins are important players in these processes (Albenne *et al.* Front Plant Sci 2013, 4: 111). *A. thaliana* has been chosen as a model plant to study the influence of the temperature on the structure and composition of cell walls. Two contrasted ecotypes have been cultivated at two different temperatures, 22°C and 15°C. Col0 is a temperate ecotype, whereas Sha is an altitudinal one originating from the Shakhara valley of Tadjikistan (altitude 3400 m). In growth chambers, differences in morphology have been observed at the macroscopic and microscopic levels, such as thickness of the leaf cuticle and diameter of stems. Cell wall proteomics studies have shown differences in protein content. A clear distribution of the identified proteins in four groups could be done depending on the ecotype (Col0 vs Sha) and on the growing temperature (22°C vs 15°C). An integrative analysis between the different data has been performed in order to identify relevant candidate proteins possibly involved in cell wall plasticity in response to temperature variations. The results of this study could apply to plant species of economical interest.

## Oral Presentation

### Transcriptomic analysis adds new insights to microscopy staining data supporting the regulation of reactive oxygen species by auxin

Ivan A. Paponov<sup>1</sup>, Vadym Budnyk<sup>2</sup>, Tatyana Khodus<sup>2</sup>, Klaus Palme<sup>2</sup>

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Plants, in contrast to animals, have a wide-ranging potential for growth adjustment in response to environmental stresses. We hypothesize that one mechanism underlying this adjustment is modulation of reactive oxygen species (ROS) by the plant hormone auxin. We tested this hypothesis by comparing microscopy staining data with data from transcriptomic analysis of gene expression induced by auxin and ROS. Microscopy staining showed that auxin differentially regulates the level of reactive oxygen species in the roots: it increases the redox potential of the plasma membrane and it decreases the cell wall levels of hydroxyl radical (OH·). Transcriptomic analysis supported the microscopy observations that auxin modulates several ROS, resulting in regulation of multiple stress responsive genes. At the transcriptional level, peroxidases are the main auxin targets among enzymes that modulate ROS levels. The absence of an over-representation of AuxRE in the promoter sequence of auxin-repressed genes indicates that the major mechanism of gene repression by auxin is independent of ARF. The auxin-repressed genes might be regulated by reductions in chloroplast superoxide, as indicated by up-regulation of many auxin-repressed genes in a mutant with enhanced superoxide levels in the chloroplast. Interestingly, 11 of the 13 down-regulated genes were expressed in chloroplasts, suggesting a novel regulation of chloroplast genes by auxin. This transcriptomic analysis added new insights to microscopy staining data regarding the effects of regulation of superoxide levels in chloroplasts by auxin.

## Oral Presentation

**Metabolomics highlights salicylate signalling pathways and the modulation of carbon, antioxidant and photo-oxidative metabolism as key drought tolerance response in oat (*Avena sativa*).**

Javier Sánchez-Martín<sup>1</sup>, Jim Heald<sup>2</sup>, Alison Kingston-Smith<sup>2</sup>, Ana Winters<sup>2</sup>, Diego Rubiales<sup>1</sup>, Luis A. J. Mur<sup>2</sup>, Elena Prats<sup>1</sup>

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Improving drought tolerance in crops is a complex task for which understanding of drought tolerance mechanisms is essential. Based on metabolomic changes in oat (*Avena sativa* L.) we define key processes occurring during water stress and the fine modulation of these processes for drought tolerance. During a time course of increasing water deficit, metabolites from leaf samples were profiled using Direct Infusion – Electrospray Mass Spectroscopy (DI-ESI-MS) and HPLC ESI-MS/MS and analysed using Principal Component Analysis and Discriminant Function Analysis. The involvement of metabolite pathways was confirmed through targeted assays of key metabolites and physiological experiments. Metabolite profiles highlighted an early accumulation of SA to influence stomatal opening, photorespiration and antioxidant defenses before any change in relative water content was observed. These changes are likely to maintain plant water status, with any photoinhibitory effect being counteracted by an efficient antioxidant capacity, thereby representing an integrated mechanism of drought tolerance in oats. The study also highlighted metabolite changes at later points consequence of the different water status in the susceptible and resistant genotypes.

## Oral Presentation

### Insights from high resolution phenotyping of *Arabidopsis thaliana* roots

Klaus Palme

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The study of root development relies on techniques for the accurate visualization of tissue and organ structure to understand cell patterning and patterns of gene expression. Currently available techniques for three-dimensional (3D) imaging are limited with respect to the thickness of roots and the resolution that can be achieved. In order to achieve a detailed functional and quantitative understanding of roots, cellular features of roots must be quantified in the three-dimensional context of cells and tissue layers. We therefore aimed, besides genetically, molecularly and functionally characterizing root development, at developing an intrinsic root coordinate system (iRoCS) as a reference model for analysis of the *Arabidopsis* root apical meristem. iRoCS can be used to rapidly parameterize image data within a single framework in a standardized way. It enables large cohorts of roots to be annotated, making statistical analyses accessible and giving an unbiased evaluation of previously hidden developmental phenotypes. iRoCS was used to study root patterning and shown to even distinguish subtle changes in the distribution of cell division in different cell layers in knock-out mutants.

## Oral Presentation

### Identification of plant stress-responsive microRNAs and isomiR variations in NGS datasets.

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MicroRNAs (miRNAs) are a class of small non-coding RNAs, which are negative regulators of gene expression in eukaryots. The processing of miRNA precursor frequently results in production of sequences which differ from the “reference” mature sequence, thus generating multiple variations known as “isomiRs”. In many cases both mature miRNA and corresponding isomiRs have been found to present in the NGS libraries. IsomiR variability can be explained by the imprecise and alternative cleavage of Dicer and Drosha during pre-miRNA hairpin processing. We have developed a tool allowing identification of miRNAs in 73 plant species using next-generation sequencing datasets. In addition the software can detect and visualize isomiRs with higher copy number relative to their mature reference sequences indexed in miRBase. Therefore the observed specific signature overabundance of particular isomiR in sample can suggest its potential role within the stress tolerant state. As additional information the software generates differential expression charts of the most dys-regulated miRNAs and IsomiRs between samples.

## Oral Presentation

### 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 treatments x 2 replicates) were analyzed by shotgun proteomics. Of 1125 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.

## Oral Presentation

### Drought stress research in crops using -omics approaches: mRNAseq and proteomics in the spot light

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We apply different models to phenotype plant biodiversity in response to a changing environment and to get insight into tolerance mechanisms. The power of -omic methods tends to be lost in crops due to the lack of genomic information or the complexity of the genome. Though technologies evolve. We are developing methods to further improve the identification rate of both RNA-seq and quantitative label free proteomics. From a pioneering drought experiment on banana, we assess that mRNA-seq was able to visualize  $18 \times 10^6$  reads belonging to 37577 different genes, with a dynamic range of ~5 orders of magnitude. The proteome analysis (QExactive/50cm-UPLC-column/6h-gradient) was able to quantify  $150 \times 10^3$  peptides, with a dynamic range of ~6 orders of magnitude. The bottle neck for proteomics is the identification of the peptides and reconstruction into proteins. Due to low abundance, only 30% of the peptides was selected for MSMS with an identification rate of 16% (1891 different proteins). The expression of 1045 genes could be characterized by both RNA-seq and proteomics, 846 were unique for proteomics and 36532 for RNA-seq. Quantitatively, 342 proteins (18%) and 3638 mRNAs (10%) were considered as differential. Eleven were in common. Analysis of an unsequenced genotype relies on the presence of a reference genome, existing libraries and on de novo sequencing/assembly. There is only a small overlap between both methods, starting again the debate about correlations between mRNA and protein. We conclude that both methods are extremely useful to characterize the phenotype of different genotypes and map allelic variations.

## Poster Presentation

### Are there parallel signaling pathways in stomatal regulation? The role of the *era1* mutation

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#### Abstract

Drought is the most relevant environmental stress affecting agricultural production worldwide. Abscisic acid (ABA) is a phytohormone, regulating plant responses to different environmental stresses with a key role in stomatal regulation. Increase in ABA concentration initiates ABA signaling and leads to stomatal closure in drought stress. Thus, several plant lines affected in ABA signaling show enhanced ABA response and drought resistant phenotypes. A mutant with an ABA hypersensitive and drought tolerant phenotype *era1* (ENHANCED RESPONSE TO ABA 1) is one of them. ERA1 encodes beta subunit of farnesyl transferase, an enzyme associated with ABA signal transduction. Thus ERA1 is a gene that has great potential to be used in genetic manipulations to achieve drought tolerant plants. We crossed *era1* with mutants in the core regulators of ABA signaling unit and analyzed steady-state stomatal conductance and stomatal response to environmental factors in double mutants. Interestingly, the lack of functional farnesyl transferase beta subunit in *era1abi1* and *era1srk2e* double mutants resulted in lower stomatal conductance compared to *abi1* and *srk2e* single mutants. However, *era1* did not rescue the stomatal responsiveness of double mutants. We discuss our results in the light of two potential signaling pathways that regulate stomatal behavior: one determining basal conductance and other determining responsiveness.

## Poster Presentation

### Evaluation of drought tolerance in bread wheat varieties

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In the context of climate changes, Mediterranean areas are increasingly exposed to drought, which is a major cause for yield reduction of many important food crops, such as wheat. The use of varieties that tolerate water deficit may contribute for adaptation strategies in farming systems. The aim of this work was to compare photosynthetic, stomatal and yield traits in four bread wheat (*Triticum aestivum* L.) varieties (Roxo, Nabão, Ardila and TE 0205) subjected to drought after anthesis. Seeds were sown in 60 L containers filled with clay loam soil, maintained under greenhouse controlled conditions. Drought was imposed by withholding irrigation for ten days, after visual assessment of anthesis (ca. 89 DAS). Well irrigated and water stressed plants were compared as regards leaf gas exchanges (net photosynthetic rate,  $P_n$ ; leaf stomatal conductance,  $g_s$ ; transpiration,  $E$ ), and water use efficiency (WUE). Subsequently, plants were maintained under a controlled irrigation (droughted plants: 50% of the water given to fully irrigated controls) until harvest, to quantify yield. Drought caused severe  $g_s$  and  $P_n$  decreases in all varieties. Grain yield components were also affected but differences were found as regards kernel yield per spike and the number of kernels per spike.

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## Poster Presentation

### Identification of drought responsive genes and promoters in *Musa* by using RNA-seq.

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With an annual production of around 100 million tons (FAOstat, 2012), banana (*Musa* spp.) is one of the most important crops in the world. However, water is the main limiting non-biological factor affecting yield in production areas. In this context, a better understanding of the biodiversity and genetic basis of drought tolerance is needed. In our lab, we have performed an RNA-seq experiment on three different banana cultivars known for their contrasted response to mild-drought stress. The mapping of the reads was performed on the double haploid *Musa acuminata* reference genome (AA; D'Hont et al., 2012) as a template. Since all three banana cultivars are triploid and have variable genome composition (AAA or ABB), mRNA-seq results needed to be analyzed in a special manner. More than 803 million out of 1.2 billion reads were uniquely mapped on the reference genome. Applying various statistical methods, we have identified a set of candidate genes differentially expressed under stress conditions. A number of them are tissue-specific and thus appropriate for identification and cloning of promoter regions able to drive expression of drought responsive genes. Currently, these candidate genes/promoters are being validated with alternative approaches (qPCR) and in different experiments carried out in the lab, greenhouse and under field conditions.

D'Hont A. et al. 2012. Nature, 488, 213-219.

## Poster Presentation

### A first glimpse on the impact of climatic changes in *Coffea* spp. assessed by RNA-seq: effect of elevated CO<sub>2</sub> and temperature

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The coffee crop is based on two species, *Coffea arabica* L. and *Coffea canephora* Pierre ex. A. Froehn, which together account for 99% of the world coffee bean production that has a huge impact on the economies of many countries from tropical regions. Coffee production has been predicted to become threatened by climate changes and global warming conditions. Still, the effective impact of enhanced atmospheric [CO<sub>2</sub>] and temperature on the biology of this crop remain to be elucidated. In this context, we aim at understanding coffee molecular responses linked to such environmental changes. Physiological and biochemical data obtain so far suggested that enhanced [CO<sub>2</sub>] mitigated the deleterious impact of under high temperatures at the photosynthetic level, promoted a higher performance of the photosynthetic apparatus and a better global status of the plants, therefore improving the plant acclimation capability. In parallel, a comprehensive transcriptomic analysis through Illumina RNA-Seq was conducted aiming at gaining insights on the main pathways affected by these environmental changes. The results are expected to ultimately be useful to assist breeding programs.

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## Poster Presentation

### Investigation of antioxidant defense parameters and retrotransposon based variations in gamma ray induced drought tolerant wheat mutants

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Wheat is the one of the staple crops for the majority of world population being used as both a human food and a livestock feed. Among abiotic stressors, drought is a major constrain responsible for dramatic yield losses under dry-land conditions. Thus, improving drought-tolerant wheat genotypes is one of the main targets for many wheat improvement programs. Physical mutagens, such as gamma-ray, have been extensively used to generate genetic variability in plant populations over the last 80 years. Advanced antioxidant defense parameters are important factors to screen plants abiotic stress conditions, such as SOD, POX, CAT and APX etc., which are stimulated for defending plant cell against stressors. These parameters are important tools to reflect genotype x environment relationship under stress conditions. Retrotransposons are the most abundant mobile elements in the plant genome and play important role from the genome reorganization to the epigenome alterations induced by environmental stressors. However, they have not been studied much like stress-induced genes. In this study, our goal is to investigate relationship between antioxidant defense parameters and retrotransposon based variations in gamma-ray induced drought tolerant wheat mutants. For this goal, we have selected in different tolerance level drought tolerant wheat mutants under *in vitro* conditions with PEG 6000 based selection using M<sub>2</sub> and M<sub>3</sub> wheat populations, which were obtained from using gamma-ray. Then, we have growth these mutants under greenhouse conditions to continue applying drought stress. M<sub>3</sub> and M<sub>4</sub> generations drought tolerant mutant lines have been used to analyze variations in antioxidant defense parameters (SOD, POX, CAT and APX) and LTR-retrotransposon based molecular markers.

## Poster Presentation

### Comparative proteomic analysis of embryogenic and non-embryogenic cell lines of *Pinus nigra*

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In our study the proteome profile of two embryogenic cell lines and corresponding non-embryogenic cell lines (*calli*) of a conifer *Pinus nigra* Arn. has been investigated. The embryogenic cell lines involved in the experiments (E 362, E366) have been initiated from immature zygotic embryos and the non-embryogenic calli (NEC 362, NEC 366) have been derived from cotyledons of plantlets (somatic seedlings) regenerated from cell lines E 362 and E 366. Bipolar somatic embryos were present in embryogenic tissues. They were composed of meristematic cells in the embryonic part and suspensor composed of long vacuolised suspensor cells. The early bipolar structures developed into somatic seedlings capable of growth in soil. The non-embryogenic calli were composed of round shaped paranchymatous cells without the presence of organised structures. No organised development has been observed in non-embryogenic calli. Proteins from embryogenic and non-embryogenic tissues were isolated using phenol-based extraction method and analyzed by 2-dimensional electrophoresis. As a preliminary result, 108 (E362-NEC 362) and 109 (E366-NEC 366) proteins spots were found differentially expressed in analysed cell lines.

#### Acknowledgements

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## Poster Presentation

### **The quest of productive variety of carvacrol chemotype of *Thymus pulegioides*: metabolomic analysis and effects of meteorological conditions**

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The species of genus *Thymus* – essential oils (EO) bearing medicinal plants in which the monoterpene phenols (thymol and its isomer carvacrol) are pharmacologically the most valuable chemical compounds. The raw material of medicinal plants must to fulfil the strong requirements on quantity of pharmacologically active compounds. The high intraspecific chemical diversity which is characteristic of many species prevents to collect the chemically homogeneous and standardized raw material in the wild populations. However, chemically heterogeneous populations are big reserve for the selection. *Thymus pulegioides*, growing wild in Baltic States, is suitable for cultivation in this region because not winterkill, the plants are enough for mechanical gathering (enough height, not woody stems). One in six *T. pulegioides* chemotypes, growing wild in Lithuania, is carvacrol (C) chemotype. Therefore the goals of study were: 1) to select the productive variety of C chemotype, 2) to evaluate the stability of amount of EO, C and its precursors under different meteorological conditions. The individual plants of C chemotype were moved from natural habitats into field collection of the Nature Research Centre (Vilnius, Lithuania). Selection of productive variety carried out by yield of total biomass, amount of EO and C. The EO isolated by hydrodistillation, metabolomic analysis carried out by gas chromatography. The influence of meteorological conditions investigated in 2008–2013. The amount of EO varied from 0.72% to 0.98% in selected variety across years and correlated with photosynthetically active solar radiation ( $r=0.89$ ,  $p<0.05$ ). The significant connections between amount of C and its precursors and meteorological conditions not established.

## Poster Presentation

### Common bean phenostressomics: Screening Portuguese common bean germplasm for disease and drought resistance

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Portugal holds a very diverse common bean (*Phaseolus vulgaris* L.) germplasm consisting of landraces that resulted from more than five centuries of adaptation and mass selection by farmers. As in the rest of Europe, and alike other legume species, national common bean production has been reduced due to yield instability of used varieties caused by biotic and abiotic stresses, namely diseases and drought. We are presently evaluating an extensive common bean collection of Portuguese landraces for drought resistance by measuring photosynthetic performance under water deprivation and resistance to important legume fungal diseases such as rust, fusarium wilt and powdery mildew. Biotic and abiotic stress resistance phenotyping is being established under growth chamber conditions with robust protocols adapted to the characterization of a large number of accessions. Simultaneously, the existing genetic diversity is being characterized using high-throughput Single Nucleotide Polymorphism (SNP) molecular markers. This will allow the future association of the detected resistances to their genetic control, with the subsequent development of molecular breeding tools to assist common bean resistance precision breeding. The agronomical, morphological and molecular diversity already observed in the common bean landrace collection indicates the presence of sources of resistance not yet explored in plant-pathogen interaction research and breeding programs. The development of common bean varieties with multiple biotic and abiotic resistances would meet farmers' expectations and needs, supporting the development of a more sustainable agriculture, by reducing the use of pesticides, fertilizers and water.

## Poster Presentation

### How can sugar sensing control growth under stress?

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Plant growth is a result of complex interaction between water, energy availability, connected with nutrient sensing. Stress is a reaction to deficiency of either of them. Two sugar pathways, the trehalose and hexokinase pathway are in the center of our interest. Both have proven to play a role in signaling and in stress. Both are also involved in stomatal closure in response to abscisic acid (ABA), a plant stress hormone. It is proposed that the origin of this particular mechanism can be found in increasingly produced sucrose content during day that causes a feedback and. The advantage of this feedback mechanism would be that stomatal closure is responsible for a higher water use efficiency and decreases the risk of excessive loss of water. Our results from the on-line phenotypical observations proves this stomatal behavior and suggests the involvement of trehalase in this mechanism. Based on clear phenotypic reaction we are sampling the leave tissues at critical time points. Final results will include the phenotypical observation, genes expression profiles from real time-PCR, protein abundance from MS/MS and enzymatic activity.

## Poster Presentation

### Proteomics to understand somatic embryogenesis in coffee

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Coffee is one of the most important agricultural products cultivated worldwide and is of high importance for the Brazilian economy. Brazil accounts for 50% of the global coffee production and is responsible for more than one third of the world's production and export. Somatic embryogenesis (SE) is a process where embryos can be regenerated from somatic tissues. However, many cells are not successful in differentiating into an embryo. Protocols for SE in coffee have been developed, but the molecular processes are still unknown. With our proteomic study we will investigate the molecular events taking place during cell differentiation that will contribute to a better understanding of totipotency. This knowledge will facilitate the production of elite genotypes of coffee. In this study we will analyze the proteomes by LC MS/MS based on a gel-free approach and compare an embryogenic and a non-embryogenic cell suspension of *Coffea arabica* L. cv. Catuaí Amarelo initiated from leaves. The challenge in this approach is the poorly annotated genome and to find the link between peptides and the original protein, since the proteins are digested before the quantification via MS. Bioinformatics tools are used in order to increase the identification rate. In this case, the identification of the proteins will be facilitated by the recently published genome and existing mRNA databases ([www.lge.ibi.unicamp.br/cafe](http://www.lge.ibi.unicamp.br/cafe)). A better understanding of the molecular mechanisms basis related to somatic embryogenesis can lead to new in vitro culture strategies for plant propagation and genetic manipulation. As the production of plants through SE can be applied on a large scale, it can open additional avenues for basic and applied research in other agricultural crops.

## Shotgun Presentation

### Phenotyping genetic diversity: developing a proteomics workflow to detect salt related allelic variability

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Salinization has an important role in soil degradation. Among other causes, irrigation systems used incorrectly is one of the biggest factors contributing to the damage of millions of hectares due to salinization. Maize is one of the most produced crop in the world and is generally classified as sensitive to salinity, despite being drought tolerant. Due to this sensitivity, it is of paramount importance to study the reactions of salt stress, specifically in relation to the detection of the stress and signaling through the plant. In this context, in the last decade there have been discoveries of (poly) peptides acting as regulatory molecules in response to stresses and thus phenotyping at cellular level may lead to the detection of novel signaling peptides during salt and osmotic stress. To achieve this goal, the genotypes Across 8023 and Across 8024, sensitive and tolerant to salt stress, respectively, and the B73, an inbred sequenced reference, will be subjected to saline conditions. Combined phenotyping at the physiological plant level and phenotyping at cellular level (proteomics and peptidomics) will be used to understand the genetic diversity and the stress responses. Several variables will be used for this purpose: monitoring transpiration, growth rate, photosynthetic efficiency based on fluorescence and stomatal behavior. Special attention will go to the monitoring of the changes in apoplastic pH to determine how the salt is perceived by the roots and the signals are transported to the leaves. These responses will be linked with the cellular phenotyping of each maize genotype during the stress by a high throughput screening of proteins plus detection of potential signaling. The proteome analysis will be performed separating and quantifying the dynamics of soluble and apoplast intact proteins by gel based approach (2DE) and gel free approach will be used to obtain peptides derived from the digested proteins. The identification of the proteins will be realized via MALDI TOF MS/MS and the peptides will be characterized via nano-2LC-Qexactive MS. This experimental setup will allow us to characterize the response of each genotype to stress at cell, tissue and plant level and we may find new forms of stress perception and signaling.

## Poster Presentation

### Unraveling seed development mechanism in *Phaseolus vulgaris* L. under water deficit: a transcriptomic approach

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Common bean (*Phaseolus vulgaris* L.) is a major staple crop in developing countries in Latin America and Sub-Saharan Africa, where water deficit (WD) is a continuous concern. Seed development (SD) plays a major role in the composition of reserves, affecting yield, quality and nutritional value of beans. Our work aims to unravel the molecular mechanisms underlying SD and how are they affected by WD. We are using global scale studies, based in Omic approaches, to investigate the dynamics in transcript and proteomic profiles during SD. We are comparing two bean genotypes with contrasting performances concerning WD resistance and productivity. The genotype SER16 stood out with increased WD resistance, as seen by its better photosynthetic and photochemical performance in comparison with the WD sensitive reference Tio Canela. Seed samples were collected from SER16 and Tio Canela from two environmental conditions (control vs WD) at four time points of grain development (10, 20, 30 and 40 days after anthesis) reflecting the four relevant stages associated with seed development. The analysis of bean transcriptome was conducted using Massive analysis of cDNA Ends (MACE) in well watered samples. Such approach allowed us to identify transcripts with differential accumulation among the major developmental phases. Future RT-qPCR studies will elucidate on how the expression of such genes is modulated by WD and reveal major pathways and regulatory mechanism with major role in common bean seed development and yield.

**Poster & Shot gun Presentation****Coffee rust modulates the leaf apoplastic proteome of *Coffea arabica* plants**

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Coffee leaf rust, due to the biotrophic fungus *Hemileia vastatrix*, causes important yield losses in coffee production, if chemical control measures are not applied. A proteomic approach was used to study the changes in the apoplastic fluid (APF) profile of coffee leaves infected with fungus (resistant and susceptible samples), at targeted stages of the infection process. The APFs were extracted by leaf vacuum infiltration and protein profiles were obtained by 2-DE. A comparative analysis of the gels allowed the detection of about 200 polypeptide spots whose volume changed in abundance upon infection (collected from 1-4 days after inoculation). Proteins, identified by matrix assisted laser desorption/ionization time of flight-mass spectrometry (MALDI-TOF/TOF-MS) followed by homology search in ESTs coffee databases, are shown to participate in the reorganization of cell wall metabolism and in the modulation of protein stability (mainly proteolysis) and stress/defense related processes. The comparative analysis of the APF proteome of healthy coffee leaves<sup>1</sup> with the infected leaves gave us clues on the proteins involved in the basal immune responses and of the R-gene mediated responses.

[1] Guerra-Guimarães *et al*, J Proteomics, 2014, 104, 128-139

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## Poster Presentation

### Exploring natural variation of Mediterranean pine (*Pinus pinaster* Aiton) in a common garden experiment using metabolomics profiling

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Natural variation of the metabolome of *Pinus pinaster* was studied to improve our understanding of its role in adaptation process and phenotypic diversity in Conifers. The metabolomes of needles and the apical and basal section of buds were analyzed in ten provenances of *P. pinaster*, selected from France, Spain, and Morocco, grown in a common garden for five years. Metabolite extraction was performed through Valledor et al. (2014) protocol, and for detection two complementary mass spectrometry techniques: GC-MS and LC-FT-MS were used to reach a higher coverage of metabolome. Metabolome, and environmental and growth data were integrated employing statistical tools to provide a comprehensive picture of phenotypic diversity. By novel mass spectrometric technologies and bioinformatics implements was possible to identify 2471 metabolites in *P. pinaster*. The analysis of the metabolome showed that differences were maintained across provenances and that the metabolites characteristic of each developmental stage are related to primary metabolism, while provenances were distinguishable when developmental stages were analyzed independently. Integrative analyses of metabolome and environmental data showed two population clusters: Atlantic and Mediterranean provenances, in relation to aridity conditions of origin, being secondary metabolites, and in particular flavonoid and terpenoid compounds, essentials to reach this differential clustering. Additionally, some metabolites such as sorgolactone, tarennoside or taxifolin were linked with specific environmental conditions and growth capability. The high quality datasets generated point to genome specialization aimed at maximizing the drought stress resistance of *P. pinaster* depending on their origin.

Valledor L et al 2014. The Plant Journal, 79(1): 173-180.

## Poster Presentation

### **Integrative omics approach to uncover the regulatory basis of phenotypic variation and local climate adaptation in plants**

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Gene expression variation responding to the surrounding environment confers phenotypic diversity within a species that can lead to consecutive adaptation and genome evolution. However, the underlying differentiations of transcriptional regulatory networks among different ecotypes of a given plant species remains largely unexplored. We have undertaken a genome scale integrative approach to identify environmental stress related *cis*-regulatory modules and the regulatory interactions crucial for local climate adaptation in multiple ecotypes of the model plant *Arabidopsis thaliana* [1,2]. For that purpose, we have analyzed next generation sequencing data from Arabidopsis 1001 genome project [3], microarray data from ERA-PG MultiStress project [4] and transcription factor binding site data from benchmarked literatures. The organisational complexities of the regulatory interactions responsible for phenotypic plasticity among multiple ecotypes of *Arabidopsis thaliana* during eleven stress conditions have been explored. Differential expression of the stress regulated genes (>3600 genes), related pathways and processes were identified. The activity profiles of the stress-specific and multiple stress-regulated transcription factors (>300 TFs) were predicted. Our work has also shed lights on the underlying differentiations of the combinatorial transcriptional regulation through homotypic and heterotypic clustering of transcription factors binding sites in *Arabidopsis thaliana* ecotypes while responding to multiple environmental conditions. Additionally, since the approach is general in nature, it could be adapted to infer networks regulating stress responsive processes in any other plant species including crops.

1. Barah P et al 2013. BMC Genomics 14: 722.
2. Barah P et al 2013. Front Plant Sci 4: 532.
3. Gan X et al. 2011. Nature 477: 419-423.
4. Rasmussen S et al 2013. Plant Physiology 161: 1783-1794.

## Poster Presentation

### Development of a novel LC-MS/MS method for the target analysis of salt stress-responsive osmolytes in *Casuarina glauca* tissues

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Salt stress, i.e., high salt concentrations in the soil, damages the plant in various ways: (i) the uptake of water and nutrients from the environment is hampered by diminishing the difference in the water potential between the soil and root cells (i.e., osmotic stress); (ii) the steady accumulation of sodium ions in plant tissues inhibits essential cellular processes, including photosynthesis, potassium ion absorption, and protein biosynthesis. Some plants have developed strategies to cope with salt stress, including adjustment of the metabolic status, for example, with the accumulation of stress-responsive osmolytes. Common osmolytes include soluble sugars (e.g., glucose, sucrose, raffinose family oligosaccharides (RFOs)), polyols (e.g., mannitol, sorbitol), amino acids (e.g., proline), quaternary ammonium compounds (e.g., glycine betaine), and polyamines (e.g., putrescine, spermidine, and spermine). Soluble sugars are highly polar compounds, and show minimal retention on typical reversed phase stationary phases. Alternative LC-MS/MS methods have been reported using a porous graphitic carbon (PGC) stationary phase for the analysis of carbohydrate-related metabolites from *Lupinus albus* stems [1] and leaves of the resurrection plant *Haberlea rhodopensis* [2] under drought stress. This work reports the development and application of a novel PGC-LC-MS/MS method for the sensitive target analysis of salt stress-responsive osmolytes that accumulate in *C. glauca* plant tissues.

[1] Antonio C et al 2008. J. Chromatogr. A 1187: 111-118

[2] Gechev T et al 2013. Cell. Mol. Life Sci. 70: 689-709

## Poster Presentation

### Uncovering signaling pathways that account for crops variability

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Cell transduction is vital to the dynamics of plant cells. Plants have many signaling transducers in common to other organisms; however, differences associated with plant species specificity are seen, in particular when compared at the gene and amino acid sequence levels. For instance, 319 of the 13,338 genes of *Drosophila melanogaster* and 437 of the 18,266 genes of *Caenorhabditis elegans* encode for protein kinases. In *Arabidopsis thaliana*, 1,049 of its 25,706 genes (about 4% of the genome) encode for these enzymes, reflecting the importance of intracellular signaling networks as prime intelligence processing systems in plants, which lack nervous system. Our goal is to address plant biodiversity, with emphasis on stress tolerance, by assessing alterations in cell signaling transducers. For this, diverse plant extracts will be analyzed using the Kinex™ Antibody Microarray, which allows to quantify the expression levels of over 850 proteins and to assess proteins phosphorylation state. We are aware that the Kinex™ Antibody Microarray was not designed for the specific identification of plant proteins, but many proteins are highly conserved between plants and humans. This experiment will be complemented with mass spectrometry analysis of the extracts, in order to cover the maximum number of proteins. The altered proteins can then be tracked in future experiments by Western blotting. Overall, the results obtained will give insights into differences in protein expression, phosphorylation mechanisms and protein-protein interactions specific of a given condition. By integrating the results with already available data (in literature and databases) we will establish key signaling transducers to pursue and validate as stress biomarkers.

## Poster Presentation

### Histological and cellular characterization of downy mildew resistance in vegetable brassicas

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Downy mildew disease in vegetable brassicas (*Brassica oleracea* L.) caused by the obligate biotrophic *Hyaloperonospora brassicae* (formerly *H. parasitica*) is a limiting factor in European main production of temperate regions. Our group has identified and characterized various sources of resistance downy mildew with breeding value. Cotyledon inoculation of 'Couve de Corte' plants with different Hb isolates expressed a resistant interaction phenotype characterized by the formation of flecking necrosis on the upper surface with no pathogen sporulation. The objective of the present research was to characterize the cellular mechanisms behind this type of resistance by histological observation of susceptible and resistant interactions. We used methods of light and fluorescence microscopy to observe the development of *H. brassicae* after inoculation and the host cells responses. A typical hypersensitivity reaction was observed at the early stages of infection in cotyledon resistance host, which contrasted with the rapid colonization of tissues and reproduction of the pathogen observed in the susceptible host. Callose deposition occurred early after pathogen penetration in both resistant and susceptible interactions and depended on extension of colonized host tissues. An analogous resistance cellular response was also observed in other host-*H. brassicae* interactions suggesting the existence of a similar mechanism of resistance to downy mildew disease.

## Poster Presentation

### Combining a dual-omics approach and biochemical analysis of bulked recombinant maize inbred lines to identify candidate genes underlying plant growth responses to water deficit

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Maize is an essential dual-used food and energy crop. However, maize has a large ecological footprint because it is one of the major recipients of irrigation water. Improvement of maize for drought tolerance is therefore essential in the context of recurrent increased risks of water stress. Here, we used a strategy based on bulked segregant analysis to gain further insights into the underlying mechanisms and genes associated with a promising genomic region exhibiting two QTLs for growth responses to water deficit (Welcker et al., 2007). Recombinant inbred lines (RILs) that were used for QTL mapping were grouped according to their allelic values in the studied QTL region. Each group was then evaluated by transcriptomic and proteomic profiling of the leaf growing zone upon well-watered and water deficit conditions. Seventy transcripts and three proteins showed significant variation between bulked RILs. Using mapping analysis and quantitative RT-PCR experiments as additional criteria, we established a candidate gene list of nine differentially expressed genes colocalizing with the QTL region. Further quantification of specific transcript and metabolite amounts support the idea that the stress-responsive ZmMYB31 gene encoding a transcriptional repressor of the lignin biosynthetic pathway (Fornalé et al., 2010) might contribute to the growth response of the maize leaf upon water deficit.

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**Poster & Shot gun Presentation****Cultivar specific symbiotic teamwork in *Pisum sativum***R. Turetschek<sup>1</sup>, G. Desalegn<sup>2</sup>, HP. Kaul<sup>2</sup> and S. Wienkoop<sup>1</sup><sup>1</sup> University of Vienna, Department of Ecogenomics and Systems Biology, Austria<sup>2</sup> University of Natural Resources and Life Sciences, Department of Crop Sciences, Austria[stefanie.wienkoop@univie.ac.at](mailto:stefanie.wienkoop@univie.ac.at)

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

## Shot gun Presentation

### Transcriptomic data: procedure for statistical analysis

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Obtaining reliable transcriptomic data involves many steps starting from the raw intensities to the selection of interesting genes. First of all the raw data must be cleaned by removing features with bad flags. The background noise can also be removed. However this step is less and less used because it gives rise to an increase of the signal variability. The aim of the next step - normalisation - is to correct the technical bias like efficiency of the labelling. Many methods of normalisation exist but one of the most used for unpaired data is the RMA normalisation which uses the quantile method. Raw intensities are log<sub>2</sub> transformed and probe signal is transformed on expression value for each gene on each array. If a block effect is observed, it can be corrected by removing the median for each block. Once data is normalized many statistical tests can be proposed for analysing gene expression. The differential analysis, based on pair-wise comparisons, allows determining up- and down-regulated genes for a comparison. For this method there are different ways for variance modelling: one variance per gene, a common variance, or groups of equal variance. In the case of multiple hypothesis tests the risk of selecting false positives increases. This is why it is important to control the false positive error rate with one of two procedures: Family Wise Error Rate (FWER) and False Discovery Rate (FDR).

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