



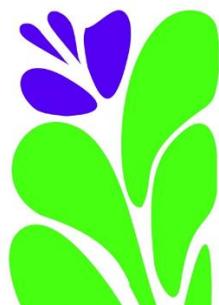
EU Cost Action FA1306 - The quest for tolerant varieties: phenotyping at plant and cellular level

Abstract book of the 3rd general meeting

Oeiras, Portugal, 27-28 March 2017

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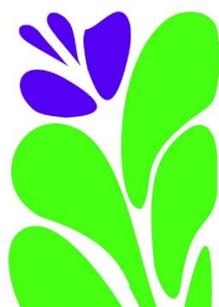
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Welcome

Welcome to the 3rd general meeting of the COST action FA1306 "The quest for tolerant varieties - Phenotyping at plant and cellular level" held at the AgroTech *campus* in Oeiras, Portugal, March 27-28th 2017. Hosted by ITQB-NOVA, the meeting has attracted over 140 participants from 27 countries.

This meeting will join experts in Phenotyping an emerging research field topic in modern plant and crop sciences that characterises plant structure and function and quantifies features such as growth and stress resilience in a way that allows linking to genetic control. Mapping genetic diversity and efficiently screen for more stress tolerance and resistant plants/crops is a major challenge for plant and agronomics sciences of the XXI century, with a large impact on food security, environmental protection and sustainability, and biodiversity preservation.

As part of this meeting, a technical visit to the *Vitis* portuguese collection, vineyard and cellars of Casal da Manteiga is included.

We sincerely hope you enjoy this event.

The Local Organisers



Scientific Programme

Monday, March 27th

8:00–9:00h Reception

9:00–9:30h Opening session

9:30–10:15h PL1 Elisa Liras (Bayer, Crop Science) - Phenotyping at Bayer HyperCare Farms

10:15-10:30h OC1 Gerrit Polder - Hyperspectral and 3D imaging for disease detection in seed potatoes

10:30-10:45h OC2 Astrid Junker - Assessment of plant performance traits in controlled environments and translation to the field

10:45-11:15h *Coffee break*

11:15-11:30h OC3 Stefanie Koemeda - Improving the phenotyping data variability

11:30-11:45h OC4 Irene Borra-Serrano - Evaluation of persistency of forage grasses in a breeding context using UAV imagery

11:45-12.00h OC5 Bernardo Duarte - Bio-optical phenotyping as tools for stress tolerance and resistance assessment in photosynthetic organisms

12:00-12:15h OC6 Onno Muller - Field phenotyping technology to measure photosynthesis via active fluorescence

12:15-12:30h OC7 Stefan Schwartz - PlantEye F500: combine 3D and multispectral information in one sensor

12:30-14:00h *Lunch*

- 14:00-14:45h PL2 Anna Kicherer (Institute for Grapevine Breeding Geilweilerhof) - Phenotyping challenges in grapevine breeding.
- 14:45-15:30h PL3 Lorenzo León (IFAPA Centro) - Olive phenotyping in breeding programs: achievements and prospects
- 15:30-15:45h OC8 Isabel Carrasquinho: Selection program in maritime pine (*Pinus pinaster* Ait.) genetic improvement for tolerance/resistance to pinewood nematode
- 15:45-16:00h *Coffee break*
- 16:00-16:15h OC9 Giuseppe Sciara: Root system architecture phenotyping reveals differential selection for a major QTL in durum wheat grown in contrasting water regimes
- 16:15-16:30h OC10 Photini Mylona: Phenotyping legume cultivars and genotypes using morphological and molecular markers
- 16:30-16:45h OC11 Marta Vasconcelos: Multispectral imaging combined with conventional analysis for early stage identification of Fe deficiency in soybean (*Glycine max* L.)
- 16:45-17:00h OC12 Diego Rubiales: Screening for ascochyta blight resistance in pea: complementation of field and growth chamber studies with histology, genomics and proteomics
- 17:00-17:15h Wrap-up of the sessions
- 17:15-18:15h General meeting of the COST Management Committee
- 17:30-19:30h *Poster session with wine & cheese*



Tuesday, March 28th

9:00-11:00h Group discussions

11:00-11:20h *Coffee break*

11:30-12:30h Conclusions

12:30-13:00h OC13 Dorri Te Boekhorst: Joint programming in phenotyping research: exploratory workshop results

13:00-14:30h *Lunch*

14:30-17:00h Technical visit with Eiras Dias (INIAV): *Vitis* portuguese collection, vineyard and Casal da Manteiga

Plenary lectures



PL1 Phenotyping at Bayer HyperCare Farms

Elisa Liras, Greta De Both, Stephanie Thepot, Randall Hess, Walid Elfeki, Raphael Dumain

Bayer, Crop Science division

The Crop Efficiency research programs of Bayer Crop Science are designed to enhance crop productivity by preserving and maximizing yield, with the primary focus on wheat, but also pursuing projects for soybean, corn and canola. These programs are using various technologies to modulate plant metabolism or improve plant nutrition. Through understanding yield formation in wheat, researchers work cross-functionally to deliver integrated solutions to farmers with yield increase and abiotic stress tolerance as prime targets. Several enabling innovative technologies are supporting the different R&D programs, and one of them is the HyperCare Farm concept, focusing on in-field precision phenotyping activities. The HyperCare Farms are field stations that have been upgraded in terms of precision phenotyping equipment. The main purpose is to measure, in various R&D trials, in a precise, automated and non-invasive manner, plant parameters (e.g. spectral reflectance, canopy temperature) as proxies for different phenotypical traits (e.g. biomass and water stress) and to evaluate the utility of new sensors under field operating conditions. Several HyperCare Farms have been established around the world. Examples of the high precision capabilities available in the HyperCare Farms are the PhenoTracker, a vehicle conceived as a mobile lab equipped with high resolution cameras, scanners and reflectance sensors and the PhenoTower, a camera system, measuring canopy temperature of the field plots. Via interpretation of all these precision phenotyped data, researchers can draw conclusions concerning the effects of the applied technologies on crop development and performance.

PL2 Phenotyping challenges in grapevine breeding

Anna Kicherer, Katja Herzog, Reinhard Töpfer

Julius Kühn-Institut, Federal Research Centre of Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof.

Grapevine breeding in general and phenotyping in particular is very time-consuming and labor-intensive. Phenotyping covers visual scoring of phenotypic traits e.g. ampelographic traits, yield, disease resistance and phenology (e.g. bud burst, flowering, veraison) as well as the use of physiological measurements of photosynthesis and water status or destructive biochemical measurements for quality assessment. However, in recent years there has been considerable progress in the development and application of non-destructive sensor based phenotyping technologies that are able to be applied at vineyards scale with the goal for high-throughput in breeding programs or for evaluation of genetic resources. Needs and requirements of phenotypic methods in grapevine breeding, the challenges we are facing will be discussed and the state-of-the-art applications that are available will be summarized. Two prototype approaches of field phenotyping platforms in grapevine breeding named *PHENObot* and *Phenoliner* will be presented. The *PHENObot* pipeline consists of several components: (1) data collection, using an automatically GPS tracked vehicle with a camera system, (2) data management established based on an image database, (3) data analysis with various automatically running programs and (4) the application for genetic analysis or selection of breeding material. The multi-sensor platform *Phenoliner* was developed from the experience gathered in the *PHENObot* approach. As a vehicle an emptied grape harvester is used as a movable tunnel to be more independent from varying field light conditions. Different sensors are installed on the platform: (1) a vertical and horizontal multi-camera-system for full grapevine height coverage (RGB and NIR), comparable to the one on the *PHENObot*, (2) a hyperspectral camera system and (3) a RTK-GPS and inertial sensor for image geotagging.



PL3 Olive phenotyping in breeding programs: achievements and prospects.

Lorenzo León and Raúl De la Rosa

IFAPA Centro "Alameda del Obispo", Avda Menendez Pidal s/n, 14004 Córdoba, Spain

An olive breeding program was initiated in the nineties in Córdoba (Spain) aiming at obtaining new olive cultivars adapted to modern olive growing. Two groups of objectives have been considered since the beginning of the breeding programs. From the agronomic point of view, the main objectives of the program are: earliness of bearing, high oil yield, oil quality, disease resistance and suitability to different growing systems and changing climatic environments. At the same time, several methodological improvements have been developed in order to shorten the juvenile period and to establish early and simplified criteria for selection. Precise phenotyping is of paramount importance for both the practical application of the breeding work (i.e. the final selection of new cultivars with improved characteristics) and to provide accurate data needed in studies for identification of molecular markers associated to traits of interest and development of marker assisted selection (MAS). Adequate techniques are needed for fast and reliable characterization of high number of samples for fruit characters, oil content and quality, plant architecture, flowering phenology and screening for disease resistance. Moreover, understanding phenotypic variation is also needed to decide optimum selection strategies in olive breeding programs regarding different possibilities of resources allocation in terms of number of replications per genotype and years of evaluation. Our breeding program is currently organized in three main steps of selection: 1) Progeny step: evaluation of single seedlings for early bearing, i.e. short juvenile period, fruit size, oil content and plant architecture for adaptation to different plantation systems, 2) Intermediate step: selected genotypes are evaluated at a second step including a limited number of replications per genotype, 3) Pre-commercial step: the most outstanding genotypes are tested in final multi environment comparative trials for a comprehensive agronomic and oil quality evaluation under different environmental conditions. Up to now, one new cultivar 'Sikitita' ('Chiquitita' in USA) was released in 2009 and is currently planted in commercial orchards in several countries. New advanced selections high yielding under different growing systems are currently under registration. The most outstanding results of the breeding program, particularly regarding phenotyping techniques, are summarized.

Oral communications



OC1 Hyperspectral and 3D imaging for disease detection in seed potatoes

Gerrit Polder, Pieter M. Blok, Dirk Otten, Pieter Kastelein, Jan M. van der Wolf

Wageningen University & Research, Plant Research, Wageningen, The Netherlands

Virus and bacterial diseases are one of the biggest problems in the cultivation of seed potatoes. Once found in the field, virus and bacteria diseased potatoes lead to rejections of the tubers resulting in a big financial loss. Much damage occurs when the disease is not detected in an early stage, which is often the case. Therefore there is a need for fast and adequate disease detection. Early detection of diseased plants with modern vision techniques can significantly reduce costs. Experiments in previous years showed that hyperspectral imaging clearly could distinguish healthy from virus infected potato plants. The classification accuracy was 84% for random selections of stem pixels and 92% for leaf pixels. Unfortunately for bacterial diseases the results are worse. Reason is that viral infections often develop into systemic infections as a means of transmission. The virus often infects many tissues, if not the whole plant. In contrast to virus, bacteria accumulates in the vascular bundles especially in lower parts of the stem, causing symptoms as black-rot in the stem, and general symptoms like yellowing, necrosis and wilting of the leaves on the affected stems. In 2016 a new experiment was set up, using 49 bacterial diseased potato plants and 20 control plants grown in the field. During the growing season, each week hyperspectral images were taken from two sides of the plants. This makes it possible to look at symptoms localized at the lower parts of the plants. In addition to the hyperspectral imaging a full 3D scan was made, as well as a top view RGB-depth scan. Preliminary results show that plants affected by bacterial diseases are distinguishable from healthy plants using a combination of the three data modalities.

OC2 Assessment of plant performance traits in controlled environments and translation to the field

Astrid Junker, Marc Heuermann, Rongli Shi, Henning Tschiersch, Matthias Lange, Daniel Arend, Jean-Michel Pape, Rhonda Meyer, Kathleen Weigelt-Fischer, Michael Grau, Andreas Börner, Uwe Scholz and Thomas Altmann

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Seeland OT Gatersleben, Germany

To meet the challenges in global food security requires the development of strategies towards the optimization of yield formation and resource efficiency of crop plants under future climate scenarios. Therefore a deeper understanding of processes underlying plant acclimation to changing environments is necessary. Using high throughput automated plant phenotyping systems at IPK, the dynamics of plant growth and photosynthetic efficiency have been studied in accession panels of *Arabidopsis* and maize under controlled conditions. This led to the identification of *Arabidopsis* and maize candidate accessions with different acclimation strategies to high light and variations in biomass yield and photosynthetic efficiency, respectively. To evaluate and enhance the prospects of lab-to field translation, candidate maize accessions have furthermore been phenotyped for root- and shoot traits under different cultivation conditions in the glasshouse and in the field. Modification of the standard cultivation procedures improved the lab-to-field translation of phenotypic trait expression which will be further optimized in the Plant Cultivation Hall currently being erected at IPK. This building will enable to run specifically designed and reproducible cultivation scenarios that mimic field conditions. In this way, trait expression similar to that in the field will be triggered and monitored using automated phenotyping installations. Investigations involving precise variation of environmental parameters will yield a deeper understanding of acclimation processes and underlying molecular mechanisms and genetic determinants and will support the prediction of idiotypes with improved performance under future climate scenarios. Furthermore recent achievements in phenotype data management, standardized metadata representation (MIAPPE), and data publication will be presented.



OC3 Improving phenotyping data variability: A comparison of spatial light homogeneity of different custom designed LED light panels in a chamber-integrated, high-throughput sensor-to-plant phenotyping system and its phenotypic effects on *A. thaliana*.

Stefanie Koemeda¹, Pedro Serrano-Drozdowskyj², Anneliese Auer¹ & Jakub Jez¹

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² *BioComp Facility, Vienna Biocenter Core Facilities GmbH, Dr. Bohr Gasse 3, 1030 Vienna*

Spatial and temporal homogeneity of light conditions are crucial in plant phenotyping, where controllable and reproducible environmental conditions are required. With light intensity decreasing inversely proportional to the distance of its source, light panels for plant research are of specific architectures. Spatial light intensity issues like edge effects and special light spectrum homogeneity are central aspects to this complex of problems. Energy efficiency, long lifetime and controllability of LEDs made this technology enter the horticulture and plant research programs, replace the traditional usage of fluorescent tubes. This study gives an overview over several custom designed LED light panels designed for an automated, chamber integrated, sensor-to-plant phenotyping system. We compared spatial light homogeneity, adjustability to the features of the surrounding space conditions and their effects on the phenotype of *Arabidopsis thaliana*. We found differences in growth rates and flowering time caused by the light conditions given by the individual panels. Our results show how light inhomogeneity of commonly used fluorescent tubes panels strongly influences the spatial phenotyping data variability and emphasizes the importance of its role amongst the other environmental parameters such as temperature, humidity and watering regime. The study highlights the challenges of LED light panel architecture for plant research and aims to stimulate discussions on light quality standards facilitating intra- and inter-laboratory reproducibility.

OC4 Evaluation of persistency of forage grasses in a breeding context using UAV imagery

Irene Borra-Serrano^{*:1}, Tom De Swaef¹, Jonas Aper¹, An Ghesquiere¹, Koen Mertens², David Nuyttens², Wouter Saeys³, Ben Somers⁴, Jürgen Vangeyte², Isabel Roldán-Ruiz^{1,5}, Peter Lootens¹

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Perennial ryegrass (*Lolium perenne* L.) is one of the dominant forage grasses in temperate regions of the world, used for grazing, hay-making or ensiling. A very important trait in the development of new perennial ryegrass varieties is persistency, defined as the capacity to survive and produce optimal yields over multiple seasons. The lack of persistency results in a rapid degradation of the sward with associated yield loss, emergence of (grass) weeds and decline of nutritive value. Breeders usually evaluate new selections 2-3 years after establishment using a visual scoring scale. This system is cheap and easy to implement but not completely free from human bias. The use of digital image analysis could avoid the bias related to the observer and provide objective quantitative data of persistency. Systems to evaluate persistency using imagery captured on-ground have already been developed, but the scale is limited to a single plot. To solve this bottleneck we propose the use of an Unmanned Aerial Vehicle (UAV) which allows to fly over the whole field and capture images of multiple plots simultaneously. UAV provides high spatial and temporal resolution, is quick, at low cost and immediately accessible. The general objective of this work was to develop a reliable methodology based on UAV imagery and derived Vegetation Indices (VIs) to estimate persistency of *L. perenne* accessions in a breeding context. Therefore, VIs data were compared to traditional breeder scoring and reference data recorded using on ground measurements (e.g. ground truth data). We have identified VIs that deliver consistent information under different environmental conditions and which display a strong correlation with ground truth data ($r = 0.89$). The degree of agreement between information of VIs generated using UAV-images and scoring for persistency has been assessed.



OC5 Bio-optical phenotyping as tools for stress tolerance and resistance assessment in photosynthetic organisms

Bernardo Duarte¹, Carla Gameiro¹, Maria Teresa Cabrita², Ana Rita Matos³, Andrei B. Utkin⁴, João Carlos Marques⁵, Isabel Caçador¹

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² Centro de Estudos Geográficos, Instituto de Geografia e Ordenamento do Território, University of Lisbon, Rua Branca Edmée Marques, 1600-276 Lisbon, Portugal.

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Adverse conditions to which photosynthetic organisms are exposed are more evidently expressed on the plant primary productivity, and can thus be assessed through light harvesting mechanisms. Pulse Modulated Amplitude (PAM) Fluorescence arises as a highly efficient non-invasive tool to address any failure or disturbance on the electronic energy transduction pathway. This technique evaluates the photonic energy capture processes and transformation to electronic energy throughout the assessment of the chlorophyll fluorescence signals. Therefore, any disturbance at the primary productivity level can be efficiently assessed by this technique. Early research showed that the laser induced fluorescence (LIF) spectra of photosynthetic organisms present two chlorophyll emission fluorescence peaks: a local maximum in the red region of the spectrum at 685 nm (F685), and an absolute maximum at the far-red region, around 740 nm (F740). The relative intensity, shape and wavelength of these peaks change with the physiological condition of the plant photosynthetic apparatus. Recently, LIF technology, showed that red/far-red emission fluorescence ratio (Fr/Ffr) is very well correlated with the maximum photochemical efficiency of PSII (Fv/Fm), estimated with continuous fluorometry. Compared to most PAM protocols, LIF has the advantage of not requiring pulses to be delivered at close range, allowing the remote analysis of a great number of individual plants in a short period of time. Additionally, these are low cost highly efficient remote sensing high-throughput phenotyping (HTP) techniques, with a non-invasive character allowing a high number of repeated measures in time or among experimental treatments. Considering this, in the present communication the potential of both PAM and LIF is discussed as effective tools to address the resistance or tolerance of different photosynthetic organisms to abiotic stresses, like thermal, hydric, osmotic and chemical stresses.

OC6 Field phenotyping technology to measure photosynthesis via active fluorescence

Onno Muller, Beat Keller, Lars Zimmermann, Christoph Jedmowski, Angelina Steier, Benedikt Janssen, Roland Pieruschka, Uli Schurr, Uwe Rascher

Forschungszentrum Jülich, IBG-2 Plant Sciences, Germany

Photosynthetic phenotyping in the field and greenhouse relies on fluorescence measurements for remote and precise estimates at plant or plot level in combination with appropriate positioning systems. For remote active measurement of fluorescence we selected the Light Induced Fluorescence Transient (LIFT) method. For positioning systems we used a rail based system moving in x,y and z direction above an experimental facility that consists of large (0.8m²) soil filled containers inside and outside the greenhouse. In the field, we use a manual operated field4cycle as first step towards the fully autonomously moving engine driven GPS steered FieldCop. We determined diurnal cycles of quantum efficiency of photosystem II (F_q'/F_m') and electron transport kinetics. The electron transport kinetics were validated (Keller et al.) and in specific we derived the time constant from Q_A to the secondary quinone (τ_1) and to the plastoquinone pool (τ_2). These novel measurement of τ_1 and τ_2 in vivo showed highly dynamic changes compared to F_q'/F_m' during the day. For barley and rapeseed a typical diurnal pattern of F_q'/F_m' inverse of the changes in PPFD was found. Whereas τ_1 and τ_2 , increased quickly in the morning and then gradually decreased depending on temperature during the day. Thus, the LIFT method provides novel highly dynamic electron transport kinetics in addition to quantum efficiency of photosystem II opening the possibility to assess novel functional parameters of photosynthetic electron transport non-invasively in greenhouse and field phenotyping.



OC7 PlantEye F500: combine 3D and multispectral information in one sensor

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PlantEye is a high-resolution 3D laser scanner that computes a robust and validated set of morphological plant parameters fully automatically. A core feature of PlantEye is that it can be operated in full sunlight without any restrictions - crucial for plant phenotyping under field conditions or if you follow a “sensor-to-plant-concept”. Phenospex has now developed a new sensor generation, which combines the actual features of PlantEye on the fly with a 7-channel multispectral camera in the range between 400 – 940nm. This unique hardware-based sensor fusion concept allows us to deliver spectral information for each data point of the plant in X, Y, Z-direction and we can compute parameters like NDVI, color index and many other vegetation indices. This new sensor generation opens a wide range of new possibilities in plant phenotyping and increases its efficiency.

OC8 Selection program in maritime pine (*Pinus pinaster* Ait.) genetic improvement for tolerance/resistance to Pinewood Nematode

Isabel Carrasquinho^{1*}, Elsa Gonçalves²

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Selection is the key part of all applied tree genetic improvement programs. It will determine how much genetic gain will be obtained in the first and succeeding generations. However, selection can only be successfully if there is enough variability in the target traits. Quantifying genetic variability for a particular trait and species should be achieved in three steps. The first is to obtain a random and representative sample of the variability within a species allowing to making inferences regarding the entire species. The second concerns to establish field trials with appropriate experimental designs containing representative genotypes. Finally, the last refers to use modelling to perform genetic data analysis. Pine wilt disease, caused by the pinewood nematode, *Bursaphelenchus xylophilus*, is a major concern to maritime pine forest in Portugal due to its economical, environmental and social impacts. It is also regarded as a major threat to European forests as this pathogen is listed as a quarantine pest. A mass selection program was initiated in 2009 in the south of Portugal. About 500 trees were selected as candidate to plus trees at Comporta, an area with one of the highest incidence of the disease. The criteria for the selection of these candidate trees were dominance, age, diameter at breast height and absence of external symptoms. Two years old half-sib progenies, obtained from 96 of those selected trees, were inoculated in greenhouse and genetic variability for resistance /tolerance to pinewood nematode was detected.



OC9 Root system architecture phenotyping reveals differential selection for a major QTL in durum wheat grown in contrasting water regimes

Giuseppe Sciara¹, Kerstin A. Nagel², Marco Maccaferri¹, Jonas Lentz², Tania Gioia², Fabio Fiorani², Silvio Salvi¹, Roberto Tuberosa¹

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This study reports the characterization of 183 elite durum wheat (*Triticum turgidum* ssp. *durum* Desf.) for root system architecture (RSA) and shoot developmental traits. Plants were grown in controlled conditions up to the 7th leaf appearance (late tillering) using the high-throughput phenotyping platform GROWSCREEN-Rhizo, a rhizo-box-based system consisting of one meter deep rhizo-boxes integrated with automatic root RGB imaging. The following RSA traits were measured: seminal root length, nodal root length, lateral root length, root system convex hull, root system width and depth distribution (twice per week). Measurements of leaf area, leaf number and tiller number were performed twice per week and SPAD measurements were collected twice during the experiment. Root dry biomass and shoot fresh and dry biomass were collected at the end of the experiment. The dynamic collection of the above-mentioned traits allowed us to model root and shoot growth and thus to disentangle the final point phenotypes in simpler and integrative phenes. A genome-wide association study (GWAS) based upon the Illumina Infinium 90K SNP assay identified many QTLs for RSA and/or shoot growth traits ($P < 0.0001$). GWAS confirmed a highly significant effect on adult plant root system width due to two major QTLs on chromosomes 6AL and 7AC previously identified on seminal roots also at the seedling stage (Maccaferri et al. 2016). Notably, haplotype frequency of the QTL cluster on chromosome 7AC was found to be significantly associated with root depth, root system width, root specific weight and shoot/root ratio revealed a strong, contrasting selection pattern between the rainfed and the irrigated breeding programs conducted at ICARDA and CIMMYT, respectively, suggesting an indirect but major role of RSA features in durum wheat breeding and environmental adaptation. The accessions used in this experiment were previously tested for yield and yield related traits in 15 different environments across the Mediterranean basin at different water and climatic regimes. The integration of field and platform data as well as the correlation analysis among traits provides valuable insights toward a better understanding and deployment of the RSA QTLome governing durum wheat yield in different environmental conditions.

OC10 Phenotyping legume cultivars and genotypes using morphological and molecular markers

Photini V. Mylona^{1*}, Michaela Sakellariou¹, George Tsanakas², Anthoula Gleridou² and Alexios N. Polidoros²

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Legumes are invaluable for sustainable agriculture and protection of the environment providing high protein food and feed, enhancing soil fertility and structure, and suspending soil erosion. Due to legume symbiotic nitrogen fixation capacity, these species can grow in infertile soils and areas that are restrictive to other crops and have been used in the past as major crops in Greece and Mediterranean countries agriculture. However, recent trends in modern agriculture have led to severe loss of cultivated legume germplasm, especially that of traditional local varieties, along with decrease in cultivation areas. Climate changes will further hinder crop yield, quality and sustainability and it is becoming increasingly important to breed productive, stress resilient legume cultivars that integrated in cropping systems will enhance agricultural sustainability and environment protection. The study is focused on phenotypic evaluation of selected legume species and genotypes to abiotic and biotic stressors under field conditions. A selection of legume species and genotypes including winter pea, chickpea, faba bean, lupin and lathyrus were studied in a randomized field plot design. Legume crops were assessed for a set of agricultural characters including yield, seed quality, fungal diseases, viruses and pests tolerance. The impact of rhizosphere associated microbes on legumes responses was also explored. Further a genetic analysis of legume germplasm centered on lentil landraces and cultivars was conducted aiming to explore the phenotypic diversity utilizing a set of morphological descriptors and current molecular markers. Statistical analysis of data revealed the genetic relationships among the samples, highlighting the importance of molecular markers in diversity conservation and breeding strategies.



OC11 Multispectral imaging combined with conventional analysis for early stage identification of Fe deficiency in soybean (*Glycine max* L.)

Marta W. Vasconcelos¹, David Araujo², Carla S. Santos¹, Susana M.P. Carvalho^{1,2}

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Iron (Fe) is an essential nutrient for several vital biological processes. However, under alkaline soil conditions, Fe is poorly bioavailable and leads to Fe deficiency, a serious condition that can hinder crop growth and productivity in about one third of the world's agricultural soils. An early detection is extremely important in order to introduce corrective measures and prevent production losses. Conventional detection methods are expensive, slow, destructive, and untimely. Here we evaluated the potential of multispectral technology and compared it to conventional phenotyping methods. Plants with differential susceptibility were grown under Fe sufficiency and deficiency and were phenotyped at different time points using multispectral imaging (covering the visible and near-infrared spectral regions), and calculating the corresponding spectral indexes. SPAD readings, organ weight, leaf area, photosynthetic pigment content, ferric reductase activity, and mineral content were also measured. Reflectance values per se were able to distinguish healthy and Fe deficient plants. For tolerant plants, two λ allowed discrimination, whereas for susceptible plants, reflectance values were discriminatory at nine λ . Reflectance values were then compared to the biochemical data, and total chlorophylls, as well as chlorophylls a and b displayed high correlations at 575 and 625 nm, indicating that multispectral imaging can be a useful tool for future field identification of Fe deficiency.

OC12 Screening for ascochyta blight resistance in pea: complementation of field and growth chamber studies with histology, genomics and proteomics

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Ascochyta blight of pea is a major disease of pea of worldwide distribution. It can be incited by various fungi, being *Mycosphaerella pinodes* the most prevalent. Only low levels of resistance are available in pea cultivars. Resistance is complex and highly affected by environmental factors and plant architecture and phenology, being the observed symptoms the consequence of a battery of resistance mechanisms acting at different phases of the infection process in combination with escape and avoidance traits. Field screenings are usually based on resistance indexes that consider only final symptoms. Screenings in seedlings are possible but do not always correlate well with adult plant responses in the field. Also, available information on levels of resistance and on the responsible mechanisms is often incomplete. Only after significant input to improve existing knowledge on biology of the causal agents and in phenotyping plant responses, will resistance breeding be efficiently accelerated. Phenotyping on *M. pinodes* resistance performed at Córdoba at the plant (field and growth chamber) and tissue level (histology, genomics and proteomics) will be presented and critically discussed.



OC13 Joint programming in phenotyping research: exploratory workshop results

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One of the driving mechanisms for strengthening the European Research Area are the European Framework Programmes. They have been very successful in focusing research and researchers across the European Union, its Associate Members and, to a lesser extent, Third Countries. Since it is estimated that roughly 80% of expenditure budgets on research and development remain at the national level, coordination mechanisms to align national research (programmes) through - in particular - public-to-public (P2P) actions have also been established. Coordination mechanisms include ERA-Nets, COST actions, Article 185 initiatives and Joint Programming Initiatives (JPI's). The JPI on Agriculture, Food Security and Climate Change (FACCE-JPI) brings together twenty-two countries committed to jointly tackle societal challenges at the crossroads of food security, climate change and agriculture. Its aim is to increase alignment of national research programmes in its remit and thus improve impact and negate unnecessary duplication of research. Adaptation to climate change is one of the central research themes in FACCE's Strategic Research Agenda. Within this theme, plant and animal breeding is regarded as a key challenge. In order to assess the current state of art, in the fall of 2016, an exploratory workshop was organized in Wageningen, focusing on phenotyping as example of the use of new techniques to identify and breed for resilient plant species in a changing climate and under extreme weather conditions. The workshop brought together scientists from research performing organisations and industry, representatives of a variety of networks and policy makers and funders. Recommendations derived from the workshop are primarily targeted at the national funders in FACCE and cover research gaps that can be addressed, as well as funding issues and knowledge exchange between different networks.

Poster communications



P1 Variation of globulin sequences as predictors of HMW glutenin genes in bread wheat (*Triticum aestivum* L.)

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Wheat has been one of the most important food grain sources for humans throughout history. In wheat, both globulin and HMW glutenin genes encode seed storage proteins, although their products belong to two different types of seed proteins with regard to their solubility. Glutenins require pH extremes or denaturing solvents for extraction and globulins are salt extractable. The HMW-GS are encoded by six genes located at *Glu-1* loci on the long arms of homologous chromosomes 1A, 1B and 1D. *Glo-2* genes are closely linked to the HMW glutenin genes at the *Glu-1* locus. In this study, detailed comparative sequence analyses of complete coding sequences of the *Glo-2* gene was obtained for each of the eight bread wheat cultivars, respectively: ‘Chara’, ‘Chinese Spring’, ‘Egret’, ‘Gabo’, ‘Glenlea’, ‘Halberd’, ‘Janz’ and ‘Suneca’. Polymerase Chain Reaction (PCR) was used to characterise by cloning and sequencing, globulin genes from the *Glo-2* locus of hexaploid wheat cultivars containing different Glu-D1 subunits (2+12 and 5+10). In order to confirm the specificity of the fragment amplified and the assignment to 1D chromosome Chinese Spring nullitrasomic lines were used and amplification product was present only in lines known to possess the 1D chromosome (‘Chinese Spring’ N1AT1B, N1AT1D, N1BT1A and N1BT1D). Comparison of globulin gene sequences obtained from the different wheat cultivars showed one single nucleotide polymorphism (SNP). HMWG 2+12 subunits carry an adenine at Glo2 position 20 whereas varieties with 5+10 subunits have a guanine residue. The D genome is particularly associated with high quality, and wheats possessing Dx5 + Dy10 genes are correlated with high quality, while the composition Dx2 + Dy12 is correlated with poor dough quality. The position and the similarity in sequences between the globulin and HMW glutenin genes indicates the presence of these two genes as part of the original duplication creating the paralogous x- and y-type HMW glutenin genes, and a paralogous *Glo-2* gene and a fragmented globulin adjacent to the Dx gene. These results may allow subsequent development of PCR-based markers to facilitate marker-assisted selection (MAS) of specific *Glo-2* alleles and HMW glutenins.

P2 Alterations in root proteome of salt-sensitive and tolerant barley lines under salt stress conditions

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Salinity is one of the most important abiotic stresses causing a significant reduction of crop plants yield. To gain a better understanding of salinity tolerance mechanisms in barley (*Hordeum vulgare*), we investigated the changes in root proteome of salt-sensitive (DH14) and tolerant (DH187) lines in response to salt-stress. The seeds of both barley lines were germinating in water or in 100 mM NaCl for 6 days. The root proteins were separated by two-dimensional gel electrophoresis. To identify proteins regulated in response to salt stress, MALDI-TOF/TOF mass spectrometry was applied. It was demonstrated that the sensitive and tolerant barley lines respond differently to salt stress. Some of the identified proteins are well-documented as markers of salinity resistance, but several proteins have not been detected in response to salt stress earlier, although they are known to be associated with other abiotic stresses. The most significant differences concerned the proteins that are involved in signal transduction (annexin, translationally-controlled tumor protein homolog, lipoxygenases), detoxification (osmotin, vacuolar ATP-ase), protein folding processes (protein disulfide isomerase) and cell wall metabolism (UDP-glucuronic acid decarboxylase, β -d-glucan exohydrolase, UDP-glucose pyrophosphorylase). The results suggest that the enhanced salinity tolerance of DH187 line results mainly from an increased activity of signal transduction mechanisms eventually leading to the accumulation of stress protective proteins and cell wall structure changes.



P3 Nondestructive detection and biochemical quantification of buckwheat leaves using visible (VIS) and near-infrared (NIR) hyperspectral reflectance imaging

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The current experimental study have been investigated possibilities of improvement methodology of nondestructive detection with hyperspectral reflectance imaging using visible (VIS) and near-infrared (NIR) based on correlation with wet chemistry quantitative analysis. Higher correlation between reflectance CRI-2 index from hyperspectral imaging analysis and total phenolics and anthocyanins contents has been observed compared to the CRI-1 index. The high concentration of total phenolics connected with high values of NDVI, NDVI 2, SAVI and LCI CRI-2 indexes from hyperspectral imaging analysis which characterize chlorophyll concentration and parameters of vegetation too. In experimental variants expressing high concentration of total phenolics have been determined lowest values of SIPI, MCA1 and NPCI indexes. ARI, mARI and CRI-2 indexes can show presence of vanillic, methoxycinnamic, cinnamic acids in the experimental plant material; or can be used for creation as new index based on the known formulas ARI, mARI and CRI-2 for vanillic, methoxycinnamic or cinnamic acids. Role of chlorogenic acid and changes of this phenolic acid content during the plant growth can be studied with NDVI, NDVI 2 and SI indexes which shown positive correlation with content of chlorogenic acid estimated by HPLC.

P4 Classification of various cold-pressed plant oils using a new semi-quantitative analytical approach

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The aim of this work has been the development of a new, semi-quantitative analytical approach in order to differentiate and classify the samples of various plant oils according to their corresponding botanical origin, by using GC/MS system and multivariate analysis. The analyzed cold-pressed oils of plant samples were obtained from the Oilseed Department of the Institute of Field and Vegetable Crops in Novi Sad, Serbia, and they included 23 oil samples of the following plant species: safflower (*Carthamus tinctorius*), flaxseed (*Linum usitatissimum*), pumpkin (*Cucurbita pepo*), sesame (*Sesamum indicum*), wild flax (*Camelina sativa*), okra (*Abelmoschus esculentus*), gopher purge (*Euphorbia lathyris*), summer savory (*Satureja hortensis*), and white mustard (*Sinapis alba*). The volume of 10 μ L of each cold-pressed plant oil sample was derivatized using TMSH solution (Trimethylsulfonium hydroxide, Merck) and analyzed on a GC/MS system under appropriate chromatographic conditions. After GC analysis, the eluting derivatized lipid components were identified using a mass spectrometer, by comparing their fragmentation spectra with mass spectra libraries. Chromatographic data of specific fatty acids were introduced into PAST program, in order to perform exploratory data analysis, i.e. hierarchical cluster analysis. The obtained dendrogram shows strong separations between investigated samples of cold-pressed plant oils using *Chord distance*, *Morista* and *Cosine* similarity measures with corresponding cophenetic correlation coefficients of 0.97, 0.95 and 0.94, respectively, thus suggesting that the presented tree represents the dissimilarities among observations very faithfully. Very high similarity was observed among the samples of flaxseed, wild flax and summer savory oil, as well as the samples of pumpkin and sesame seed oil, with the gopher purge oil being the most separated of all analyzed samples. It is worth mentioning that among the analyzed plant oil samples, gopher purge represents the only one being non-edible and toxic. Obtained results show that it is possible to classify plant oil samples of various oilseed species, according to the corresponding botanical origin, in a rapid way, on a GC/MS instrument with contemporary data analysis tools, successfully avoiding the application of analytical standards and time-consuming qualitative and quantitative determinations.



P5 Exploring the miRNAome in *Haberlea rhodopensis* – a model plant for drought tolerance.

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Haberlea rhodopensis is a paleolithic tertiary relict species best known as resurrection plant with remarkable tolerance to desiccation. *H. rhodopensis* exposed to severe drought stress shows ability to maintain the structural integrity of the photosynthetic apparatus which reactivates easily upon rehydration. Profiling of miRNAs by NGS has progressed rapidly and is a promising field for applications in RNAi. Here, we present the small RNA-seq analysis of 3 libraries derived from control (HRH), dehydrated (HRD) and rehydrated (HRR) plants. So far, the miRNAome of *Haberlea rhodopensis* remains unknown. We have identified 131 conserved miRNAs in the NGS datasets based on homology search with miRBase database. Most abundant miRNAs in the libraries were miR159a, miR396a-5p, miR159a, miR166a-3p and miR166e. The differential expression analysis showed a specific miRNA profiles upon dehydration and rehydration stage and revealed changes in expression level of several miRNAs, which are observed also in other plant species as key players in abiotic stress response. For example, miR396a-5p was found in tobacco to act as stress-responsive gene by increasing the tolerance to drought and cold, which we also confirmed to be upregulated in the process of dehydration in *Haberlea rhodopensis*. Upon rehydration process, most deregulated miRNAs were miR396a-5p, miR159a, miR168a and miR168b. Furthermore, we are going to experimentally validate selected miRNAs which can hold potential as drought tolerance biomarkers or can be even used as an artificial siRNA in plant molecular engineering.

P6 Use of infrared thermography for detection of drought stress in potato and tomato

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Leaf temperature can be a good indicator of leaf stomatal conductance and thus of plant physiological condition. Infrared (IR) thermal imaging (also known as IR thermography), is a specialized branch of IR thermometry. This imaging technique permits to measure IR radiation emitted by objects at ambient temperature over a large amount of points and processing those measurements to form a thermal map of the target surface, including leaf surface. Thermal imaging has been used not only as tool to monitor plant/crop physiological condition, but also to phenotype novel genotypes of relevant crops and to support breeding programs for drought resistance. The aim of presented study was to test the use of thermal imaging as a non-destructive and fast method to monitor drought stress and support irrigation management in potato and tomato crops. Another aim was to use the technique to characterize different genotypes with regards to their response to water stress. The preliminary results show that IR imaging can be used as a tool for both irrigation scheduling and to test the response of potato and tomato genotypes to drought. However, there are still limitations posed by crop architecture. To validate thermal imaging data the leaf gas exchange methodology has been used in presented experiments. Experiments were carried out at the Faculty of Agriculture as part of a collaborative research project between Serbia and Portugal.



P7 Deciphering the mechanisms of biochar induced plant performance and fusarium suppression 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 plant growth promotion and soilborne-disease suppression are not fully understood. This study tested the effects of biochars at concentration of 0-3% (w:w) on tomato plant growth and physiology, 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% and improved the plant growth and plant physiological status (Photosynthesis, Transpiration, Chlorophyll contents, etc). 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 taxonomic composition between biochar-amended and control soils. Nevertheless, biochar amendment caused a significant increase in microbial taxon and functional diversity (Shannon's diversity, 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 and physiology observed in the presence of biochar.

P8 Water quality tolerance of citrus rootstocks with contrasting stress sensitive is improved by root zone aeration via selective uptake and transport of ions

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The effects of root zone aeration in combination with water quality (WQ) treatments were studied in two rootstocks with contrasting stress tolerance to determine if aeration could reduce the negative effects of water quality stress. In plants exposed to saturated root zone aeration or normal conditions supplied with fresh water (FW), treated waste water (TWW), or fresh water with similar salt concentration as TWW (FW+NaCl). Measurements were conducted in Volkameriana and 639-Citron for ionic and water relations, photosynthetic features, growths and morphology and tissue specific membrane properties, activity and sugar and starch contents. 639-Citron was less affected while Volkameriana was highly sensitive to WQ under normal condition. Surprisingly aeration had minor influences under any WQ on 639-Citron while Volkameriana had significant positive effects. Aeration increased leaf photosynthesis and gas exchange, water and nutrient (K) uptake and increased biomass which plays compensative effects to WQ. We propose that there is a balance between photosynthesis, and water and nutrient use and increased biomass accumulation.



P10 Suspension cultures of *Pinus nigra* Arn.- cell system for the study of extracellular proteome

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Suspension cultures of several conifer species have been established to study physiological, biochemical as well as structural aspects of cells in liquid culture system. In recent years it has also been shown this culture system is convenient for study of proteins secreted by plant cells into extracellular space. Extracellular proteins play crucial role during plant development. The aim of our study was to establish suspension cultures from embryogenic tissues of *Pinus nigra* and following to analyse the cell secretome to identify extracellular proteins involved in somatic embryogenesis. Six cell lines different in embryogenic capacity have been selected. At day 8 of growth cycle 2.5g embryogenic tissue was resuspended in 25 ml of proliferation medium (DCR) and the suspension was cultured on rotary shaker (VWR) at 70 rpm. During the cultivation period the sedimented cell volume (SCV) of suspension was used as non-destructive quantitative measurement of growth. Media changes occurred in 10-days interval by pipetting 3 ml of SCV to 22 ml of liquid medium to reach final volume 25 ml. The maturation capacity of early somatic embryos was also monitored. The proliferation and survival of cells in liquid medium was cell line dependent. Cell lines (E420, E456, E477) reached SCV from 10 to 16 ml and were able to proliferate in this culture system for several weeks. Other cell lines (E451, E476) showed poor proliferation (SCV from 4 to 6 ml) and stopped proliferation after three weeks of cultivation. Somatic embryo maturation was also cell line dependent. Cotyledonary somatic embryos from suspension cultures with capacity to regenerate somatic seedlings have been obtained on semisolid medium.

P11 Phenotyping and genotyping *Phaseolus* sp. germplasm for cold tolerance

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Phaseolus beans are one of the most important food legume species in the world. Beans are grown in spring, autumn and in winter for their grains in the field and fresh pods in greenhouses. Cold stress (temperatures below 10 °C) is primary stress factor limiting the growth and production. Breeding cold tolerant beans may ensure safe and extended growth season. Previous screening tests indicated that variability existed within bean germplasm, which can be utilised for breeding cold tolerant cultivars for more reliable bean production. A core collection of 130 *Phaseolus* sp genotypes previously selected for their chilling tolerance (+5 °C) was tested under controlled environment conditions for frost tolerance (-3 °C) at seedling stage. Cold damage was assessed on the bases of visual damage scale, cell membrane leakage, chlorophyll measurement and leaf colour. The molecular characterization of core collection of 20 genotypes was determined by the Sequence Related Amplified Polymorphism (SRAP) method. Results were discussed for breeding cold tolerant bean cultivars that may provide safer crops in chilling conditions.

This project is supported by The Scientific And Technological Research Council of Turkey (TUBİTAK) (Project no. 114 O 806 as part of COST Action No. FA1306 The quest for tolerant varieties: Phenotyping at plant and cellular level)



P13 Application of a mass spectrometry-based metabolomics approach to study *Casuarina glauca* salt stress tolerance

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Casuarina glauca is a model actinorhizal plant characterized by its ability to establish symbiosis with nitrogen-fixing *Frankia* bacteria. This plant species grows naturally in coastal zones and is able to thrive under extreme salinity environments. *C. glauca* tolerance to high salinity is associated to biochemical and physiological adjustments such as low tissue dehydration, osmotic adjustments, and high membrane integrity. Mass spectrometry (MS)-based plant metabolomics has emerged as a powerful tool to address biological questions related to plant environment and agriculture. To date, there is almost no information on the the *C. glauca* metabolome. In this study, a modern metabolomics approach that combines two MS-based analytical platforms, namely LC-QIT-MSn target analysis and GC-TOF-MS metabolite profiling, is being applied to study the impact of salt stress in symbiotic and non-symbiotic *C. glauca* plants. Our most recent results, presented here, agree with those previously obtained from morpho-physiological analysis, and provide new knowledge on the primary metabolome of *C. glauca*, its symbiosis with *Frankia* Thr, and its metabolic readjustments under increasing salt concentrations. Furthermore, the divergent metabolite responses particularly found in the amino acid metabolism suggest root and root nodule specific metabolite responses, and support the fact that from 200 mM NaCl upwards, symbiosis was turned off. Based on these results, a second independent biological experiment is currently ongoing to assess, at the physiological and metabolite levels, the performance of non-symbiotic *C. glauca* plants under a combined salt and heat stress.

P14 The FLaxMixomics project: how to integrate multiple -omics data sets?

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As part of the "NoStressWall" project aiming to better understand how flax plants, with high economical interest (textiles, bio-composites, isolation), are able to cope with water deficit, transcriptomic's data sets (3704 genes), proteomic's (396 proteins), metabolomic's (40 metabolites) and morphological one (8 variables) were generated for leaves, stems and roots, during a 10 days kinetic (d1, d4, d10). In order to achieve a precise understanding of the adaptation mechanisms of flax to such stress, we considered these datasets together and not separately as is traditionally done so far. This type of 'integrative biology' approach makes possible to consider gene, protein and metabolic expression simultaneously in relation to morphological variables. To this end, we used 'mixOmics', a package of R (<http://mixomics.org>) developed in particular in collaboration with the Toulouse Mathematical Institute. By using the SPLS (Sparse Partial Least Squares) function, we were able to simultaneously integrate and select the relevant variables from 2 datasets and thus generate biological molecule networks - morphological parameters for each organ. Moreover, by multi-block analyzes (ie integration of more than 2 datasets) with the `block.spls` function (to include a supervised aspect in the analysis) of mixOmics, we aim to study the relationships between the different data sets and to identify in each block which subsets of 'active' variables are in strong relationships with the other blocks.



P15 Evaluation of drought tolerant mutant lines under saline environment based on some agronomic and biochemical parameters

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Salinity is a major abiotic stress, which greatly reduces the photosynthetic capacity of plant leaves and increases the production of reactive oxygen species, and as a result of all of this, the loss of product and yield in agricultural plants occurs. It is a problem in many regions worldwide. Therefore, the development of varieties with an increased salt tolerance is one of the strategies to meet the growing food demand. Mutation-assisted breeding has been proven to be an effective way of improving cultivars with a high resistance/tolerance to various abiotic and biotic stresses. In this study, the morphological and physiological responses of M₅ generation gamma irradiation- induced drought-tolerant Sagittario mutant lines under salinity have been studied and which line(s) are more suitable for the Advanced National Breeding Program in terms of releasing new bread wheat cultivar(s) tolerant to both drought and salinity has been determined. For this purpose, the previously detected threshold NaCl concentration has been applied to screen one control and five candidate drought tolerant lines against salinity under greenhouse conditions based on some of agronomic (Flag Leaf Area, Main Shoot Length, Spike Length, Weight of Grains per Spike, Number of Grains per Spike and 100 Grain Weight) and biochemical (Total Chlorophyll, Carotenoid, Proline, SOD, CAT, POX, Carbonylated Protein Content and K⁺/Na⁺) parameters.

P16 Finding the invisible at the sub-cellular level – Pathogen-specific answers of disease-susceptible plants

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Disease symptoms are phenotypic reactions of plants to pathogen attack. These reactions often become obvious only days after infection occurred, possibly causing fatal delays in the implementation of control measures. Gene transcriptional changes can provide information on the plant response to biotic and abiotic stresses well before the visible symptoms develop. Detection of disease-induced gene expression could thus enable early recognition of a beginning infection shortening the timely gap when the disease is present, but not detectable. This work aims at understanding the differing gene transcriptional changes in tomato leaves following pathogen attack. Pathogen- and mock-inoculated tomato leaf samples were subjected to Illumina transcriptome sequencing using 3-fold experimental replicates. Whole transcriptomes of the early (24 hours post inoculation) interaction of susceptible tomato (*Solanum lycopersicum*) leaves with its major pathogens *Phytophthora infestans*, *Oidium neolycopersici* and *Botrytis cinerea* led to the identification of disease-specific gene regulation patterns. Running a standard pairwise differential expression analysis and EBSeq – an empirical Bayes hierarchical model comparing all disease- and mock-inoculated samples – we identified 28 up-regulated and 22 down-regulated genes in tomato leaves inoculated with *B. cinerea* as well as 18 up-regulated genes in leaves inoculated with *P. infestans*. Leaves inoculated with *O. neolycopersici* appeared healthy both in terms of visible symptoms as well as at the gene expression level. The level of differential gene regulation and their putative function will be discussed.



P17 The role of symbiotic root nodule sulfate transport of *Lotus japonicus* for the protein biosynthesis of *Mesorhizobium loti* bacteroids

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Lotus japonicus establishes nitrogen-fixing root nodules in symbiosis with *Mesorhizobium loti*. Only the rhizobacteria, surrounded by a peribacteroid membrane (PBM) derived by the plants plasma membrane, express the nitrogenase and are able to reduce molecular nitrogen. The PBM, the structural and functional interface between plant and bacteroid, plays a central role in the metabolic exchange between the symbionts. Besides nitrogen and carbon, the sulfur metabolism plays an important role for the nitrogen fixation process. Several studies support this since the symbiotic sulfate transporter (SST1) is one of the most abundant PBM proteins and the nodules contain large amounts of reduced sulfur compounds. However, the role of sulfate during nodule protein-synthesis and–functional regulation remains unclear. We used ^{34}S -metabolic labelling and mass spectrometry to analyse the incorporation of reduced sulfur into nodule (plant and bacteroid) proteins. Samples were taken 24, 48 and 72 hours after pulse labelling. Sulfur turnover dynamics and the incorporation into key proteins for symbiotic nitrogen fixation are being presented and sulfur in the context of nodule function and protein biosynthesis discussed.

P18 From an *in vitro* to a greenhouse model: the role of glycolysis and fermentation during drought stress in a polyploid crop

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Banana (*Musa* spp.), a non-model crop, ranks among the top ten staple foods, with a total production over 145 million tons in 2013 (FAOstat). Bananas need vast amounts of water and even mild-drought conditions are responsible for considerable yield losses. In a previous *in vitro* experiment using 5% PEG for 3 days, we characterized the general reaction to osmotic stress via transcriptomics (Zorrilla *et al.*, 2016). Therefore, we performed large-scale mRNA sequencing on three triploid genotypes that represent important subgroups of cultivated bananas. First results from plants grown under autotrophic conditions in a growth chamber pointed to an increased metabolic flow through the glycolytic-fermentative pathway when oxygen becomes limiting in fast growing tissues. To validate this outcome in a physiologically more relevant context, we performed a greenhouse experiment with the same genotypes subjected to water deprivation (stop of irrigation) until the soil pF value was equal or higher than 2.8. By RT-qPCR we analyzed the expression pattern of thirteen key genes (and their paralogs) involved in glycolysis, fermentation and (an)aerobic respiration, and compared it to the expression patterns obtained in the lab model. Our results confirmed the shift from a more aerobic to a more anaerobic metabolism under drought stress in fast growing tissues. However, they also showed the complexity of dealing with polyploid genomes and gene families composed by paralogous copies, and the challenge of comparing results from lab and greenhouse models.



P19 Non-destructive analysis of biomass accumulation in grasslands using UAV

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Grasslands are important ecosystems in Europe and form the basis of dairy and meat production. Permanent grasslands have, via spontaneous adaptation, adapted to the local geographical and climatological circumstances in which they evolved. However, previous research has shown that present-day grassland populations are not capable of maintaining current levels of biomass yield under unusual weather circumstances, associated with climate change. GrassLandscape, a FACCE-JPI ERA-NET+ project¹, aims to deliver knowledge about the natural biodiversity of one of the main component species of temperate grasslands, *Lolium perenne* (perennial ryegrass), to support future grasslands in coping with climate change, including periods of drought and more extreme weather. This knowledge can then be used to increase the resilience of grasslands under changing natural climatological conditions. We are presently evaluating agronomic and ecophysiological aspects in more than 500 populations of perennial ryegrass, originating from all over Europe, North Africa and the East in a multilocation field trial. Each population has been sown in small plots (1.8 x 0.8m²) in two replications on three locations with contrasting environments (France, Germany and Belgium). At one of these locations, ILVO (Melle, Belgium), the plots are also being monitored using a UAV equipped with a visual camera several times over the growing season. Based on the images, vegetation indices and height maps are calculated to estimate parameters such as emergence, sod density and biomass accumulation. Correlations between the UAV-derived data and on-ground measurements are determined to check the usefulness of UAV-based phenotyping methods.

¹www.facejpi.com/Research-Themes-and-Achievements/Climate-Change-Adaptation/ERA-NET-Plus-on-Climate-Smart-Agriculture/GrassLandscape

P20 *Quercus suber* phellogen: what INFERNO reveals

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Cork is a highly valuable, and renewable, non-wood forest product, and plays an important role in the Portuguese economy. Not all trees produce cork of good quality (GQC), but only GQC is valuable for stoppers production. At the molecular level, we have discriminated between GQC and bad quality cork (BQC; Teixeira et al 2014; Machado et al 2015). However, at the proteomic and transcriptomic level, the differences between GQC and BQC are not abounding. Of the 355 detected proteins, none was found to be exclusive to a given type of cork quality. However, by using InfernoRDN v1.1.6044, former DanTE (Data Analysis Tool Extension, Popitiya *et al.* 2008), it was possible to relate 14 proteins with cork quality. On the other hand, only 60% of the phellogen proteins were identified. A possible reason lies in the lack of genome sequence information and insufficient functional annotation. Significant sequence matches were found against UniProtKB, and the annotation was derived from the collinear InterPro signature pre-computed hits. These findings points out to a fine regulation of the metabolic activity with a substantial impact on cork quality. Our next goal is to build a model for phellogen biochemistry at the cellular level and the impact on cork quality based on proteins. The main goal is to increase our knowledge on the phellogen production and how distinct types of cork are built.

Teixeira *et al.* 2014 Journal of Experimental Botany 65, 4887-905. doi10.1093/jxb/eru252

Machado *et al.* 2015. COST FA1306,

1st General Meeting (www.itqb.unl.pt/~pinheiro/Abstract_Book_17_07_2015.pdf)

Polpitiya *et al.* 2008. Bioinformatics, 1556-8. <https://doi.org/10.1093/bioinformatics/btn217>



P21 Physiological studies on different black poplar hybrids in the context of a site-clone trial for assessment of aboveground biomass as dependent from age, density and coppicing

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Some photosynthetically related parameters were studied in the course of a field trial aimed at assessment of productivity potential in different black poplar hybrids. The experiment was established in a randomized block design with four poplar clones (I-214, Luiza Avanzo, MC and I-37/61). In March 2014 cuttings were planted in twin rows 0.5 m apart and with 1.8 m between each set of twin rows at three different densities (0.5, 0.75 and 1.0 m). The net photosynthetic carbon assimilation rate differed insignificantly between the poplar clones, with the highest value being measured in clone I-214 ($15.5 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). The genotype (clone affiliation) significantly affected the rest photosynthetic characteristics followed. Clones I-214 and Luiza Avanzo demonstrated both higher stomatal conductance and transpiration rate, which considerably exceeded the respective values in clones I-37/61 and MC. The last two poplar clones showed higher water use efficiency (WUE), with values significantly surpassing those measured in clones I-214 and Luiza Avanzo.

P22 Application of prognostic equation overcomes barriers to field phenotyping and reveals novel phenotypic variation

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Selection in plant breeding acts on phenotypic variation. Thus, conditions that maximize phenotypic variation and its expression range are desirable and lead to the sought-after percentile increase in progress through selection. The negative effects of interplant competition and soil heterogeneity on phenotypic expression are effective barriers to the efficiency of field phenotyping for crop improvement. This is the case for both qualitative and quantitative characters and applies to a broad range of crops, as has been demonstrated in research trials with barley and cowpea, respectively, through the application of prognostic equation. In barley, a species well-known for its spike morphological variability, we were led to the recent discovery of a novel spike morphology phenotype, not previously described, that is shown to be consistently associated with increased yield after detailed field characterization. In cowpea, application of prognostic equation within a popular landrace was used for selection of higher pod biomass under various fertigation regimes, without any introduction of external genetic variation. This led to a previously undetected range of phenotypic variability, manifested as new and distinct classes to the phenotypic distributions, and resulted in increasing pod biomass more than 100%.



P23 Assessment of plant drought tolerance by combination of traditional physiological approaches with modern image-based phenotyping

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Lettuce is one of the most important species of leafy vegetables in the world. For comprehensive study of plant activity, the measurements of classical physiological characteristics are often time-consuming and frequently destructive. Thus, modern high-throughput phenotyping facilities can be successfully used for evaluation of growth dynamics, as well structural and functional states of vegetable plants. In this study, the adaptive responses to progressive drought in twelve leafy lettuce genotypes, differing in leaf color, have been studied in a pot experiment at controlled conditions of PLANTSCREEN platform. Plant structural responses were simultaneously complemented with measuring of biochemical, photochemical, hyperspectral reflectance and leaf water status parameters. Imaging analysis of optical signals from water-stressed plants revealed opening leaf rosette and progressing senescence of the oldest leaves. Genotypes with higher drought tolerance maintained the high level of chlorophyll content and have increased content of carotenoids, anthocyanins and flavonoids in leaves, as well higher level of photochemical efficiency. Combination of high-throughput phenotyping with traditional methods of drought tolerance mechanisms study provide a more complete information about plant behavior under drought.

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P24 Physiological and metabolic responses of citrus associated to tolerance to combined heat stress and drought conditions

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According to all predictions, climate change will increase the incidence of damaging environmental cues such as high temperatures and water scarcity with a severe negative impact on agriculture. The physiological and biochemical responses of citrus to heat stress and drought applied alone or simultaneously have been investigated to identify specific responses to the stress combination. Two citrus genotypes differing in stress tolerance were used in order to identify responses involved in tolerance. High temperatures increased stomatal opening and transpiration to refrigerate leaf surface thus protecting the photosynthetic apparatus whereas drought reduced these parameters in order to minimize water loss. When the two abiotic stress conditions were applied simultaneously, citrus exhibited an intermediate response balancing water loss through transpiration and evaporative cooling. This response appeared to be more efficient in the tolerant genotype than in the sensitive one, showing also a diminished impact to photosystems and reduced oxidative damage. Moreover, tolerance was correlated with biochemical changes: the sensitive genotype exhibited a complete metabolic reconfiguration comprising the activation of energy pathways (glycolysis and TCA cycle) and the induction of protective secondary metabolites (phenylpropanoids, flavonoids and triterpenoids). On the contrary, the tolerant genotype induced metabolic pathways involved in recycling of reduced co-factors and ATP produced in photosynthesis, preventing oxidative damage. Combining physiological phenotyping and metabolite profiling allowed the identification of physiological and biochemical signatures associated to tolerance to combined stress conditions in citrus.



P26 Interaction between hydrogen peroxide (H₂O₂) and gibberellin (GA₃) in elongation dynamics of the first internode of deep-sown wheat, *Triticum aestivum* cv. Tir

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Plants develop several mechanisms to cope with limited water 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 mesocotyl or first internode. There are only a few reports about the phenotypic plasticity of internode elongation in the literature. In one of these studies, it was suggested that internode elongation of deep-sowing tolerant wheat cultivars is more sensitive to gibberellin. Moreover, in the recent years, reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂) also have emerged as important regulators of plant growth and development. However, there is no study the role of hydrogen peroxide in the gibberellin induced-first internode elongation. With this aim, we tried to characterize the interaction between GA₃ and H₂O₂ in the internode elongation of deep sowing tolerant wheat, Tir. Therefore, wheat seeds were sown depths to 2 cm and 10 cm of soil. For GA₃ and Uniconazole treatment (GA₃ biosynthesis inhibitor), they dissolved in dimethyl sulfoxide was added to the 1 % agar medium at a concentration of 2.89×10⁻⁶ M and 2×10⁻⁷ M, respectively. To determine the effect of H₂O₂ on the first internode elongation, H₂O₂ treatment was achieved by soaking the seeds for 24 hours in 0.05 μM H₂O₂. At 10 days, the contents of H₂O₂ and GA₃ and the activities of antioxidant enzymes in the first internodes of Tir were determined. On the other hand, expression levels of the some of the semidwarfing genes and GAMyB transcription factor were determined. To our knowledge, this is the first study that concerns the relationship between GA₃, H₂O₂ and antioxidant defence system in the first internode elongation under deep sowing.

P29 The relationship between drought tolerance in maize in vegetative and reproductive stage

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Twenty maize inbred lines, 15 tolerant and five susceptible to drought stress, were chosen for the experiment. The genotypes were defined either as drought tolerant or drought susceptible according to their response to drought during early reproductive stage. The plants were grown in a greenhouse under optimal conditions until they reached V6 growth stage. From V6 stage they were subjected to the limited water and/or nitrogen supply and imaged for four weeks at the automated phenotyping and imaging platform at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). The experiment consisted of four treatments: 1) control - optimal watering (75% of field capacity) and optimal fertilizing; 2) nitrogen limited (N) - optimal watering, no nitrogen fertilizer added; 3) water limited (W) - 30% of field capacity, optimal fertilizing; and 4) nitrogen and water limited (N+W). Leaf number and chlorophyll content in the last fully developed leaf were manually measured two times a week. At the end of the experiment fresh (FW) and dry (DW) biomass weight, water use efficiency and relative water content were measured. Under optimal and N limited conditions susceptible lines had higher FW and DW on average, while under W and N+W limited conditions these parameters were higher for tolerant genotypes. Tolerant lines showed faster leaf development under W and N+W limited conditions than susceptible ones. Chlorophyll content under optimal and N limited conditions was similar for both groups of genotypes, but tolerant genotypes had somewhat lower chlorophyll content at the end of the experiment in W and N+W limited conditions. The preliminary results indicate positive relationship between tolerance to water limiting conditions in vegetative and reproductive stage.



P30 Fatty acid composition in phenotypic characterization of grapevine resistance against *Plasmopara viticola*

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Grapevine downy mildew, one of the most devastating diseases in viticulture, is caused by the biotrophic oomycete, *Plasmopara viticola*. Current strategies to prevent this disease rely on the intense use of phytochemicals which are not efficient and are very harmful for environment and human health. One of breeders' goals is the introgression of resistant traits, in order to create hybrid that are resistant to this pathogen while conserving fruit and wine properties. However, in grapevine breeding, the selection of plants that present the resistant trait is a long and laborious process. Our principal aim is to find biomarkers that allow distinguishing resistance from susceptibility in an early stage of grapevine development. Our previous studies have shown that membrane lipid composition has an important role in defense against *Plasmopara viticola* since linolenic acid levels increase in the resistant *Vitis vinifera* hybrid 'Regent' and are associated with Jasmonic acid synthesis. Our preliminary data demonstrate constitutive differences in the content of C18:1 and C18:3 between resistant (*Vitis riparia*, *Vitis vinifera* 'Regent') and susceptible varieties (*Vitis vinifera* 'Trincadeira' and 'Pinot noir' cultivars) that may be considered as biomarkers for resistance. Our next approach will consist in analyzing the fatty acid composition of a large number of *Vitis* species and cultivars, in order to find evidences that the fatty acid profile allows the discrimination between resistant and susceptible grapevine plants.

P31 Phenotypic characterization of *Chlamydomonas reinhardtii* mutants that perform a deficient CO₂ assimilation

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is an enzyme responsible for atmospheric CO₂ assimilation. In this study we are performing a phenotypic characterization of the I58W3 *Chlamydomonas reinhardtii* mutant that has three Trp residues close the entrance of the central channel of RuBisCO enzyme. It was previously found that this mutation caused a deficient CO₂ assimilation due to a decrease in the carboxylation rate of RuBisCO compared with the control strain. In mixotrophic conditions, at 25°C, I58W3 mutants grow slower and have reduced amounts of chlorophylls and proteins than the wild type control cells, as previously observed in another RuBisCO mutant (Y67A). Oxygen evolution measured with a Clark-type O₂ electrode demonstrated that the mutant have altered photosynthetic and respiratory rates. Photosynthetic electron transport rate was also measured as the capacity to reduce the 2,6-dichlorophenolindophenol (DCPIP), a synthetic substitute of NADP⁺. Staining living cells with Nile Red indicates that I58W3 cells accumulate more neutral lipids than the wild type. From the data gathered it appears that I58W3 cells have an altered photosynthetic performance, related to the closure of the solvent channel of RuBisCO. These mutants are however capable of accumulate increased amounts of storage lipids, which can be an advantage for biotechnological purposes.



P32 Quantification and identification of allele specific proteins in a polyploid non-model crop: proof of principle in 3 banana genotypes/phenotypes

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Polyploid crops are governed by various allelic copies of genes arising from their chromosome redundancies. Edible *Musa* cultivars are typically triploid hybrids: their morphology and genetic background is determined by the differential contribution of ancestor species carrying A genome alone or in combination with the B genome. In an *in vitro* screening with three contrasting genotypes grown under mild osmotic stress, the drought tolerant cultivar showed enhanced root growth. Our aim was to investigate a causal relationship between observed phenotypes and alleles. 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 cannot be retrieved. However, using an integration of transcriptomics and LC-MSMS proteomics, the allele specific product - revealed by unique tryptic specific peptides - is separated and quantified. 234,000 spectra were aligned and quantified, resulting in 6801 identified peptides. By alignment to cultivar specific sequence data 415 amino acid polymorphisms were picked up. Additionally using multi- and univariate statistics, we selected 87 peptides more abundant in the drought tolerant cultivar and 22 with increased abundance in the drought susceptible cultivar. Both methods rendered proteins correlated with enhanced root growth under mild osmotic stress. The function of these proteins, independent of selection method, include general osmotic stress responses. By transcriptome read alignment at the specific location of SNP we assessed the homeoallelic contribution indicating the genomic background of the allele. This information is crucial for subsequent analyses where genome contribution and drought stress tolerance must be validated in more cultivars. Only then these allelic isoforms can be used as proxy for drought tolerance in biodiversity mining and breeding applications.

P33 Phenotyping epigenetic inheritance in Arabidopsis

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It is an open question and a matter of debate whether environmentally induced epigenetic states can be transmitted to the offspring and if they have any adaptive value. Plants are great model organisms for experimentally addressing such questions, as their germ line is much less fixed than in most animals and much more exposed to environmental influences. We designed multigenerational phenotyping experiments to address the following questions: To what extent can exposure to environmental stress in one generation influence growth and development of the next generation? Do some of these effects have the potential to be heritable over several non-stressed generations? Initial data revealed an abundance of parental effects with surprising magnitude. To go beyond parental effects and to improve phenotyping resolution, we used a platform with a robot camera taking images of the same plants over a period of several weeks.

Two different *Arabidopsis* ecotypes had been subjected once, twice or three times to either short intense heat stress (37°C for 24 h) or prolonged mild heat stress (30°C for 10 days), followed by two, one or no non-stressed generation. We could not detect any significant heat-induced phenotypic changes in the 3rd non-stressed generation. This indicates that environmentally induced chromatin changes either do not occur in the stem cells of the shoot apical meristem or are reset in the germ line. However, negative findings leave always doubts. To refine the analysis to the molecular level, we are setting up systems that allows switching epigenetic states and monitoring their maintenance and studying the epigenetic configuration directly in the plant's germ line cells.



P34 Analysis of the lipid content and photosynthesis in *Glycine max* under drought stress

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United Nations projections claim that nearly half of the world's population will be living in arid, semiarid and dry sub-humid regions in 2030. Therefore, understanding how crops react to drought is of major relevance. Since soybean is an economically important crop, besides being a model legume its study is crucial to avoid productivity losses related to water deficit. Membrane lipids are major targets of drought and changes occurring during stress affect plant growth and productivity. In the present work we used the *Glycine max* cultivar Williams 82, which has the genome sequenced. Two types of drought stress were imposed: fast desiccation of detached leaves and slow dehydration of whole plants, by suppression of irrigation. Leaf fatty acid and glycerolipid composition were studied by gas- and thin layer chromatography. Pigments and lipid peroxidation products were quantified spectrophotometrically and chlorophyll fluorescence was measured by Pulse-amplitude modulation (PAM) and by Laser induced fluorescence (LIF). Both stresses result in changes in fatty acid and glycerolipid composition with a particular negative impact in linolenic acid (C18:3) and plastidial lipids. In contrast, lipid peroxidation products and neutral lipids were shown to accumulate in response to drought. Both methods used for photosynthetic analyses proved to be useful for stress evaluation in a non-destructive manner and discriminated between adaptation mechanisms under moderate stress from negative impacts of severe stress. Future experiments will include the analysis of expression of lipid metabolism genes possibly involved in the drought-induced changes observed.

P35 Profiling of the polar lipidome of *Salicornia ramosissima* and *Halimione portulacoides* to valorization of autochthonous halophyte plants

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Halophytes have the ability to cope with saline environments. These plants grow in a wide variety of saline habitats, from coastal intertidal sediments and supralitoral saline soils to lands of arid and semiarid regions, and agricultural fields. This represents a huge advantage considering that unprecedented rate of salinization of soils formerly used for agriculture is a world-wide issue, with salt stress being one of the most serious environmental factors limiting the productivity of conventional crop plants. In addition, with the decline of salt works in mainland Portugal, the sustainable production of autochthonous halophytes has been considered as a potential solution to reverse the ecodecline of these unique habitats. Halophyte plants have evolved several physiological traits that enable them to thrive under these stressful conditions. Halophytes lipid composition change in response to changes in the environment to improve resistance of plants against salt stress. Therefore, it is important to understand the deviation of lipidome and the best conditions, which halophytes growth more efficiently to produce good quality food for human consumption. In this work we studied the lipidomic profile at molecular level of two edible autochthonous halophytes: *Salicornia ramosissima* and *Halimione portulacoides*. Through the modern methodology of high resolution, HILIC-LC-Q-Exactive-MS and MS/MS it was possible to identify over two hundred molecular species in both plants, although *S. ramosissima* and *H. portulacoides* have a distinct lipidome profile, both plants have high omega-3 levels and the same major lipid classes: phosphatidylcholines, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidic acid, monogalactosyldiacylglycerol, digalactosyldiacylglycerol, sulfoquinovosyldiacylglycerol and hexosylceramide, which can contribute to their valorization as food and feed. The results on the lipid profiling of saltmarsh halophytes for human consumption are expected to contribute with science-based knowledge regarding halophytes biology and the environment. Results will contribute to the valorization of halophytes as a food resource, and open a door for potential future applications of halophytes bioactive compounds at national and international levels.



P36 Metabolic characterization of grapevine leaves: phenotyping for resistance

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Grapevine (*Vitis vinifera* L.) is the most important fruit crop in the world. Unfortunately, all the *V. vinifera* cultivars used for wine making are susceptible to fungal associated diseases, like downy and powdery mildews, which cause a massive damage and destruction of the plant, resulting in significant economic losses. The alternative strategy for disease control is either the application of powerful pesticides, with high environmental impacts, or the development of breeding programs between wild *Vitis* species (resistant to pathogens) and *V. vinifera* (susceptible) towards the development of new cultivars with good berry quality and a high degree of resistance. These programs require an understanding of the innate resistance of cultivars against these pathogens and the identification of resistance-related biomarkers. To understand the innate resistance/susceptibility of cultivars, the metabolic characterization of grapevine leaves is of utmost importance, since plants contain a unique metabolome that change upon pathogen infections. We developed a metabolite extraction method for grapevine leaves, performed a large scale metabolic characterization using Fourier Transform Ion Cyclotron Resonance MS (FTICR-MS) and identified several metabolic entities that enable a constitutively discrimination between resistant and susceptible plants. Further studies are being conducted in order to validate possible biomarkers.

P37 Consequences of global warming and metal pollution on the metabolism of omega 3 and 6 fatty acids of *Phaeodactylum tricorutum*

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Global warming and metal pollution impact marine ecosystems and interactions between the living organisms. Alterations in the fatty acid composition of primary producers will influence the energy and matter that reach higher trophic levels of the food chain. The diatom *Phaeodactylum tricorutum* has recently gained attention due to its ability to produce the long chain polyunsaturated fatty acid eicosapentaenoic acid (C20:5, EPA), an omega 3 fatty acid with many positive effects in human health. This study aims at finding alterations caused by a temperature increase and exposure to metal pollutants on the growth rate, fatty acid composition, more specifically EPA, photosynthetic parameters, pigment composition and gene expression of the diatom *Phaeodactylum tricorutum* using techniques like cell counting in Neubauer chamber, gas chromatography, spectrophotometry, pulse amplitude modulated chlorophyll fluorescence and qPCR. Results obtained indicate that both temperature increase and nickel stress, imposed separately and combined, result in significant decreases of the relative amounts of EPA. Changes in growth rates, pigment composition and photosynthetic parameters were also observed in metal-exposed cells. Further analysis will include the study of the effects of other metal pollutants present in marine systems as well as gene expression studies in order to get a better understanding on the effects of stress on the regulation of EPA production in *Phaeodactylum tricorutum*.



P38 Metabolic characterization of *V. vinifera* cv. Trincadeira in response to *Plasmopara viticola*

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Grapevine (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crops in the world, mainly due to the wine industry. In Portugal, one of the most important and widely used cultivar in wine making is *Vitis vinifera* cv. Trincadeira. However, it is susceptible to many fungal infections. Nowadays, one of the most threatening diseases that affect entire crops is the biotrophic oomycete *Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni affecting the leaves, shoots and fruits, reducing berry quality and yield, with significant production losses. Currently, the most widely used strategies for pathogen control rely on the preventive of powerful fungicides or the development of new hybrid grapevines that combine good berry quality and high degree of resistance. Since plants have a unique metabolome that change upon pathogen infection, in this study, we have characterized the metabolome of *V. vinifera* cv. Trincadeira leaves after inoculation with *Plasmopora viticola* by Fourier Transform Ion Cyclotron Resonance MS (FTICR-MS). This approach allowed the identification of several metabolic entities that not only may differentiate infected and non-infected grapevine leaves but also allow to discriminate different stages of infection.

P39 Phenotyping the disease progress of *Fusarium* head blight in summer wheat using hyperspectral imaging

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Fusarium head blight (FHB) is a destructive fungal disease of wheat and small grains, causing high yield losses and quality reduction. Mycotoxins, generated by these fungi, pose a serious threat to food safety and animal health. Resistant cultivars are effective tools to avoid damages caused by FHB. Achieving resistant cultivars requires precise and innovative methods to phenotype disease symptoms and host-pathogen interaction. Within this context proximal sensing techniques are promising methods to automate resistance phenotyping under controlled conditions. The hypothesis of this study is that the symptoms caused by FHB differ according to the host susceptibility, resulting in detectable differences in the optical properties of the host plant according to its resistance type. It is expected that specific regions of the electromagnetic spectrum are influenced depending on the susceptibility of the host genotype. Hyperspectral cameras (VINIR 400-1000 nm and SWIR 1000-2500 nm) were used for monitoring and phenotyping FHB symptoms on wheat cultivars with different degrees of susceptibility. The progress of FHB was monitored in a time series experiment (4, 6, 8, 10, 13, 17, 21, 30 days after spray inoculation (dai)) to phenotype type I resistance (resistance to the initial infection (penetration)). The spectral signature of infected spikelets of susceptible varieties changed considerably compared to resistant varieties and non-inoculated control in parallel to infection development. Spectral vegetation indices (SVIs) (i.e. Normalized differences vegetation index (NDVI), Photochemical reflection index (PRI), Pigment-specific simple ratio (PSSRa, PSSRb, PSSRc) and Water index (WI)) were calculated on hyperspectral images in order to investigate the potential of specific wavebands in detecting FHB. Early symptoms on glumes were associated with changes in values of vegetative indices as early as 3 dai. Hyperspectral imaging is a promising method to phenotype FHB symptoms on wheat under controlled conditions.



P40 Effect of meteorological factors on percentage of linalool in *Thymus pulegioides* growing in same field conditions

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Chemical polymorphism is characteristic of essential oil bearing medicinal and aromatic species *Thymus pulegioides* (Lamiaceae). Linalool, the main chemical compound of linalool chemotype, can reach up to 80% in *T. pulegioides* linalool chemotype and determine possibilities of use of this chemotype in cosmetic products, food and pharmaceutical industry. *T. pulegioides* is suitable for cultivation in Baltic States region, therefore, is actually as same meteorological factors can influence on composition of essential oils of linalool chemotype. The aim of this work was to establish the effect of some meteorological factors on percentage of linalool in essential oil of *T. pulegioides* linalool chemotype growing under the same field conditions. *T. pulegioides* linalool chemotype was vegetatively propagated and grown in open ground under same field conditions. Aerial parts were annually collected at full flowering stage and dried at room temperature. Essential oils were isolated by hydrodistillation and analysed by GC/MS. Meteorological data were obtained from meteorological bulletins of closest station of meteorology of Lithuanian Hydrometeorological Service under the Ministry of Environment. Results showed, that amount of essential oil varied from 0.55% to 1% across years and significantly correlated with sunshine duration ($r=0.83$, $p<0.05$). Amount of linalool was quite stable ($CV=9\%$). Significant connections between amount of linalool and meteorological conditions not established.

P41 Plant phenotyping at the Central European Institute of Technology (CEITEC)

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CEITEC is an independent University Institute at Masaryk University which currently comprises more than 200 scientists associated in 5 research centers, 10 core facilities and 35 research groups. Plant Sciences Core Facility of the CEITEC is a newly established facility which offers scientific services and access to the research infrastructure for phenotyping analyses. It serves as a focal point for broad-based cutting-edge biological research. The core facility operates 16 computer assisted plant growth chambers (phytotrons) and provides defined environment for controlled plant growing. Controlled parameters are: temperature (from +4°C to +40°C), humidity (from 30% to 80%), light intensity (from 0 to 250 $\mu\text{mol}(\text{photon})\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) with independent selection of photoperiods. For optimum plant growth light-emitting diodes (LEDs) as a sole light source are used. Thus provides excellent spectral quality with high irradiance for plant physiology applications. The following light wavelengths is possible to combine: UV_{365,385}, blue_{400,450}, green₅₂₅, amber₅₉₀, red₆₆₅ and far-red₇₃₀. A full range of "day/night" cycles with "dawn/dusk" and "cloudy sky" effects can be programmed. In some chambers different gas conditions (e.g. ethylene and CO₂) can be adjusted. This allows simulation of various environmental stress conditions. Support personnel provide cultural care, pest management, and maintenance of the entire core facility. Beside the environmental simulation the following services provided by the Core Facility are available: low-throughput screening for phenotyping of several crops, phenotyping of roots and seedlings on agar plates and phenotyping of seeds. Collaboration with the Photon Systems Instruments Company makes it possible to test and implement innovative technologies in plant cultivation, imaging and phenotyping. Thanks to the in house Cellular Imaging Core Facility at the CEITEC, documentation of plant phenotype may be supplemented by detailed analysis at cellular and subcellular levels. Collaboration with Vienna Biocenter Core Facilities, GmbH in Vienna (VBCF) supported by the program INTERREG V-A Austria-Czech Republic (2014-2020) under the project RIAT-CZ (2016-2019) allows to share best practices in managing core facilities, and to share knowledge and expertise leading to improvement of the provided services.

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P42 Jasmonic acid and ethylene-related genes retrieved from transcriptomic data show differential expression during coffee – *Colletotrichum kahawae* interactions

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Coffee Berry Disease, caused by the hemibiotrophic fungus *Colletotrichum kahawae* (Ck), is the major constraint for Arabica coffee production in Africa, with yield losses reaching 80% if no chemical control is used. Understanding the molecular genetic basis of coffee resistance to Ck is of high priority to support breeding strategies. A high throughput RNA sequencing approach (Illumina sequencing) was used to study the transcriptional profile during key steps of Ck's infection process (24, 48 and 72 hours after inoculation) in the variety Catimor (which exhibits field resistance to Ck in Kenya) and in the susceptible variety Caturra. A set of genes from the salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) pathways was selected and analysed by qPCR aiming to elucidate the phytohormones involvement in coffee-Ck interactions. Results suggest a more relevant role of JA and ET phytohormones than SA in this pathosystem. An earlier and stronger induction of the JA pathway was only observed in the resistant genotype suggesting that JA maybe responsible for the successful activation of defense responses. In the susceptible genotype, ET seems to be involved with the tissue senescence observed at the beginning of the necrotrophic fungal growth. As far as we know, this is the first attempt to unveil the role of phytohormones in coffee-Ck interactions, thus contributing to deepen our knowledge on the complex mechanisms of plant signaling and defense.

Acknowledgments:

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P43 Effect of G × E interaction on oil and protein content in wheat (*Triticum aestivum*, L.)

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Oil and protein content in wheat grain are quality indicators important for food processing, cosmetics and pharmaceutical industries. These and many other quality parameters could be significantly influenced by genotype, growing conditions and cultivation practices, especially fertilization. The objective of this study was to investigate effects of genotype, growing season, nitrogen (N) fertilization and their interactions on the oil and protein content in 24 varieties of winter wheat (*Triticum aestivum*, L.). Field trials with two N rates (low nitrogen N₄₅ and high nitrogen N₁₁₀) were conducted at the location of Rimski Šančevi, Serbia, during two growing seasons. The oil was extracted from wheat bran obtained by laboratory mill MLU 202. Classical Rushkovsky method was used to determine oil content, while protein content was determined by the ICC 105/2 method. Significant variability was found among the genotypes for both analysed traits. In two growing seasons and at different N rates, the oil content varied from 2.02% to 5.58%, with the average value of 3.96% and coefficient of variation (CV) 9.2%. The protein content ranged from 10.7% to 17.7% with the CV of 3.1%. All sources of variation (genotype - G, year - Y and N fertilization - F) and their interactions had significant effects on oil and protein content, except the Y × F interaction on the latter. Regarding the oil content, the effect of the year was stronger than the effect of nitrogen. In contrast, protein content was more affected by the N fertilization than by the year. Principal component analysis was used for grouping genotypes according to their stability and reaction to different growing environments. Cvs. Cipovka, Dragana and Simonida were identified as very stable with high oil content in different growing conditions, while cvs. Bankut 1205 and Banatka were identified as potential sources of high protein content. The identified genotypes can serve as parents in wheat breeding for higher oil and protein content.

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P44 Study of cms stability in NS rapeseed line

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Using stable cytoplasmic male sterility (cms) systems in rapeseed is essential in hybrid creation. This task is challenging since during conversion to sterile form some fertile individuals can be found in cms lines that can be determined by flower observation in the field. Improving the process of identifying atypical plant individuals is essential in both breeding and seed production. Obtaining accurate phenotypic evaluation is crucial in development of easy and quick laboratory tools for early detection of cms and (fertility restoration) Rf genes. Consequently, we examined rapeseed lines which are a part of Novi Sad breeding program and in which Ogu INRA cms was introduced. We analyzed anther development and microsporogenesis on cytological level and observed rudimentary developed anthers in 16 cms lines. Interruption in microsporogenesis occurred most often after the phase of tetrads. Additionally, we used cms-p molecular marker for evaluation of the presence/absence of cms gene in 12 experimental cms lines (15 individuals per line). By performing accurate field phenotyping and developing quick methods, such as cytogenetic and molecular markers for gene identification, obtaining rapeseed cms lines for hybrid creation can be significantly accelerated. However, for identification of the best suited molecular markers for marker assisted selection, reliable and time-efficient phenotyping is imperative.

P45 Field phenotyping of leaf traits in relation to hyperspectral data in wheat genebank collection

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In field phenotyping aimed at evaluation of genetic resources, the hyperspectral analysis represents an alternative technology to characterize the different properties of crop canopies. The wheat germplasm is characterized with a broad phenotypical diversity, including leaf traits. In this respect, the open question is the reliability of hyperspectral indices in the estimation of leaf properties when applied in a broad spectra of genotypes differing in plant and leaf morphology, anatomy and chemical composition of leaves. For this purpose, the hyperspectral field records as well as the subsequent leaf analyses were made in more than 100 wheat genotypes in the collection of National Genebank in Piestany (Slovakia). The traits of the fully developed flag leaves (chlorophyll and carotenoid content per leaf area and per dry mass unit, chlorophyll a to b ratio, chlorophyll to carotenoid ratio, leaf thickness (measured as specific leaf weight, SLW, leaf area, SPAD value, etc.) were correlated with 132 hyperspectral indices developed to estimate different properties of crop aboveground biomass. The selected genotypes provided relatively high diversity in all observed traits (thick vs. thin leaves, high vs. low chlorophyll concentration; very small vs. very large leaves), providing good background for correlation analyses. The results indicated that numerous parameters designed for estimation of specific leaf traits (e.g. chlorophyll content, canopy structure) expressed only poor correlation with measured data; anyway, we also identified the parameters with relatively good correlation across the entire collection of wheat genotypes, which can be regarded as more reliable and universal, useful for the use in phenotyping of wheat genetic resources. The study represents one of the initial steps of the program aimed at phenotyping of wheat germplasm, towards developing the methodological approaches to assess the genotypes, including traits related to adaptability, plasticity and tolerance to abiotic stress factors.

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P46 Photosynthetic parameters in phenotyping of wheat genotypes for tolerance to drought and high temperature

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The study was aimed at the evaluation of several photosynthetic methods and technical approaches in assessment of tolerance to drought and high temperature in wheat genotypes. The post-anthesis drought stress was induced by withholding of irrigation under a rainout shelter. The set of physiological parameters was monitored regularly, including the leaf water content and osmotic adjustment. Using the values of CO₂ assimilation and transpiration, the leaf transpiration efficiency was calculated; moreover, the stomatal conductance was measured by porometer. The numerous measurements of rapid chlorophyll a fluorescence kinetics in wheat leaves were recorded and the drought stress-associated changes at the chloroplast level were analysed. This technique was used also for assessment of photosynthetic thermostability. The drought tolerance of genotypes was classified according to harvested grain yield in non-stressed and stressed conditions. The correlations of physiological traits with yield-based drought tolerance of genotypes were assessed. Our results indicated that the drought-tolerant genotypes had a higher transpiration efficiency due to a lower stomatal conductance in non-stressed conditions, but the higher stomatal conductance associated with higher osmotic adjustment in drought. The analysis of chlorophyll fluorescence kinetics based on numerous measurements enabled to identify parameters, which well reflected the drought stress level and hence, they correlated well with genotypes' drought tolerance. In contrary, the commonly used fluorescence parameters were fully insensitive until the sub-lethal drought level. The simple methodical approach enabled to identify also the significant genotypic differences in heat resistance. The results confirmed the link between the leaf transpiration efficiency and yield in conditions of post-anthesis drought stress in wheat. Moreover, the rapid, non-invasive, easy-to-do measurements of chlorophyll fluorescence kinetics in wheat leaves were shown to be useful in assessment of drought stress severity and the drought stress tolerance.

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P47 Leaf parameters can accurately discriminate between *Quercus x hispanica* and their parental species *Q. cerris* and *Q. suber*

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Oaks are important forest tree in the Northern Hemisphere with high ecological and economic interest. Oak sympatric species can intercross leading to a significant amount of natural hybrids which have the remarkable capability of being fertile, being therefore very useful to study the heredity patterns of distinct phenotypes. Oak hybrids can display intermediate phenotypes for certain traits and parental specific ones for other characteristics. The differentiation between both parents' phenotypes can be very challenging, especially in an open pollinated hybrid population composed of several different yet undetermined generations. In this study we used a segregating artificial hybrid population of *Q. cerris* X *Q. suber* (*Q. x hispanica*) settled in Vimeiro, Portugal for more than 80 years composed of 13 “mother” hybrid, 22 half-siblings, descendants of “mother” hybrid #2, two *Q. cerris* and six *Q. suber* individuals to study discriminant leaf morphometric parameters. To find the best discriminants we have phenotyped more than 30 leaf parameters through image analysis in several adult leaves of each tree. The obtained metrics were used in analysis of variance and multivariate analysis. Parameters such as leaf area, perimeter, circularity, width, maximum length, number of indents, indent width mean and median, and indent depth mean discriminate between the “mother” hybrids and *Q. suber* and *Q. cerris*. Moreover, “mother” hybrid #2 was found significantly different from ten of its descendants. These results will be used to support further genetic association studies.



P49 The life-cycle of *Porphyra dioica*: a lipidomics approach

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Porphyra spp. (Rhodophyta) is one of the most economically important seaweeds for food application in the Atlantic. It has a unique trimorphic life history, comprising a macroscopic blade (gametophytes), a carposporophyte stage and a filamentous conchocelis phase (sporophyte). To our knowledge, the only commercial scale cultivation of the species is done in Portugal by ALGAplus, using a land-based integrated multitrophic aquaculture system. In this work, the lipid profile of the conchocelis phase (indoor nursery) and young blades (outdoor cultures) of *Porphyra dioica* was established for the first time, by using hydrophilic interaction liquid chromatography electrospray ionization-mass spectrometry. Glycolipids, phospholipids, phosphosphingolipids (inositephosphoceramides) and betaine lipids were observed for the two distinct phases of *Porphyra dioica*, but with dissimilar lipidomic signature. Overall 108 lipid molecular species were identified in the lipidome of leaf *Porphyra dioica* and 125 molecular species were identified in the lipidome of conchocelis phase. The blade was found to contain higher number of glycolipids species within galactolipids and sulfolipids and also lyso-sulfolipids, not observed for conchocelis. Predominant species of galactolipids and sulfolipids bear 16:0, 20:4 and 20:5 fatty acids. Regarding phospholipids, both stages included phosphatidylcholines (PC), phosphatidylglycerols (PG), phosphatidylinositols, phosphatidylethanolamines (PE) and lyso-PE, and phosphatic acid with predominant species combining 18:0, 18:1, 20:4, and 20:5 fatty acids. A dissimilar phospholipidome pattern was observed since conchocelis contains a higher number of PG and PE species, while lyso-PC and lyso-PG were observed exclusively for the blade stage. Moreover, six diacylglycerol neutral lipids were identified in the lipidome of conchocelis. This first approach on the lipidome profile of the gametophyte and sporophyte stages of *Porphyra* by using mass spectrometry-based approaches allowed discriminating distinct life-cycle stages of macroalgae and hypothesise about the potential preservation of polar lipids signature of chloroplastic membranes such as glycolipids during development stages.

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P50 Complex interplay of hormonal signals occurring during ripening of Trincadeira and Syrah grapes and during grape defense towards *Botrytis cinerea*

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Growth regulators play an important role in grape ripening and grape defense (Fortes et al. 2015 *Molecules*; Agudelo-Romero et al. 2015 *J. Exp. Bot.*). By using an innovative approach (Trapp et al. 2014 *Front. Plant Sci.*), we have evaluated the hormone of Trincadeira and Syrah grapes during regular ripening and upon infection with *Botrytis cinerea*. Quantifications using an Iontrap mass spectrometer revealed that ABA increased tremendously in both cultivars until the onset of ripening (*veraison*) supporting a major role of this hormone in the regulation of ripening of this non-climacteric fruit. The content in all the hormones analyzed decreased however, at harvest stage but hormone-related signaling processes as assessed by microarray and real time-PCR are very active at this stage. ABA also increased in infected green berries and *veraison* berries in particular in Trincadeira grapes. Regarding grape defense, a very interesting result was that Syrah green healthy berries present a higher basal content in SA, IAA, OPDA and JA-Ile but accumulate less than Trincadeira when infected. Gene expression analysis revealed that in Trincadeira indeed jasmonic acid synthesis is stimulated upon the infection but salicylic acid signaling is inhibited, confirming that jasmonates are involved in response to necrotrophic pathogens. Taking together the results revealed a complex interplay of hormonal signals occurring during grape ripening and defense and provided novel information regarding the factors that may be related to the higher susceptibility of Trincadeira grapes towards infection with *Botrytis cinerea*.



P51 The effects of salt stress on biomass, grain yield and photosynthetic parameters are synergistically enhanced by water limitation in wheat

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Drought and salinity are two widespread environmental abiotic stress factors in many regions. Breeding of crops for tolerance to both drought and salinity stresses is a significant target in agriculture and needs proper understanding of physiological characteristics and natural variations. We used a high-throughput shoot phenotyping platform to analyze salt and drought tolerance of 14 wheat genotypes of different geographical origin (5 from Serbia, 4 from Austria, and 5 from Azerbaijan). Salt stress (0.2 NaCl/kg soil) induced retardation of water uptake, especially under water limited conditions (20% field capacity). Green biomass and grain yield were not significantly affected by salt stress under well watered conditions (60% field capacity). However, when salt and drought stress was applied together green biomass and grain yield decreased to ca. 30 and 10-25 % of the well watered no salt control, respectively. Interestingly the ratio of the grain yield and dry biomass remained practically constant even under the combined effect of salt stress and water limitation, showing that carbon partitioning to grains is not affected specifically by the salt + water stress. Net photosynthesis rate and accumulation of the osmoprotectant proline responded also most significantly to the combination of salt stress and water limitation. These data indicate that the effect of salt stress is synergistically enhanced by water limitation.

P52 Sources of resistance to downy mildew disease in wild rocket crop

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Wild (*Diplotaxis tenuifolia*) and cultivated (*Eruca sativa*) rocket are used in Mediterranean cuisine in fresh cut salads, being part of packaged ready-to-eat products and garnish for food. Baby-leaves are associated with a healthy diet style, showing twice more beneficial compounds when compared with other leaf types, and ensure the prevention of several diseases. Downy mildew (DM) is a foliar disease caused by the oomycete *Hyaloperonospora* sp. pathogen, which represents an increasing threat to rocket production in different regions of the world. DM attacks the plants in all stages and is a great problem for Portuguese producers. The high susceptibility observed in wild rocket commercial types reduces crop production, which becomes often destroyed before harvest. The use of resistant / tolerant varieties will allow to increase crop yields with great benefits for producers. Besides, it also represents clear advantages for environment with reduction of pesticide treatments, providing consumers with a better quality food. The two main objectives of this study were (1) to develop a method for cotyledon and leaves DM evaluation in controlled conditions, and (2) to screen different genotypes to identify resistance responses. Fourteen-day plants from ten genotypes were inoculated by pulverization with a fresh field *H. parasitica* inoculum. Twelve days later, the cotyledon and the first two leaves were visually evaluated in terms of DM resistance in a total of 90 plants of each accession, on three separate replications, and using six interaction-phenotype classes. Four accessions were very susceptible and have no interest as resistant sources, four were moderately resistant, and two showed good DM resistance with clear advantages to be used.



P53 Brillouin light scattering (BLS) spectroscopy and correlative fluorescence brillouin imaging (FBi) based non-invasive mechanical phenotyping of *Arabidopsis* mutants

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We present an all optical non-invasive mechanical phenotyping approach for *Arabidopsis* mutants based on their characteristic high-frequency mechanical storage moduli as determined using Brillouin Light Scattering (BLS) Spectroscopy. We also introduce fluorescence emission–Brillouin scattering imaging (FBi): which allows for the all-optical parallel measurement of the mechanical properties and the fluorescence intensity with sub-micron resolution within live cells. Using FBi and BLS we find: 1. A region of enhanced stiffness of the cytoplasm extends over several microns in the vicinity of the extracellular matrix (ECM) for onion and *Arabidopsis* epidermal cells. 2. A change in the cellular hydrostatic (turgor) pressure can significantly modulate the mechanical properties of the plant ECMs. 3. The “stiffness” of plant ECMs as measured by BLS is symmetrically patterned in *Arabidopsis* hypocotyl cells undergoing directional growth. In particular, the measured high frequency longitudinal storage modulus measured of “long” cell walls are significantly larger than those of adjacent “short” ones. This is in contrast to AFM measurements of the tensile modulus which has recently been shown exhibits the opposite trend. These opposing trends are explained by considering the different inherent constraints of the measured moduli, and can qualitatively explain the observed directional (elongated) growth of hypocotyl cells. Consistent with this no asymmetry in the ECM’s mechanical properties is observed in symmetric root cells. 4. *Arabidopsis thaliana* with photoreceptor mutants have a significantly reduced ECM stiffness, suggesting red/far-red light signals are essential modulators of ECM elasticity. Our results suggest BLS and FBi offer not only a powerful tool for investigating mechanosignaling pathways in *Arabidopsis*, but can provide a new paradigm for non-invasive all-optical mechanical and correlative mechanical- chemical phenotyping of plants.

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P54 Thermal patterns in the vineyard to support management

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Irrigated viticulture expanded fast in Southern European countries such as Portugal in order to optimize berry yield and quality and increase vine's longevity. However, irrigation practice in South Portugal increases pressure over the available water resources. In addition, row crops such as grapevine, are more vulnerable to heat stress due to the effect of soil heat fluxes, which can be harmful to canopy leaves and berry thermal condition. Therefore, a better understanding of grapevine responses (diurnal and seasonal) to environmental factors (air temperature, soil water) and agronomic practices (deficit irrigation, soil management) are on demand by the wine industry. Ground based thermal imaging has been used to monitor canopy and soil temperature patterns along the day/season as a method to assess vine water status and predict risks of heat stress damage. In the frame of the EU-INNOVINE project, field trials were carried in 2014 and 2015 in Alentejo (South Portugal). Diurnal and seasonal responses of two *Vitis vinifera* varieties, Aragonez (syn. Tempranillo) and Touriga Nacional, were evaluated. Vines were subjected to sustained deficit irrigation (SDI 30-40% Etc), and regulated deficit irrigation (RDI, ~ 50% of the SDI). Diurnal canopy temperature (T_C) and soil surface temperature (T_{soil}) were assessed with a thermal camera. Punctual measurements of leaf temperature (T_{leaf}) with thermocouples, leaf water potential and leaf gas exchange were also done. T_C values were above the optimal temperature for leaf photosynthesis during the daylight period (11-14h to 17h) in the most stressful days (high VPD, high T_{air}) and especially under RDI conditions. T_{soil} was on average 10 to 15°C higher than T_C . In both years of observation we found a strong correlation between T_C derived from thermal imaging and the leaf water potential and stomatal conductance to water vapour. Moreover, the good correlation between T_C and T_{leaf} derived from thermocouples validates the thermal imaging approach. Our results suggest that T_C can be explored as a simple but robust thermal indicator of crop performance, and eventually, as a parameter to feed future growth models for the grapevine crop.



P55 Metabolomics: A robust bioanalytical tool for the dissection of plant metabolism and prediction of complex traits

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Being closer to the phenome compared to other “omics”, metabolomics can be applied for the study of the metabolism of organisms and the discovery of the missing links between genotypes and phenotypes. Focusing on plants, it can be employed for the dissection of their responses to biotic and/or abiotic stimuli and the corresponding metabolism regulation. The in-depth understanding of the undergoing biochemical changes in such interactions could lead to the discovery of reliable plant metabolites-biomarkers. The latter could be further exploited in biomarker-assisted selection programs of cultivars and species based on desired traits or genetic engineering of highlighted biosynthetic pathways. Here, the “proof-of-concept” of the use of metabolomics for the discovery of validated biomarkers of plants’ resistance to biotic and abiotic stimuli is presented in detail for certain species. The content of plants in various metabolites and the up- or down-regulation of key-pathways could be used as reliable indicators of their physiological state and the *in silico* projection of traits. Among others, phytoalexins, signaling molecules, and other metabolites with biological roles, as well as biosynthetic pathways such as, the phenylpropanoid, isoflavonoid, and alkaloid could serve as such. Upon the development of validated metabolomics models, the approach can be adapted in the high-throughput study of traits of concern, contributing towards the reduction of the required time and costs.

P56 The quest for climate smart varieties: phenotyping the banana biodiversity

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Bananas (*Musa* spp.) are giant perennial herbs that are produced in more than 120 tropical and subtropical countries. They are the largest fruit crop in the world with an annual production of 141 million tonnes [for comparison, global apple production was 81 million tonnes in 2013] (2013, FAO). Only 15 % of global banana production, mostly “Cavendish bananas”, is exported; the vast majority of bananas produced (85%, including Cavendish) are locally traded and/or consumed. The Bioversity International *Musa* Germplasm Transit Centre (ITC), hosted by the Catholic University of Leuven (KULeuven), houses a unique collection of banana diversity. It now holds over 1500 banana accessions, as a global heritage for the benefit of current and future generations. The proposed project aims to expand ITC’s services and boost the use of its conserved germplasm by establishing an automated *Musa* phenotyping and nursery facility to increase the ITC’s capacity to screen the conserved material for beneficial traits and to multiply on-site healthy plant material in African fields. The project has teamed up with the Belgian private sector to develop and install a climate-controlled container (Farm Flex) that will provide a more dynamic setup for ITC research, which can be easily and rapidly rearranged to fit the specific needs of an experiment. The initial focus will be on screening the banana diversity for drought tolerance. The phenotyping facility will allow the work to be automated and therefore performed faster and with higher levels of accuracy/high throughput. The Farm Flex will also be adapted to be used as an in-field nursery in Africa to rapidly and cheaply supply healthy plant material.



P57 The small RNAome from *Pinus pinaster* Ait under drought stress

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The profiling of small non-coding RNAs (sncRNAs) in plants under specific conditions such as drought stress, may provide important information regarding the molecular responses of genotypes with contrasted behaviours, which can be useful for selection/breeding purposes. These small RNA molecules are widely recognized as important regulatory elements, shown to play essential roles in several biological processes. In this study we aimed at the identification of small non-coding RNAs and their target genes involved in maritime pine drought response in root, stems and leaves (needles). Genotypes with contrasted response to drought were used to identify differentially expressed sncRNAs potentially involved in different behavior during water stress response. Twelve small RNA libraries were prepared from root, stem and needle tissues, and sequenced using Illumina HiSeq2500. Sequencing data were analysed using miRPursuit, an open source workflow for the analysis of plants small RNAs (<https://github.com/forestbiotech-lab/miRPursuit>), and a total of over 11,000 non-redundant sequences were identified as potential *Pinus pinaster* small RNAs. A group of high confidence sncRNAs was identified showing differential expression, including conserved miRNA from over 30 different miRNA families, novel miRNA and tasi-RNAs. Potential sncRNA target genes were also identified showing GO term annotations related to “Response to stress” and “Response to abiotic stimulus”. In conclusion, we provide a first characterization of the small non-coding transcriptome of *P. pinaster* under drought stress conditions representing an important step to uncover specific regulatory networks controlling drought adaptation in pine.

P58 Stress tolerance in pre-conditioned plant populations through Pulse Amplitude Modulated (PAM)

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Different plant populations of the same species can be exposed to different environmental stresses which can precondition plant tolerance and resistance responses to other stresses. Thus, it is important to identify and assess functional traits in which environmental variability has a significant role in how the species respond to global and local change. The effects of abiotic and biotic stresses in plants are generally revealed by chlorophyll fluorescence analysis, a non-invasive measurement of photosystem II (PSII) activity. Pulse-amplitude modulated (PAM) fluorometry is one of the most common techniques used to study the induction and quenching of chlorophyll fluorescence in physiological studies. Using PAM to measure heat-stress induced changes in two halophytes populations with different pre-conditioning histories (heavy metal contaminated versus non-contaminated environment) showed significant physiological variances in the PSII photochemistry regarding response and resistance to the same stress which shows intraspecific variation probably due to environmental variation. The finds were supported by biochemical analysis of the leaf fatty acid composition presenting a significant variance in linoleic acid and linolenic acid concentration and between the ratio of unsaturated to saturated fatty acids which expresses the same variability between populations. In the near future, it will be important to increase long-term studies on natural populations in order to understand plant response to environmental factor including climate change.



P59 Metal nanoparticles toxicity in marine photosynthetic organisms assessed through biophysical phenotyping: a first approach for stress evaluation

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The effects of dissolved heavy metals pollution on marine photosynthetic key planktonic organisms has been widely reported in various studies. Nevertheless, the rising concerns about the increasing presence of metal nanoparticles in the environment raises new questions regarding their toxic effects. Photobiology is highly sensitive to metal pollution, showing different responses in dissolved or in nanoparticle form. The application of PAM fluorescence lets to analyse photosynthetic processes, under different culture conditions, highlighting alterations in various parameters along the energy transduction pathway with a high-throughput non-invasive way. PAM phenotyping performed in metal-treated *P. tricornutum* cells points out to a differential effect of Cu and Zn in their dissolved or nanoparticle forms in the photosynthetic energy transduction pathways. Differences in photosynthetic parameters were more evident in organisms exposed to nano-Cu, if compared to Zn nanoparticles treatment. Initial analysis on pigments data on Cu clearly showed a higher CuChl substitution grade in cultures treated with copper nanoparticles compared to its dissolved form. Analysis of fatty acid composition showed a gradual reduction of the major membrane polyunsaturated fatty acid EPA with increasing concentration of nano Cu. Nevertheless, deeper analysis still needs to be done to support chronic exposure and to obtain an overall and more general sign of stress caused by these new contaminants.

P60 The relationship between dehydrin accumulation and winter survival of winter wheat and barley

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Low temperatures induce an active plant acclimation response which is associated with enhanced accumulation of several stress-inducible proteins including dehydrins. Dehydrins present a distinct biochemical group of late embryogenesis abundant (LEA) proteins characterised by the presence of a lysine-rich amino acid motif, the K-segment. They are highly hydrophilic, soluble upon boiling, and rich in glycine and polar amino acids. In controlled conditions, a significant correlation between dehydrin protein or transcript relative accumulation and plant acquired frost tolerance (determined as LT50) was found. Moreover, a correlation between wheat WCS120 and barley DHN5 dehydrin proteins and wheat WDHN13 transcript accumulated in plants grown under field in autumn and their winter survival were found. Dehydrin proteins and transcripts can be thus used as reliable markers of plant frost tolerance not only in controlled growth conditions, but also in field conditions.

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P61 Phenotypic variance of grape berry according to cluster position and irrigation management

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The impact of water deficit on berry quality has been extensively investigated during the last decades. Nonetheless, there is a scarcity of knowledge on the performance of varieties exposed to a combination of high temperatures/water stress during the growing season and under vineyard conditions. The objective of this research was to investigate the phenotypic variance of grape berry subjected to two irrigation regimes, sustained deficit irrigation (SDI, 30% ET_c) and regulated deficit irrigation (RDI, 15% ET_c) and of two cluster positions (east- and west-exposed sides) on berry ripening in red Aragonez (Tempranillo) grapevines. The study was undertaken for two successive years monitoring different parameters: pre-dawn leaf water potential, berry temperature, sugars, polyphenols, abscisic acid (ABA) and related metabolites. The expression patterns for different transcripts encoding for enzymes responsible for anthocyanin and ABA biosynthesis were also analyzed. In both years anthocyanin concentration was lower in RDI at the west side (RDIW- the hottest one) from *véraison* onwards, suggesting that the most severe water stress conditions exacerbated the negative impact of high temperature on anthocyanin. The down-regulation of *VviUFGT* expression revealed a repression of the anthocyanin synthesis in berries of RDIW, at early stages of berry ripening. At full-maturation, anthocyanin degradation products were highest at RDIW. This suggests that the negative impact of water stress and high temperature on anthocyanins results from the repression of biosynthesis at the onset of ripening and from degradation at later stages. Irrigation regime and berry position had small effect on free-ABA concentration. However, ABA catabolism/conjugation process and ABA biosynthetic pathway were affected by water and heat stresses. This indicates the role of ABA-GE and catabolites in berry ABA homeostasis under abiotic stresses. In addition, a thermal disruption of the anthocyanins/ABA relationship among the different irrigations and cluster positions was observed, being the RDIW showing the larger difference, suggesting the higher susceptibility of RDI to high temperature as compared with SDI.

P62 *In vitro* phenotyping of seminal root traits in wheat

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Root system characteristics are essential for acquisition efficiency of soil resources and shoots anchoring to the ground. The root growth angle (RGA) is a principal component of root system architecture and determines direction of root elongation. It has been strongly associated with water and nutrients uptake ability in many crop species and may be considered as a potential trait to improve crop production. The aim of this work was to perform the screening of root traits at the seedling stage in a set of 73 genotypes of *Triticum aestivum* L. from different origins. Sowing was performed in square Petri dishes filled with agar, incubated at 21°C in the dark. RGA and seminal roots were analysed at 3 and 6 DAS. A large variability was found in RGA values that ranged from 66 ° to 147 °. Differences were also observed as regards the number and length of seminal roots. Narrow root angle and a higher number of seminal roots are considered proxy traits for selection at early growth stages in wheat breeding programs. Evaluation of genetic variability in root traits (RGA and seminal root features) allows to take advantage of naturally occurring diversity to select for more adapted varieties as regards environmental constraints. It will also contribute to breeding strategies for Portuguese Wheat Breeding Program under Mediterranean climate conditions, where unpredictable heavy rainfall as well as drought episodes are expected to increase.



P63 Using the chlorophyll fluorescence signal and machine learning techniques to automatically identify *Quercus* species: preliminary results

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A combination of chlorophyll fluorescence and machine learning was used to develop a non-invasive method for the automatic identification of different tree species. The rapid kinetics of PSII fluorescence emission in a dark-light transition was recorded *in situ* in leaves from three oak species (*Quercus rotundifolia*, *Quercus suber* and *Quercus canariensis*) with a Handy PEA (Hansatech) chlorophyll fluorometer. Both the entire data set of the fluorescence induction curves and three calculated fluorescence indexes (Fv/Fm, PI and C-Area) were used to construct classifiers based on decision trees. The percentage of success in the automatic classification reached 80% when the entire data set (Fluorescence Vs Time) of the curves was used, being slightly lower when the calculated fluorescence indexes were used. Changing the setting parameters of the decision trees (depth, maximum number of leaves per tree and minimum number of samples per leaf) failed to improve the percentage of success of the automatic classification, when compared with the classifier's default settings. Other artificial intelligence algorithms will be tested in an attempt to improve the success in the automatic classification of the species.

P64 Annotation of woody plant phenotypic data

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Woody plants are a major natural resource in Europe, with a huge ecological and economical impact, supporting millions of jobs across diverse industries (e.g. wine, fruit, olive oil, coffee, paper, timber and cork) and strongly contributing to the European GDP and therefore are a central research domain in academia and industry. Massive sequencing and genotyping of woody plants is generating large quantities of molecular data. Phenotypic data collected for each plant genotype, often in multiple locations and field conditions, has also been generated within different breeding programs. Moreover, novel approaches are being implemented to identify and collect large sets of quantitative phenotypes and to explain the genetic basis of important traits. The Portuguese ELIXIR node ([link](#)) is involved in the effort of making plant phenotyping data interoperable through the development of controlled vocabularies for annotating plant phenotypic data. The use of controlled vocabularies, to define the material assayed, the form of the experiment and the observed phenotypes, is critical to enable interrogation and comparison of datasets. Therefore, we have been working on establishing or improving existing ontologies and guidelines for ontology usage when annotating plant data, focusing on woody plants, and more specifically on forest tree species. A brief overview of this work, developed in the frame of the ELIXIR- EXCELERATE European project will be presented. The use of such standards will facilitate the sharing of structured information between different users as well as between software tools. This effort will contribute to build a framework that is of added-value to the woody plant user communities including both industry and academia.

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P65 Two non-destructive techniques to quantify smaller-than-chamber leaf area for leaf gas exchange measurements in photosynthesis systems

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The combination of leaf gas exchange and chlorophyll fluorescence measurements is critical for studies emphasizing on leaf photosynthesis and plant phenotyping. Several systems measuring such photosynthetic parameters in leaves of almost any plant species are available today. However, certain leaf variables, such as leaf area, make the use of these valuable systems not easy. These systems are mostly quantifying gas (CO_2 or H_2O) exchange per unit leaf area based on the gas exchange taking place on the maximal measured leaf area (MMLA) set by the leaf (measuring) chamber dimensions (e.g. 2 cm^2) per unit time. Therefore, the gas exchange per unit leaf area of leaves with measurable area smaller than MMLA cannot be accurately quantified and if not considered, gas exchange per unit leaf area will always be underestimated. We hereby tested whether two easy-to-use, non-destructive techniques can be used to quantify the measured leaf area (MLA). The first technique was based on imaging while the second was based on a single leaf width measurement and leaf shape geometry. The first true leaf of young maize, barley, hard and soft wheat plants was used for gas exchange, chlorophyll fluorescence and leaf area measurements. Our findings show that both techniques can be used to safely and accurately quantify/estimate MLA. The imaging technique can be used to quantify MLA of leaves of any shape while the leaf width – based technique can be accurately used to estimate leaf area in monocot species.

P66 Are chlorophyll fluorescence and machine learning techniques able to automatically classify *Arabidopsis* water stress status?

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A combination of chlorophyll fluorescence and machine learning was used to develop a non-invasive method for the automatic identification of plant water stress status. The rapid kinetics of PSII fluorescence emission in a dark-light transition was recorded in leaves from *Arabidopsis thaliana*, subjected to different levels of water stress, with a Handy PEA (Hansatech) chlorophyll fluorometer. The relative water content (RWC) of each leaf at the moment of the fluorescence measurement was determined. Both the entire data set of the fluorescence induction curves (Fluorescence Vs Time) and three calculated fluorescence indexes (Fv/Fm, PI and C-area) were used to construct classifiers based on decision trees, aimed to automatically assign each leaf to the corresponding RWC class. Four RWC (%) classes were considered: [100-80], [80-50], [50-30] and [30-0]. When the entire data set of the curves was used the percentage of success in the automatic classification reached 80%, being slightly lower when the calculated fluorescence indexes were used. Interestingly, changing the setting parameters of the decision trees (depth, maximum number of leaves per tree and minimum number of samples per leaf) showed that the percentage of success of the automatic classification was slightly higher (85%) at low depth (3-4) than at higher depth (80%). Other artificial intelligence algorithms will be tested in an attempt to improve the success in the identification of plant stress status.



P67 Portuguese traditional olive tree (*Olea europaea* L.) identification using endocarp characteristics and SSR markers

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Portugal has a large genetic patrimony of olive tree (*Olea europaea* L.) represented by many “old” local cultivars, some of restricted distribution. Traditional olive orchards represent 80% of the olive growing area in Portugal and it is recognized they are important repositories of genetic variability. They have potential value for olive breeding in what concerns tolerance and adaptation to many biotic and abiotic stresses (expected to be intensified by global climatic change). Dominant cultivars in the Alentejo traditional orchards are the local “Cordovil de Serpa” and “Verdeal Alentejana”, but other local cultivars are always present, although in small numbers. Due to the emergence of modern olive growing systems, identification, classification, and conservation of autochthonous olive cultivars in traditional orchards is a priority in order to avoid genetic erosion. The objective of this work was to inventory, characterize and identify the richness of the olive trees in traditional orchards of the Serpa region (Alentejo). Thirty two selected accessions (based on local farmers’ knowledge) were studied by means of the endocarps morphological characteristics. A set of seven microsatellite *loci* were also used to evaluate the genetic diversity and the relationships existing among accessions. The PCA analysis of the olive tree endocarps discriminates the accessions on the basis of endocarp size and distribution of vascular bundles on the surface. For the SSRs analysis (by means of Identity, MSAT and PCoA) a total of 56 alleles were identified using the 7 *loci*, a high genetic variability was revealed and all the accessions were discriminated. Both methods identified cases of mislabeling, while only SSRs identified synonymies.

P68 Advanced imaging tools for plant phenotyping

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High throughput plant phenotyping, also called plant phenomics, is an emerging and fast growing research field that aims to bridge the existing gap between genomics and plant breeding, by solving the so-called phenotyping bottleneck. Moreover, it can supply highly detailed information and tools for the advancement of both plant physiology and agronomy. Plant phenomics takes advantage from the recent developments in the fields of imaging, computer vision and sensor technologies, allowing the nondestructive detection of phenotypic characters. Plant phenomics ranges from basic science to applications in breeding and precision agriculture, combining studies performed under both controlled environments and in the open fields. Last year, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA) joined the Italian Plant Phenotyping Network (ITA-PPN) which gathers the national research centers and universities active in this field. CREA has developed in the last ten years advanced skills for the development of analytical methods for phenotyping, mainly imaging-based. The high-throughput character of our proposed phenotyping methods should help to improve the detection of important plant traits in large field trials as well as help us to reach a better understanding of underlying yield physiological processes and facilitate the genotype-phenotype associations. In particular, we developed the following analytic tools for: shape analysis 2d or 3d using landmarks (geometric morphometrics) or outline methods; quantitative color analysis from RGB images (we developed specific algorithms to standardize colors using colorchecker; 3D Thin-Plate Spline); punctual spectrophotometry and hyperspectral imaging; dynamic thermography imaging based; stereovision (multi camera systems for 3d reconstruction). Moreover, we developed an open source conveyor belt prototype multi-sensorized for rapid characterization of experimental wheat field plots. CREA developed advanced analytical approaches based on multivariate methods of prediction and classification (linear approaches and approaches based on artificial neural networks) applied in multi-parametric and multi-sensor metrology for an innovative support in phenomics. All these tools has been developed in Matlab environment, but could be easily exported in open source environments in order to realize highly customizable systems within the phenomics framework.



P69 Looking at biochemical responses following cork harvesting in *Quercus suber*

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Quercus suber L. (cork oak) woodlands occupy about 1.7 million ha in the western Mediterranean Basin. They constitute valuable ecosystems, hosting high levels of biodiversity, whilst supporting the cork industry and acting as a source of income for rural populations. It is generally assumed that *Q. suber* is well adapted to Mediterranean-type climate conditions. However, a general trend of decline (in occupied area and tree vitality) has been observed during the last decades. Despite its importance and the number of studies focusing the species, there are still knowledge gaps, especially in relation to the potential impacts of cork harvesting (the major economic activity related to these woodlands). As cork is harvested from late May to late August, usually coinciding with hot, dry periods, marked by water stress, trees might be subjected to an increased level of physiological stress after harvesting. To address this issue, we set up an experiment at a cork oak woodland located in Central Portugal (Coruche region), where two similar pairwise sets of cork stripped and unstripped (control) trees were monitored during 2015, a hydrological dry year (with about 50% of the long-term precipitation average). Measurements were carried out for 4 months, before and after cork harvesting, and included tree water status (predawn and midday leaf water potentials and leaf gas exchange) and daily sap flow. Additionally, after cork harvesting, leaves and phloem tissue of all trees were also sampled at intervals with increasing duration for biochemical determinations, e.g. total phenolic compounds and sugars, to evaluate the physiological response of individual trees to cork harvesting. Preliminary results show that, after cork harvesting and as summer progressed, phenolic compounds in the phloem increased more in cork stripped than in control trees. This difference was higher when, in each group, the analysis was restricted to the trees that were experiencing higher levels of water stress and considered producers of cork of good quality. Sucrose content in the phloem tissue of control trees increased steadily in the weeks after cork harvesting, being much higher than in treatment trees by the end of the summer. This trend may be reflecting differences in phloem capacity to translocate sugars, following cork harvesting, and in phellogen activity throughout the summer in response to water stress. So far, our results suggest that cork harvesting is likely triggering a biochemical response, however, summer drought seemed to be the main driver for the physiological stress experienced by *Q. suber* trees.

P70 Rapid ground-based phenotyping methods for canopy cover and canopy reflectance estimates in a durum wheat diversity panel

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There is increasing demand for simple, rapid methods to evaluate crop plants growing under field conditions to separate and identify the interacting effects of genetics, environment and management on crop growth and development. We assessed canopy cover using digital images and canopy reflectance using spectral data collected from a tractor-mounted phenotyping system for a diversity panel of 260 accessions of durum wheat (*Triticum turgidum* Desf.), which was grown in 2016 at the Maricopa Agricultural Center in Arizona. The diversity panel lines originated from different countries across the wheat-growing areas, and was previously genotyped using a high-density single-nucleotide polymorphism (SNP) assay. We manually photographed (Lumix DMC digital camera) each plot at two positions within the plot, using a custom-built metal A-frame stand designed to ensure a nadir view from a constant height and providing a field of view matched to the 0.76 m wide rows. Using a tractor-mounted sensor array system, we measured canopy reflectance with a multi-spectral crop sensor deployed on the front boom. Canopy cover was estimated from each digital camera image using a batch script implemented in ImageJ (1.49V), with analysis of 600 images requiring approximately one hour. Spectral reflectance measurements were recorded with a data logger and the data corresponding to the red (670 nm) and NIR (780 nm) wavelengths were used to calculate the normalized difference vegetation index (NDVI). Plot-level estimates of canopy cover and NDVI were assessed at different times during crop growth. Comparisons of the two phenotypes showed good agreement but also high-lighted potential confounding effects of plant population. Both methods show value as simple, low-cost options for field-based phenotyping to facilitate genotype-phenotype association studies and better understand the impacts of abiotic and biotic stresses on crops.



P71 Interplay between chromatin remodeling and DNA repair during seed germination: a case study in *Medicago truncatula*

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Fast and uniform seed germination is a priority for gaining high crop yields. The availability of molecular hallmarks of seed vigor is expected to positively impact seed technology, providing innovative tools to overcome conventional priming protocols (Paparella *et al.* 2015, *Plant Cell Rep.* 34:1281-1293; Macovei *et al.* 2016, *Plant Cell Rep.* doi: 10.1007/s00299-016-2060-5; Araujo *et al.* 2016, *Front. Plant Sci.* 7:646.2016). This work explores the effects induced by the histone deacetylase (HDAC) inhibitor trichostatin A (TSA, 10 mM and 20 mM) during *Medicago truncatula* seed germination. The *MtTRRAP* (transformation/transactivation domain-associated protein) gene is known in human cells as a key player in the recruitment of histone acetyltransferase (HAT) complexes to chromatin during DNA repair but it is so far poorly investigated in plants. Time- and dose-dependent up-regulation of *MtTRRAP* gene and predicted TRRAP interacting partners were observed during *M. truncatula* seed imbibition as well as in seeds collected at the radicle protrusion phase and 4-day old seedlings. Alkaline Comet assay performed on radicles isolated at the protrusion phase and cotyledons excised from 4-day old seedlings revealed dose-dependent DNA damage accumulation. Up-regulation of *MtOGG1* (8-OXOGUANINE GLYCOSYLASE/LYASE) was indicative of the repair response activated towards trichostatin A-induced oxidative DNA damage.

P72 Preliminary results on the use of reflectance spectroscopy and artificial intelligence techniques to automatically identify *Vitis* species

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Enhancing the resistance of cultivated grapevine to fungal infection, while keeping a good berry quality, constitutes a major goal for breeders. Breeding approaches are quite time consuming and resource-intensive until the expression of the trait is observable in the progeny. Moreover, the selection of resistant offspring is based on controlled inoculations, where thousands of plants have to be tested. Thus, discovery of methodologies that would allow a quick and accurate identification of the seedlings that inherited the resistant trait would have a major impact for grapevine breeding. Diagnostic assays based on optical techniques have the advantage of being non-invasive and time- and cost-effective, being therefore effective in high-throughput plant phenotyping. In this work we gathered data from *Vitis vinifera* and *V. riparia* leaves with reflectance spectroscopy. Collected data was used to construct classifiers based on decision trees. Preliminary results suggest that reflectance spectroscopy is a promising technology to distinguish between the *Vitis* genotypes, mainly in the near-UV band of the spectrum. The percentage of success in the automatic classification reached 90%, and the spectroscopic conditions may still be improved. Taken together, the results suggest that the conjugation of spectroscopic techniques with artificial intelligence may contribute to accelerate the breeding of *V. vinifera* cultivars resistant to downy mildew.



P73 PhenomicsNL, the Dutch Plant Phenotyping Network

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The global population is growing and fossil fuels are becoming scarce. This is why it is important to grow crops that can be cultivated efficiently and have high yields, whether these are grains, vegetables, fruits or raw materials for bioplastics. But this is easier said than done. The agricultural sector is experiencing the results of climate change all across the globe; from floods in Bangladesh and droughts in the Horn of Africa, to the arrival of new plant diseases in Europe. Research groups at Wageningen University & Research are currently studying the behaviour of plants at different levels: from model and individual plants to the growth of crops in greenhouses and on the field.

Speeding up developments: Combining all knowledge, expertise and facilities in the field of phenomics into one single PhenomicsNL platform speeds up developments which enable chain partners to strengthen their position on the international market. Through this platform, Dutch companies will be informed about join large-scale initiatives. Wageningen University & Research has established several public-private partnerships and has been a partner in notable European initiatives such as SPICY, the COST action Phenomenall and the European Plant Phenotyping Network (EPPN). With PhenomicsNL, Wageningen University & Research is involved in many new programmes including those of the International Plant Phenotyping Network (IPPN), EMPHASIS and EU-project EPPN 2020. The EMPHASIS initiative develops and/or improves new and existing facilities and makes them widely accessible so that a clear image of the response of crops to climate change can be created through collaboration. EMPHASIS is part of Roadmap 2016 created by the European Strategy Forum for Research Infrastructures (ESFRI).

P74 Phenotyping as a tool to screen the plant growth and symptom expression in sensitive, tolerant and resistant potato genotypes infected with potato virus Y

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Potato virus Y (PVY) is of extreme economic importance as it is responsible for yearly losses in production of crops from family Solanaceae in Europe, and thus the subjects of investigation in many research groups all over the world. The tuber necrotic strain of Potato virus Y (PVYNTN) causes potato tuber necrotic ringspot disease (PTNRD) in sensitive potato (*Solanum tuberosum* L.) cultivars that is responsible for great losses in crop industry. Sensitive cultivars of potato infected with PVYNTN show growth inhibition, faster senescence and leaf drop, chlorotic ring-spots and/or spot necrosis on inoculated leaves, crinkles and mosaics on systemic infected leaves and necrotic ring spots on tubers. Viruses from PVYN-Wi group can also cause severe symptoms on potato. Symptom development and their severity depend on the isolate of PVY, potato cultivar, environmental conditions and other factors. In our studies, differences in growth inhibition, senescence, leaf drop and symptom appearance were monitored on four potato (*Solanum tuberosum* L.) genotypes (two sensitive, one tolerant and one resistant) after the infection with two different isolates of Potato virus Y, PVYNTN and PVYN-Wi. Results were collected in a time series after infection. The results were complemented with the analyses on the cell level, e.g. quantitative real-time PCR analyses of differentially expressed genes.

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P75 High-throughput plant phenotyping at the Vienna Biocenter Core Facilities (VBCF)

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The VBCF Plant Science Facility (PlantS) is one out of ten facilities forming the Vienna Biocenter Core Facilities GmbH (VBCF). It is a publicly funded non-profit research institute, situated at the Vienna Biocenter (VBC). While servicing in-house plant research, we offer access to our infrastructure and scientific services to external collaborators as well. The VBCF Plant Sciences Facility operates 22 state-of-the-art plant growth chambers providing highly adjustable environmental conditions i.e. low temperature (frost), high temperature (up to 50°C), water logging, different light intensities and spectra (LED: UV₃₇₅, blue₄₀₅, blue₄₅₀, white and red_{660,730}) and different gas conditions (e.g. CO₂). Plants additionally has a fully chamber-integrated sensor-to-plant camera system at its disposal allowing high-throughput phenotyping experiments with *A.thaliana*. Phenotyping can therefore be combined with precise environmental simulations across different climate zones and various abiotic stress conditions. Very recently the phenotyping chamber was upgraded with adjustable high-tech LED illumination improving and extending the phenotyping service by high-light stress and adjustable spectral conditions. Subsequent image analysis runs on LemnaTec OS software, allowing reproducible high-throughput screenings. The software also facilitates analysis of customized phenotyping experiments i.a. low-throughput (side-view) phenotyping of crop plants, screening of seedlings (agar-plates), root phenotyping (agar-plates), phenotyping of seeds and confocal microscopy image analysis. Being part of VBCF enables us to offer combined plant research services also including attendances of other VBCF core facilities.

P76 Regulation of the Hemp hypocotyl lignification

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Lignin and lignans are two macromolecules deriving from the monolignol pathway. Despite the similarity of their building blocks, they fulfil different functions *in planta*. Lignin strengthens the tissues of the plant, while lignans are involved in plant defence and growth regulation. Their biosynthesis are tuned both spatially and temporally to suite the development of the plant (water conduction, reaction to stresses). It was previously shown that the growing hemp hypocotyl is a valid system to study secondary growth and the molecular events accompanying lignification. The present work confirms the validity of this system, by using it to study the regulation of lignin and lignan biosynthesis. Observations at the microscope, lignin analysis, proteomics, together with targeted RT-qPCR, *in situ* laccase and peroxidase activity assays were carried out to understand the dynamics of lignan/lignin synthesis during the development of the hemp hypocotyl. We suggest a role for the hemp dirigent and dirigent-like proteins based on phylogenetic analysis and targeted gene expression. The complementary approach adopted identifies the main players (genes and proteins) involved in the biosynthesis of monolignols and their oxidative coupling (class III peroxidases and laccases), in lignin deposition (dirigent-like proteins) and the stereo-conformation of lignans (dirigent proteins). Our work sheds light on how the growing hemp hypocotyl regulates, at the transcriptional and proteomic level, the provision of the precursors needed to synthesize the aromatic biopolymers lignin and lignans.



P77 Chlorophyll a fluorescence – measuring principles and protocols

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Chlorophyll fluorescence (CF) is a versatile non-invasive method to monitor a variety of plant stresses. The simplest fluorescence parameters to measure are the quantum yield of PSII, $\Phi_{PSII} = F_q'/F_m'$, and electron transport (ETR). It only requires a saturating light pulse and is measured in light. The most used parameter, though, is probably F_v/F_m , which estimates the maximum photochemical efficiency of PSII. It requires 20-30 minutes dark adaptation of the leaf and can monitor stress created by e.g. light, low or high temperature, or nutrient deficiencies. If the leaf is dark-adapted first, $\Phi_{PSII} = F_q'/F_m'$ measured in light can be complemented with a full quenching analysis where the non photochemical quenching (NPQ) and the redox state of PSII (qL) are part the energy balance of PSII, all affected by stress. The use of CF in high-throughput phenotyping by fluorescence cameras is limited by the technical difficulties to apply and detect a saturating pulse from a 3D plant, and it will only give a snapshot of photosynthesis. Monitoring-PAM (Moni-PAM) is a new tool for medium-throughput phenotyping, where CF can be measured on up to seven leaves simultaneously. It can follow the development of stress *in situ* and can be used to design measuring protocols for high-throughput phenotyping. Even though CF is a non-invasive method the saturating pulse does affect photosynthesis and may contribute to down-regulation of PSII through increased NPQ if applied too frequently. Therefore, the measuring protocol should be designed with care. For full quenching analysis dark adapted values of the initial F_o and saturated maximum F_m are needed. This can be achieved by applying a saturating pulse during the night. The default control mode of the Moni-PAM is by triggering the saturating pulses by a clock, which means that the leaf will be exposed to several saturating pulses during the night, dependent on chosen sampling frequency. The effect of the CF sampling frequency and examples of how several species respond to naturally fluctuating light and high temperature will be shown. Both the fluctuating nature of daylight and the sampling frequency have implications for how protocols for high-throughput phenotyping by CF should be designed when the technical development reach the stage where the technique can be used on 3D crop plants.

P78 Lipidomics approaches to study plant phenotype : applications and future perspectives

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Plants are, nowadays, important sources of food and feed for humanity and livestock. They are rich in fibers, proteins and contain lipids with high nutritional value, such as omega-6 and omega-3 fatty acids, and with health beneficial effects. Lipids are major components of cell membranes and chloroplast and as signaling molecules regulating plant metabolism. Plants modulate the lipid metabolism using an orchestrate of enzymes to maintain a specific composition of lipids in the different parts of plants. But lipid metabolism is adjusted in response to nutritional, environmental and stress-induced conditions, and also during developmental phases with change in plant phenotype. In the last years, mass spectrometry based approaches new perspectives in the understanding of the role of lipids in plant biochemistry. It can provide clues concerning the roles of enzymes and genes involved in lipid metabolism. We will give an overview of the modern analytical strategies based on mass spectrometry in plant lipidomics and how they can be exploited to assess variation in plant lipidome during growth or plant adaptation to stress. Some examples will be presented to illustrate how lipidomics was employed in the identification of polar lipids fingerprinting macroalgae from distinct phyla or to detect lipid changes in the adaptation of some plants to nutrients deprivation and temperature and light. Lipidomics will contribute to boost the valorization of plant biomass and their based-products.



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