



PLANT BIOLOGY EUROPE FESPB/EPSO 2014 Congress

Abstract Book Poster Presentations

**22nd - 26th June 2014, Dublin, Ireland
Convention Centre Dublin**

www.europlantbiology.org

info@europlantbiology.org

Tweet us @fesp_b_eps_2014



P101

Differential Selection of Sodium and Potassium Ions by Two Closely Related HKT1 Homologs, AtHKT1 and TsHKT1;2 under Salt Stress

Akhtar Ali, Rashid Aman, Hyeong Cheol Park, Bohnert Hans. J, Dae Jin Yun
Gyeongsang National University, Jinju, GyeongsangNamdo, Republic of Korea

Maintaining uptake of potassium ions under salinity stress is crucial for plant survival, and this fact has, for decades, led to the emphasis on identifying and characterizing salinity-related plant-specific genes and pathways. We tested the function of HKT1;2 from the Arabidopsis-relative extremophile *Thellungiella salsuginea*. *T. salsuginea* contains at least two HKT genes. TsHKT1;1 is expressed at very low levels, while the abundant TsHKT1;2 is transcriptionally strongly up-regulated by salt stress. TsHKT-based RNA interference in *T. salsuginea* resulted in Na⁺-sensitivity and K⁺-deficiency. The *athkt1* mutant lines over-expressing TsHKT1;2 proved less sensitive to Na⁺ and showed less K⁺ deficiency than lines over-expressing AtHKT1. TsHKT1;2 ectopically expressed in yeast mutants lacking Na⁺ or K⁺ transporters revealed strong K⁺-transporter activity and selectivity for K⁺ over Na⁺. Altering two amino acid residues in TsHKT1;2 to mimic the AtHKT1 sequence resulted in enhanced sodium uptake and loss of the TsHKT1;2 intrinsic K⁺ transporter activity. We consider the maintenance of K⁺ uptake through TsHKT1;2 under salt stress an important component supporting the halophytic lifestyle of *T. salsuginea*.

P102

Elevated CO₂ Mitigates the Impact Drought on *Arabidopsis thaliana*: Growth and Genome-Wide Analyses

Hamada AbdElgawad, Gaurav Zinta, Marios N Markakis, Malgorzata A.Domagalska, Gerrit T.S. Beemster, Han Asard
Antwerp University, Antwerp, Belgium

Future climate scenarios indicate increasing, CO₂ levels in combination with more frequent drought spells. Recent studies suggest that elevated CO₂ reduces the effect of drought stress, but the mechanisms underlying the reduced stress effects remain unclear. Therefore we investigated the growth, physiological, biochemical and genome-wide transcriptional responses of *Arabidopsis thaliana* rosette to mild (40%SWC) and severe drought (45% SWC) under ambient and elevated CO₂ (360 and 620 ppm respectively). By means of kinematic analysis we found that drought reduced leaf growth by inhibiting cell division and elongation. Elevated CO₂ reduced the impact of mild drought on growth by increasing both cell number and size, and the impact of severe drought by increasing cell size only. At physiological level, drought inhibited rates of photosynthesis and increased the levels of reactive oxygen species and oxidative stress parameters. Elevated CO₂ mitigated these detrimental effects, particularly under severe drought. Consistently, the transcriptome analysis showed that photosynthesis genes were most strongly overrepresented among the down-regulated genes in response to drought under ambient CO₂ levels. The transcriptome implicates the antioxidant defense metabolism in protective effect of elevated CO₂, with up regulation of ascorbate peroxidase, glutathione reductase and superoxide dismutase and down regulation of phenols, peroxidase and dehydroascorbate reductase genes were observed. Additionally, we observed that elevated CO₂ alleviated the stress induced-H₂O₂ generation processes (photorespiration and mitochondrial electron transport chain). We therefore conclude that elevated CO₂ mitigates the effects of drought in plants by altering the antioxidant defense metabolism and by reduced hydrogen peroxide production.

P103

Physiological and Growth Responses to Salinity Stress in the Bioenergy Crop *Miscanthus x giganteus*

Evangelia Stavridou, Richard Webster, Paul Robson

Institute of Biological Environmental & Rural Sciences, Aberystwyth University, Aberystwyth, Wales, UK

Salinity, both natural and secondary, is a severe abiotic stress especially in the Mediterranean region with sodium chloride (NaCl) being an abundant salt species. Salts inhibit plant growth, have a negative effect on crop productivity potential and may result in death. High salinity land may provide an alternative resource for the cultivation of dedicated biomass crops for renewable energy and chemicals as this would avoid competition with food crops. A leading biomass crop is the giant grass *Miscanthus*. The response of the commercial *M. x giganteus* to salt stress is largely unknown. *M. x giganteus* was grown under 9 different levels of salinity. Increased salinity effected plant elongation, biomass production, WUE, leaf stomatal conductance, leaf ash content and proline accumulation. Based on these results two salinity levels that corresponded to the two phases of growth response due to the osmotic and the ionic toxicity effects of salinity were chosen for further detailed studies. *M. x giganteus* treated with the two different NaCl concentrations under controlled conditions were analysed for morphological and physiological measurements, and for differences in the antioxidant enzymes activity. Photosynthesis measurements of the A/Ci response were performed and analysed to identify how the photosynthetic pathway was affected under stress. The objective of this study was to distinguish the two phases of the salt stress, determine the salinity level to which plants tolerance is altered and identify key tolerance mechanisms that ultimately may lead to potential novel applications of *Miscanthus*.

P104

Revealing the Molecular and Physiological Responses to Water Deficit in the Bioenergy Crop *Miscanthus x giganteus*

Jennifer Ings, Luis A.J Mur, John Clifton-Brown, Maurice Bosch, Paul Robson
Aberystwyth University, Aberystwyth, UK

High yielding perennial biomass crops of the species *Miscanthus* are widely recognized as one of the most promising lignocellulosic feedstocks for the production of bioenergy and bioproducts. *Miscanthus* is a C4 grass and thus has relatively high water use efficiency. Cultivated *Miscanthus* comprises primarily of a single clone, *Miscanthus x giganteus*, that is high yielding and expresses many desirable traits required in an ideal biomass crop; however, it responds poorly to low water availability. Despite this, we have little knowledge of the mechanisms involved in the drought response and recovery from drought in *M. x giganteus*. To address this, we are implementing integrative approaches, combining physiological, genetic and metabolic analysis, using *M. x giganteus* plants exposed to water-deficit stress in pot experiments. Withholding water resulted in marked changes in plant physiology with growth-associated traits among the first affected, the most rapid response being a decline in the rate of stem elongation followed by a reduction in photosynthetic performance. Determining the ability of the plants to regrow after a period of water deficit has enabled identification of the critical drought point of the rhizome and given further insight into the balance between withstanding water deficit and biomass accumulation. Leaf tissue samples were taken at key drought stages (intermediate and terminal) for RNA sequencing analysis and metabolite profiling. These analyses identified metabolites and genes associated with drought and provide potential targets for improved drought-tolerance of the *Miscanthus* bioenergy crop.

P105

Cell Wall Polysaccharides and Toxic Metal

Zuzana Vatehova^{1,2}, Karin Kollarova², Anna Malovikova², Desana Liskova^{1,2}

¹*Department of Plant Physiology, Faculty of Natural Sciences, Comenius University, Mlynská dolina B-2, 842 15 Bratislava, Slovakia,* ²*Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia*

Cadmium is a well-known environmental pollutant with distinctly toxic effects on plants. To minimize the detrimental effects of cadmium exposure and its accumulation, plants have evolved detoxification mechanisms. Such mechanisms are mainly based on chelation and subcellular compartmentalization. The plant cell wall is an important cell feature that performs numerous essential functions. As the first barrier it prevents the input of toxic metals into the cell and plant organ, but this defense mechanism is still fully unknown. In this connection the effect of toxic cations on cell wall polysaccharides composition in roots of maize has been studied.

Zea mays L. as a member of the Poales, has peculiar primary cell walls of the type II distinguished by higher content of hemicelluloses (arabinoxylan, arabinogalactan, glucuronoarabinoxylan) and lower content of pectin compared to dicots. A combination of extractions (hot water and alkali), chemical (content of uronic acids, phenolics, proteins) and analytical (FTIR, HPLC) methods for the characterization of cell wall composition in plants treated with cadmium cations were used. The results indicate that the cell wall functions not only as a sink for toxic metal accumulation, but is also modified under metal stress.

Better understanding of plant cell wall function in defense against toxic metals could point out plants utilizable as a green technique, phytoremediation, for cleaning up toxic metal-contaminated soils and water.

This work was supported by the Slovak Research and Development Agency, contract No. APVV-0140-10, Slovak Grant Agency for Science VEGA 2/0083/14.

P106

Molecular Mechanism of Cd Accumulation in *Nicotiana glauca*

Kristina Majsec, Mirjana Pavlica

Department of Molecular Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

Cadmium is one of the most dangerous substances on Earth and toxic for most organisms. Surprisingly, some plants can be hypertolerant and accumulate Cd in significant amounts. Researching this phenomenon is important for our understanding of metal hyperaccumulator biology. *Nicotiana glauca* is a tobacco plant growing in Negev desert on contaminated soil, with elevated metal content in the tissue. In this study we examine its Cd-accumulating properties and underlying molecular mechanisms in comparison with non-accumulating *N. tabacum*.

Plants were grown on MS media with 15 μ M Cd for 4 weeks, harvested and dried to constant weight. Cd content was determined by ICP-OES and showed 3-fold higher shoot-to-root ratio for *N. glauca* in comparison with *N. tabacum*. 10 week-old plants were treated with 15 μ M Cd. After 3 days, RNA was isolated, reversely transcribed and qPCR was performed with actin gene as internal control. CIT1 showed higher constitutive expression in *N. glauca* roots, NRAMP-1 in shoots and NAS in the whole plant. Upon Cd treatment expression of NAS, NRAMP-1 and PCS further increased. NAS (nicotianamine synthetase) appears to have high importance for Cd-accumulation by chelating metals throughout the plant. Additionally, CIT1 (citrate synthase) possibly fosters Cd-xylem loading, while NRAMP-1 (metal transporter) participates in Cd storing into vacuoles in leaves.

Our results show that *N. glauca* is tolerant to Cd and preferably accumulates Cd in shoots. Our future research plan includes NRAMP-1 localization assay in order to verify its role in Cd-accumulation, and investigating expression and localization of other potentially important genes for these properties.

P107

The Role and Regulation of the Only DELLA Protein of Rice in Abiotic Stress

Nuno Gonçalves¹, M. Margarida Oliveira¹, Isabel A. Abreu^{1,2}

¹*Instituto de Tecnologia Química e Biológica, Oeiras, Portugal*, ²*Instituto de Biologia Experimental Tecnológica, Oeiras, Portugal*

DELLA proteins are the “Green Revolution genes” encoding gibberellin response modulators. They have been involved in plant growth and development and also in stress responses in Arabidopsis. According to our preliminary gene expression results performed in rice submitted to drought, we hypothesize that crop DELLAs are also involved in abiotic stress responses, and propose rice as a model crop to study the tolerance mechanism. While Arabidopsis has 5 different DELLA proteins, rice, as most crops, only has one (SLR1) suggesting possible accumulation of specific functions, for which we intend to test by Arabidopsis heterologous SLR1 expression. Our data points for a SLR1 regulation by drought-stress occurring mostly at the protein level, via ubiquitin/proteasome degradation or stabilization by other post-translational modifications (PTMs). We are currently using reverse-genetics and proteomics to evaluate the relevance of SLR1 at the transcript and protein levels during stress response in wild-type, knockdown, knockout rice mutants and also tolerance-contrasting genotypes by performing multiple stress screenings. Meanwhile we are producing both complementation and over-expression rice transgenic lines as tools for further stress-induced proteome characterization in order to identify which pathways are influenced by SLR1 during imposed stresses and also determine its interactome with a SLR1-specific antibody. Furthermore, we intend to understand the fine tuning of its regulation by several PTMs. The knowledge gathered will help to understand stress tolerance mechanisms in cereal crops, and also to identify bottlenecks in crop adaptation to stress conditions.

P108

The Role of Arabidopsis Non-Specific Phospholipase c1 in Heat Stress

Zuzana Krckova^{1,2}, Jan Martinec¹, Olga Valentova²

¹Institute of Experimental Botany AS CR, v. v. i., Prag, Czech Republic, ²Institute of Chemical Technology, Prag, Czech Republic

Non-specific phospholipase C (NPC), recently discovered plant enzyme, catalyzes hydrolysis of phosphatidylcholine to diacylglycerol and phosphocholine. In Arabidopsis genome six NPC genes (*NPC1* to *NPC6*) were identified. It was shown, that NPC plays significant role in response of Arabidopsis to hormones and/or abiotic stress: NPC3/NPC4 in brassinolide signalling, NPC4 is salt/osmotic stress, NPC5 in phosphate starvation.

The aim of this project was to study a role of NPC in heat stress (HS). Based on preliminary results we focused to NPC1. We used Arabidopsis seedlings, wild type (WT), knockout T-DNA insertion line (*npc1*) and overexpressing line NPC1-OE. Plants were tested either for basal thermotolerance (BT, 42 °C, 45 min) or for acquired thermotolerance (AT, 37 °C, 1 h; 22 °C, 2 h; 45 °C, 105 min).

Thermotolerance in *npc1* was impaired in both BT and AT compared to WT. There were significant decreases in survival rate, root length and chlorophyll content in comparison to WT. By contrast NPC1-OE was more resistant to BT compared to WT. Using fluorescently labelled PC as a substrate we determined the increasing activity of NPC in Arabidopsis after 15 and 30 min of HS to 150% and 200% respectively. We observed reduced level of NPC1 expression (up to 40%) after HS. No changes in expression of heat marker genes in *npc1* were detected.

Our results show for the first time that the non-specific phospholipase C1 play significant role in Arabidopsis response to heat stress.

This work was supported by Czech Science Foundation project no. P501/12/1942.

P109

Tolerance to Drought Stress of a *Medicago truncatula* Tobacco-Retrotransposon (Tnt1)-Insertion Mutant

Amaia Seminario¹, Yun Kang², Esther M. González¹, Michael Udvardi²

¹*Public University of Navarra (UPNA), Pamplona, Navarra, Spain,* ²*The Samuel Roberts Noble Foundation, Ardmore, Oklahoma, USA*

Drought is a major environmental stress factor that affects the growth and productivity of plants via changes in metabolism, cell division and other processes. Greater drought tolerance in plants is likely to become more important in future as the share of water allocated to agriculture declines. A Tnt1-insertion mutant population of *Medicago truncatula* ecotype R108 (Tadege et al., 2008) was screened for drought stress tolerance. Subsequently, a mutant line (NF3103) was selected because of its highly induced expression under drought stress in both *M. truncatula* and alfalfa. This line was known to be mutated in a nucleoredoxin gene (Mtr.42918.1.S1_at) which may be involved in signalling cascades controlling, among others, cell division and growth and maintaining cell redox homeostasis. In the current study, we aim to characterize physiologically the drought response of this mutant.

M. truncatula Gene Expression Atlas reports an increased expression of this gene in both shoot and root under drought conditions. Four weeks-old *M. truncatula* plants will be subjected to progressive drought stress by withholding water. Biochemical and physiological responses to drought will be characterized. Biomass, water potential, photosynthesis, chlorophyll content and root anatomy will be determined. Additionally, the content of different key metabolites of the carbon/nitrogen primary metabolism will be analysed in order to ascertain photoassimilate distribution at the whole plant level.

References:

- Tadege et al. (2008) Large-scale insertional mutagenesis using the Tnt1 retrotransposon in the model legume *Medicago truncatula*. *Plant Journal* 54: 335-347

Acknowledgements:

- Spanish Ministry of Economy and Competitiveness (AGL2011-23738) and Public University of Navarra.

P110

A Specific Role of Hydrogen Peroxide in UV-B Exposed Leaves

Gyula Czégény¹, Petra Majer², Min Wu³, András Dér⁴, Leif A. Eriksson³, Åke Strid⁵, Éva Hideg¹
¹*Institute of Biology, University of Pécs, Pécs, Hungary,* ²*Institute of Plant Biology, Biological Research Centre, Szeged, Hungary,* ³*Department of Chemistry and Molecular Biology, University of Gothenburg, Göteborg, Sweden,* ⁴*Institute of Biophysics, Biological Research Centre, Szeged, Hungary,* ⁵*Department of Science & Technology, Örebro Life Science Center, Örebro University, Örebro, Sweden*

Ultraviolet (280-400 nm) radiation is an important regulator of plant growth and development. Our study is focussed on the UV-B (280-315 nm) regulation of the antioxidant - pro-oxidant balance using either prolonged exposure to low doses of supplemental UV-B or short treatments with monochromatic UV-B.

Tobacco and Arabidopsis plants were grown under 150-250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Mature leaves were exposed to low doses of supplemental UV-B for one week, which resulted in a 10-15% loss of photosynthetic activity compared to control plants kept under PAR only. Increase in ROS specific antioxidant capacities contributed to the acclimation to UV-B and defence against H_2O_2 had the largest increase among these.

Short (1-6 min) treatments of 1 cm^2 leaf areas with a 308 nm laser showed that UV-B alone is capable of increasing cellular H_2O_2 concentrations and that UV-induced prompt decrease of photosynthetic electron transport depends on leaf antioxidant levels.

In vitro ROS experiments showed that H_2O_2 is photo-cleaved by UV-B into hydroxyl radicals ($\bullet\text{OH}$), with an action spectrum peaking at 290-300 nm. Calculations confirmed this and showed that $\bullet\text{OH}$ was the only radical product of this photo-reaction.

Our results suggest that the effect of UV-B is two-fold, the first being the generation of H_2O_2 and the second a partial conversion of this H_2O_2 to hydroxyl radicals. As various abiotic stresses (e.g. drought, high PAR) are known to increase cellular H_2O_2 levels, this may explain why ambient solar UV-B enhances the effect of these.

Supported by the Hungarian Scientific Research Fund OTKA NN-85349.

P111

Light Quality and Temperature Dependent CBF Expression in Cereals

Aliz Novák^{1,2}, Ákos Boldizsár¹, Éva Ádám³, Gábor Galiba¹

¹*Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary,* ²*University of Pannonia, Veszprém, Hungary,* ³*Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary*

Winter hardiness of cereals depends on many factors, such as temperature, light intensity and day length. The CBF genes, encoding transcription factors are induced by short cold exposure. As transcription factors, they regulate other genes, thus initiating processes that lead to the formation of frost tolerance. Because of their high yield, winter cereals are economically important, so it is necessary to understand the whole frost acclimation process thus helping the work of molecular breeders. In *Arabidopsis* it was proven that light-quality influences CBF expression, leading to increased level of frost tolerance.

In our experiment effect of monochromatic blue, red and far-red light was examined first on the winter habit *Triticum monococcum* 'G3116' line. The expression level of several CBFs and a cold-inducible effector gene, *Cor14b* was detected by quantitative real-time PCR method. Our results prove that there is interaction between the CBFs and the light-quality in cereals as well.

In the second experiment, frost tolerance was tested on the winter barley 'Nure', the winter wheat 'Cheyenne' and the winter einkorn 'G3116'. Beside white light, additional red or far-red or blue light was given to the plants and the membrane injury of the leaves was investigated by electrolyte leakage measurement at different freezing temperatures. Expression levels of several target genes were also detected. Whole plants were frozen at the end of the treatments and regeneration ability, survival rates were also calculated. It was obviously proven that the far-red light treated plants showed higher frost tolerance and expression level of several frost-related genes.

P112

***HVP* (Vacuolar H⁺-PPase) Genes in Barley: Chromosome Location, Sequence and Gene Expression Relating to Na⁺ Exclusion and Salinity Tolerance**

Yuri Shavrukov

Australian Centre for Plant Functional Genomics, University of Adelaide, Waite Campus, Urrbrae, SA 5064, Australia

Three vacuolar H⁺-pyrophosphatase (*HVP*) genes for a proton pump have been identified in barley (*HVP10*, *HVP1* and *HVP3*). The first gene, *HVP10*, has been mapped to chromosome 7HS and showed increased gene expression in both roots and in shoots under salt stress. In the current study, a second gene, *HVP1*, was mapped to chromosome 7HL with different expression profiles in studied barleys. *HVP1* appears to be involved in both adjustment to osmotic stress and management of toxic Na⁺ concentrations in shoots. *HVP1* gene has eight exons and seven introns. The predicted amino acid sequences of *HVP1* in studied barleys were identical, although there were a number of SNPs within the coding regions. This suggests structures of both *HVP10* and *HVP1* are very conserved among barleys, and it supports a hypothesis that promoter regions of both genes are involved in the regulation of gene expression. A third gene, *HVP3*, has been previously described but it was mapped in chromosome 1H in current study, and deduced that the gene contains four exons and three introns. In our experiment *HVP3* was expressed neither in roots nor in shoots of any of the barley genotypes tested. Based on published EST data, it is hypothesised that *HVP3* is expressed only during seed development and is not linked to abiotic stress responses. Nucleotide sequence analysis indicated that during evolution there may have been insertions/deletions occurring within two highly conserved domains shared by *HVP1/HVP10* and *HVP3* genes.

P113

***TaDREB3* Transgene Transfer, Inheritance and Expression in Different Genetic Backgrounds for Improving Drought Tolerance in Bread Wheat**

Yuri Shavrukoy, Sergiy Lopato, Peter Langridge

Australian Centre for Plant Functional Genomics, University of Adelaide, Urrbrae, SA 5064, Australia

We have previously transformed bread wheat cultivar Bobwhite with a construct for improving drought tolerance, which contained wheat *TaDREB3* gene driven by the drought inducible maize *Rab17* promoter. The aims of this study included: (1) transgene transfer into local elite bread wheat cultivars by conventional cross-hybridisation with transgenic line, (2) analysis of transgene inheritance and stability of expression across several generations, and (3) improvement of stress tolerance of hybrid plants to drought. A T₃ transgenic line (BW8-9-10-3) of cv. Bobwhite with multiple transgene copies, excellent phenotype and increased drought tolerance was used as a pollinator in crosses with four elite bread wheat cultivars: IGW-2971, Bonnie Rock, Wyalkatchem and Magenta. A single plant with confirmed transgene presence was selected from each hybrid combination for three rounds of backcrossing, using the same elite wheat cultivar as the maternal, recurrent parent. PCR and Northern blot analysis of F₁BC₃ hybrid plants demonstrated a close to expected (1:1) transgene segregation and drought-responsive transgene expression for progeny with IGW-2971 and Bonnie Rock backgrounds. Although presence of transgene was detected in three from six F₁BC₃ hybrid plants based on cv. Wyalkatchem, inducible expression of the transgene was not observed in any of these plants. Under well-watered conditions, the phenotypes and grain yield components of the F₂BC₃ transgenic plants were similar to their corresponding recurrent parents and null-segregants. Under severe drought conditions, all tested hybrids demonstrated 12-18% improvement of survival rates compared to their corresponding recurrent parents and nulls. Yield analysis is currently being in progress.

P114

Applied and fundamental research in ABA Signalling. Generation of drought-tolerant transgenic barley by expressing mutated versions of the Arabidopsis PYL4 ABA receptor

Pedro L. Rodriguez, Miguel Gonzalez-Guzman, Lesia Rodriguez, Laura Lorenzo-Orts, Marta Peirats-Llobet, Maria A. Fernandez
CSIC-UPV, Valencia, Spain

ABA plays a crucial role for regulation of plant growth and development as well as plant response to both biotic and abiotic stress. Genome sequencing in crop plants has paved the way for the identification of core ABA signalling components in commercial crops. Sustainable production of food faces several key challenges, particularly the generation of new varieties with improved water use efficiency and drought tolerance. Different works have shown the potential applications of *Arabidopsis* PYR/PYL/RCAR ABA receptors to enhance plant drought resistance. We demonstrated that 35S:PYL4^{A194T} plants showed enhanced drought and dehydration resistance compared to non-transformed or 35S:PYL4 plants. In order to demonstrate the efficacy of mutant ABA receptors in crop plants, we generated barley (*Hordeum vulgare*) transgenic plants that over-express mutant versions of *Arabidopsis* PYL4 receptors. The demonstration of PYL4 technology in barley would be invaluable in pointing the way for other cereal crops of huge agricultural value, such as maize, wheat and rice. As a result, we found that barley transformed plants that express PYL4^{A194T} or PYL4^{H82R V97A} showed enhanced drought tolerance and therefore, we describe a novel technology to cope with water stress. We have also identified the full set of tomato (*Solanum lycopersicum*) ABA receptors, which were functional in *Arabidopsis* plant cells and over-expression of two monomeric-type receptors conferred enhanced drought resistance, which could not be achieved by over-expression of a tomato dimeric receptor. Gene expression analyses, chemical and transgenic approaches revealed distinct properties of tomato PYR/PYL/RCAR ABA receptors that might have biotechnological implications.

P115

Role of Mitochondrial and Endoplasmic Reticulum in Calcium Signaling, Using Genetically Encoded Probes in *Arabidopsis thaliana*

Giovanna Loro^{1,2}, Maria Cristina Bonza², Smrutisanjita Behera³, Andrea Wong¹, Ilaria Drago¹, Jörg Kudla³, Tullio Pozzan¹, Alex Costa², Michela Zottini¹

¹University of Padua, Padua, Italy, ²University of Milan, Milan, Italy, ³Universität Münster, Munster, Germany

Plants react to changing environmental conditions quickly inducing signal transduction pathways. A wide variety of signals, including abiotic, biotic, and developmental stimuli, were observed to evoke specific spatiotemporal calcium transients, further transduced into a transcriptional and metabolic response by Ca^{2+} sensor proteins. So far in plants, most of the research on Ca^{2+} signalling has been focused on the transport mechanisms for Ca^{2+} into and out of cytoplasm. Recent advances demonstrate how different organelles are involved in the process of Ca^{2+} signaling and their role in stress perception has been highlighted. In this work, we investigated the role of subcellular compartments in the calcium signature formation using the FRET-based genetically encoded probes Cameleons. Different lines of *A. thaliana* expressing Cameleon YC3.6 targeted to the mitochondria (4mt-YC3.6), and the D4 targeted to the endoplasmic reticulum (CRT-D4) were generated. The generation of lines expressing the YC4.6 Cameleon in the chloroplast is now underway. The correct localization of the probes and their constitutive expression were verified. In our experiments, seedlings roots were treated with different stimuli known to give calcium transients, such as extracellular ATP, glutamate and salt stress. Calcium dynamics were monitored in the cytoplasm, using the NES line, in the mitochondria and into the ER. An increase in calcium concentration, both in mitochondria and ER, was measured, meaning that the probes work correctly and their affinities are appropriate to Ca^{2+} levels. Our results suggest that these compartments play a role in the recovery of the $[\text{Ca}^{2+}]_{\text{cyt}}$.

P116

Differential Responses of Three Solanaceae Species to a Temperature Drop under Continuous Light

Tatjana Shibaeva, Elena Sherudilo

Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk, Karelia, Russia

The responses of tomato, eggplant and pepper plants to a temperature drop when grown under continuous light (CL) during transplant production were investigated in relation to physiological disorders, floral development and fruit yields. During first 5 (tomato) or 7 (eggplant, pepper) weeks control plants were grown under 16 h photoperiod and temperature 26/20°C (day/night); plants under 24 h photoperiod were grown at constant temperature of 26°C or treated daily by 2 h temperature drop to 10°C (CL+DROP). Afterwards plants were transplanted into plastic greenhouses and grown from May to August under natural photoperiod. Exposure of seedlings to CL resulted in leaf chlorosis in tomato, leaf chlorosis and necrosis in eggplant, and leaf deformities in pepper plants. CL significantly reduced dry weight and leaf area of tomato transplants and subsequently early fruit yield of greenhouse grown tomatoes. Eggplant transplants despite severe leaf injuries developed under CL had higher dry weight and leaf area compared to control plants. CL hastened flowering and fruit set and increased early and total fruit yields in eggplant. Transplant performance and fruit yield of pepper was also increased by CL despite delayed flowering and fruit set. A temperature drop treatment alleviated physiological disorders in plants, but CL+DROP plants of all three species did not show benefit in fruit yields compared to control plants, although DROP almost doubled early yield in CL-treated tomato plants. Possible mechanisms of differential responses of three Solanaceae species to CL and a temperature drop are to be discussed.

Supported by RFBR, research project 14-04-00840a.

P117

The Actin Cytoskeleton Is a Target for NaCl and Hydroxyl Radicals in *Arabidopsis thaliana* Root Cells

Gregory Pozhvanov¹, Sergei Medvedev¹, Kris Vissenberg², Vadim Demidchik³

¹Saint-Petersburg State University, Saint-Petersburg, Russia, ²University of Antwerp, Antwerpen, Belgium, ³Belarusian State University, Minsk, Belarus

The effect of NaCl on root cells is a prime cause of plant damage by salinity. In *Arabidopsis thaliana* root cells, early effects of NaCl (20-400 mM) include the elevation of cytosolic free Ca²⁺, generation of hydroxyl radicals and K⁺ efflux, which together trigger adaptation programmes, metabolic adjustment or a programmed cell death (Demidchik et al., 2014, DOI: 10.1093/jxb/eru004). Using *GFP-FABD2 Arabidopsis* roots and confocal microscopy, we have found that the cytoskeleton is another prime target for NaCl. Here, we have demonstrated that 100 mM NaCl delays or stops root growth and induces actin polymerization in the root elongation zone within 10 min. Microfilament angle distribution deviated from an initial orientation (predominantly axial) to a broad spectrum with peaks at 15°, 45° and 90°. This effect was prevented by addition of polyamines (spermine and spermidine), blockers of Ca²⁺-permeable cation channels and scavengers of hydroxyl radicals. Therefore, it was probably related to Ca²⁺ influx and hydroxyl radical generation. Addition of hydroxyl radical-generating mixture, containing 1 mM Cu²⁺, 1 mM L-ascorbic acid and 1 mM H₂O₂, resulted in qualitatively similar cytoskeleton rearrangements, which however were about ten times more rapid (as compared to NaCl-induced effect). Treatments with hydroxyl radical scavengers, polyamines, EGTA or by cation channel blockers delayed the hydroxyl radical-induced effect on the root cell cytoskeleton. These ameliorating treatments also rescued root growth arrest induced by NaCl or oxidative stress. We propose a hypothetical model that relates the observed cytoskeleton rearrangements with other early stress-induced physiological processes in plant cells.

P118

Cadmium-Induced Ethylene Production and Responses in *Arabidopsis thaliana* Rely on ACS2 and ACS6 Gene Expression

Kerim Schellingen¹, Dominique Van Der Straeten², Filip Vandenbussche², Els Prinsen³, Tony Remans¹, Jaco Vangronsveld¹, Ann Cuypers¹

¹Hasselt University, Hasselt, Belgium, ²Ghent University, Ghent, Belgium, ³University of Antwerp, Antwerp, Belgium

Industrial and agricultural activities have contributed to the dispersion of toxic metals, such as **cadmium** (Cd), in all ecosystem compartments. Growing on contaminated soils, plants can take up and accumulate Cd through their root system and transport it to the aboveground plant parts. This ultimately leads to the introduction of Cd into the food chain, eliciting threats to the public health.

Cadmium is a highly phytotoxic element that affects phytohormones, which play important roles as signal molecules in responses to environmental stimuli. **Ethylene** is involved in multiple molecular, biochemical and physiological processes during the entire lifecycle of the plant and is often considered as the 'stress hormone'. It is known that an increasing ethylene production can inhibit plant development and accelerate senescence and abscission processes.

The effects of Cd exposure on the production of ethylene and its precursor 1-aminocyclopropane-1-carboxylic acid (ACC), and on the expression of the ACC Synthase (ACS) and ACC Oxidase (ACO) multigene families were investigated in *Arabidopsis thaliana*. Increased ethylene release after Cd exposure was directly measurable and confirmed with enhanced levels of ACC and higher mRNA levels of ethylene responsive genes. ACC synthesis is the rate-limiting step in ethylene biosynthesis, and transcript levels of both ACS2 and ACS6 showed the highest increase and became the most abundant isoforms after Cd exposure, suggesting their importance in the Cd-induced increase of ethylene production. This was confirmed in *acs2-1acs6-1* double knockout mutants, which showed a decreased and delayed induction of ethylene production and expression of ethylene responsive genes.

P119

CO₂ Transport by PIP2 Aquaporins in Barley

Jiye Rhee^{1,2}, Izumi C. Mori², Maki Katsuhara², Jiri Santrucek¹

¹Faculty of Sciences, University of South Bohemia, Ceske Budejovice, Czech Republic, ²Institute of Plant Science and Resources, Okayama University, 2-20-1 Chuo, Kurashiki, Japan

CO₂ permeability of PIP2 aquaporins of *Hordeum vulgare* L. was investigated. Five PIP2 members were heterologously expressed in *Xenopus laevis* oocytes. CO₂ permeability was determined by decrease of cytosolic pH in CO₂-enriched buffer using a hydrogen ion-selective microelectrode. HvPIP2;1, HvPIP2;2, HvPIP2;3 and HvPIP2;5 facilitated CO₂ transport across the oocyte cell membrane. However, HvPIP2;4 that is highly homologous to HvPIP2;3 did not. The isoleucine residue at position 254 of HvPIP2;3 was conserved in PIP2 aquaporins of barley except HvPIP2;4, which possesses methionine instead. CO₂ permeability was lost by the substitution of the isoleucine-254 of HvPIP2;3 to methionine, while water permeability was not affected. These results suggest that PIP2 aquaporins permeate CO₂ and the conserved isoleucine at the end of the E-loop is crucial for CO₂ selectivity.

P120

Understanding Drought Response in Maize: Characterization of a Novel Transcription Factor Related to ABA Signaling

Elena Nájjar, Belmiro Vilela, Victoria Lumbreras, Montserrat Pagès
Centre for Research in Agricultural Genomics - CRAG, Barcelona, Spain

Abscisic acid (ABA) is the major phytohormone that mediates drought responses in land plants. ABA triggers several signaling transduction pathways that lead to an altered gene expression with effects on stress response and acquisition of tolerance. Protein kinases and phosphatases are of dominant importance in ABA signal transduction and among these proteins, a subgroup of the SnRK2s, including OST1, has been shown to be a major hub on the regulation of this pathway. When ABA levels increase, SnRK2 becomes active affecting other proteins and cellular processes, such as the activation of ABF transcription factors by phosphorylation affecting gene expression.

However, a full description of the ABA-induced transcriptional regulation may also include the identification of alternative transcriptional regulators SnRK2 dependent. We started this work cloning a maize SnRK2 gene aligned in the ABA activated group next to arabidopsis OST1 gene which is essential for the ABA dependent closure of stomata under drought stress. To identify new transcriptional targets affected by SnRK2 activity and involved in the regulation of drought inducible promoters we took a yeast two-hybrid approach using SnRK2 as bait against a library of drought stressed leaves. This technique yielded different potential targets and we started to further characterize a Zn-finger-type transcription factor with homology to an Arabidopsis protein, which is a regulator of proton responsive gene expression. We are currently characterizing the Zn-finger-type transcription factor interaction with SnRK2 and evaluating their function in ABA and in other abiotic and biotic stress responses.

P121

Characterization and Regulation of ZmOST1 Substrate: ZmAKS Transcription Factor

Agnese Rabissi, Belmiro Vilela, Victoria Lumbreras, Montserrat Pagés, Dolores Ludevid
CENTRE FOR RESEARCH IN AGRICULTURAL GENOMICS, Barcelona, Spain

Drought is the most limiting abiotic factor for plant growth worldwide and abscisic acid (ABA) is the major phytohormone mediating drought responses in land plants.

SnRK2 (sucrose non-fermenting 1-related protein kinases 2) proteins are plant specific kinases that together with the ABA ligands PYR/PYL/RCAR and the type 2C protein phosphatases constitute the central core of ABA perception and signal transduction. In Arabidopsis there is a growing number of reports describing SnRK2/OST1 (Open Stomata 1) function, and a conservative mechanism of ABA response between plant species has been proposed.

In our lab we previously cloned and characterized the OST1 from Zea mays and established its role on ABA signaling. By yeast two-hybrid using ZmOST1 as bait against a library of drought stressed leaves we identified a bHLH transcription factor (TF) that is a potential target of ZmOST1. Here we describe the work we have been performing in the characterization of this TF, which is homologous to the Arabidopsis AKS2 (ABA Responsive Kinase Substrate), involved in stomatal movements and regulated by OST1.

We established that ZmAKS is a nuclear protein capable of dimerization, that it interacts with and is a substrate of ZmOST1, that it can bind to an E-box like nucleotide sequence, and that it promotes stomatal aperture. We are currently studying its promoter sequence to define ZmAKS expression in the whole plant. Finally we have generated transgenic plants to carry out functional and phenotypic analysis.

Our results suggest a novel OST1 regulatory pathway during osmotic stress signaling.

P122

What Enzyme Activity Is Managed by Phospholipase C?

Jitka Brouzdova, Zuzana Krckova, Jan Martinec
Institute of Experimental Botany AS CR, Prague, Czech Republic

Phospholipases are key components of the plant phospholipid signalling network. Stimulation of the signalling network is involved in many responses of plants to both biotic and abiotic stress. The new member of phospholipases family is nonspecific phospholipase C (NPC), which was recently described in plants. Six putative isoforms (NPC1-NPC6) has been identified in *Arabidopsis*. Only two of them, NPC4 and NPC5 have been further characterized to date. They are phosphatidylcholine hydrolysing enzymes that cleave common membrane phospholipids such as phosphatidylcholine (PC) to phosphocholine and diacylglycerol (DAG). The objective of this project is to further investigate this protein family by functional characterization of NPC1, NPC2 and NPC6.

Coding regions of AtNPC cDNAs was ligated in-frame into expressing vector pET30 (Novagen) by PCR mediated introduction of appropriate restriction sites. Constructs coding for fusion proteins with His6 tag on C or N-terminal was introduced into *E.coli* BL21 cells. Expressed recombinant protein was purified by affinity chromatography. Fluorescently labelled substrates was used for determination of NPC activity, formation of fluorescently labelled products was measured after separation on HP-TLC.

First we prepared NPC4, which activity has been already published, but it serve as a positive control of our method. We observed the expected conversion of PC to DAG, but also the emergence of unknown product, which is ultimately stronger than DAG. We also successfully produced NPC1, 2 and 6 expression protein. Biochemical characterisation of these proteins will be presented as well.

P123

Molecular Characterization of Fleshy Fruit Responses to Low Oxygen

Dubravka Cukrov, Stefano Brizzolara, Francesco Licausi, Pietro Tonutti
Institute of Life Science, Scuola Superiore Sant'Anna, Pisa, Italy

A decrease of oxygen concentration may occur inside the fruit in correspondence of the last stages (ripening) of fruit development. Additionally, in order to prolong commercial life, some fruits (e.g. apples) can be stored under extreme low oxygen conditions (Controlled Atmosphere) and the modulation of the oxygen level is crucial for a successful storage and the maintenance of quality. Very little is known on the metabolic reset and the molecular regulatory mechanisms activated in fruit tissues under hypoxic conditions. The effects of prolonged hypoxic treatments (0.4 and 0.8 kPa oxygen) applied after harvest have been compared in Granny Smith apples. A transcriptome-wide analysis using RNA-seq (Illumina) revealed that, in cortex tissue, the different hypoxic conditions selectively affect the expression of several hundred genes including those involved in primary metabolism (glycolysis, fermentation). Based on the transcriptomic analysis, secondary metabolic pathways (e.g. phenylpropanoid) also seem to be altered by low oxygen treatment. A biochemical survey indicated that different hypoxic conditions affected the accumulation of specific metabolites, such as ethanol, alanine, asparagine and aspartate, which thereby represent possible markers. Members of the ERF-VII transcription factors (TFs) family displayed differential expression suggesting their involvement in the modulation of hypoxic responses, as observed in model species. Using tomato plants, a reverse genetics approach aimed at affecting the stability of these TFs has been undertaken to elucidate their role in fruit development in general and, more specifically, in the onset and evolution of ripening on- and off-plant.

P124

Characteristics of Carbon and Nitrogen Metabolism in Barley Genotypes under Different Nitrogen Supply and Drought Conditions

Szilvia Veres¹, Marko Petek², Brigitta Tóth¹, Zed Rengel³

¹*Crop Science, Debrecen, Hungary*, ²*Plant Nutrition, Zagreb, Croatia*, ³*School of Earth and Environment, Perth, Australia*

The interaction between carbon and nitrogen assimilation and their efficiency are key importance for plant production. Applying optimal nitrogen amount at total genetic potential, more carbon assimilation per unit nitrogen would increase biomass. The supply of nitrogen is crucial for leaf growth and the role of nitrogen in agricultural production is intimately connected with photosynthesis. Photosynthetic rate correlates closely with leaf nitrogen content in several plants. At the same time low photosynthetic potential per gram of leaf does not directly cause the slow growth of nitrogen deficient plants. Previous studies focused on investigating mainly the intensity and products of dark reaction of the photosynthetic pathway. Less result have about light reaction affected by genotypic and nitrogen supply variations, mainly under stress conditions.

The main goal of this research was to investigate some physiological parameters of barley genotypes under two nitrogen contents and water deprivation conditions at anthesis. Dry weight of plants was determined with thermo-gravimetric method. Relative chlorophyll content measurement was applied to follow the total chlorophyll contents of leaves. The water status of plants was established by measuring relative water content (RWC) of leaves. Chlorophyll fluorescence induction method was used to examine potential photochemical activity (Fv/Fm). Additionally, parameters of slow phase of chlorophyll fluorescence induction curve also were determined, such as 1-qP/NPQ. The values of 1-qP/NPQ characterised the intrinsic ability of PSII to balance photochemical and non-photochemical quenching. Measuring this parameter is a good tool for get information about stress plasticity of plants under non-optimal conditions.

P125

Age and Ecotype Override Effects of Abiotic Stressors on Extractable Antioxidants of *Sonchus oleraceus* L.

S.M.M.R. Mawalagedera^{1,2}, Kevin S. Gould¹

¹*School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand,*

²*Department of Plant Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka*

Leaves of *Sonchus oleraceus* L., a traditional component of the Māori diet in New Zealand, are unusually rich sources of assorted phenolic compounds that show high antioxidant activity *in vitro*. Extractable antioxidant activities vary considerably among plants from natural populations, though the underlying causes of this variation are unknown. To study possible ontogenetic and/or environmental causes of this variation, plants of different ages from two New Zealand ecotypes (ACB and OAM) were exposed to chilling, salinity or the combination of both. Antioxidant activities, ascorbate levels and total phenolic contents were quantified, and HPLC profiles obtained for methanolic extracts of treated leaves. All measurements were consistently higher in older than in younger plants, irrespective of ecotype or treatment. ACB was more concentrated in hydroxycinnamic acids and ascorbate, and displayed higher antioxidant activities compared to OAM: a trait that was maintained across generations. For the younger plants, the imposition of stressors effectively augmented levels of chlorogenic acid in leaves, but did not increase the extractable antioxidant capacities. The data indicate that plant age and ecotype, rather than exposure to abiotic stressors, are the primary determinants of antioxidant potential in *S. oleraceus*.

P126

Patterns of Night-Time Water Use Are Interrelated with Leaf Nitrogen Concentration in Shoots of 16 Deciduous Woody Species

Priit Kupper, Gristin Rohula
University of Tartu, Tartu, Estonia

Although the impact of nitrogen availability on the night-time water relations of plants has received a lot of attention during the last decade, knowledge of how these two traits are interrelated is contradictory and still limited. The aim of the current study was to investigate the impact of leaf nitrogen concentration on night-time (E_n) and daytime (E_d) transpiration rate, nightly water-use percentage of daytime water use (NWU), and increase in night-time transpiration rate (INT) in artificial predawn hours in the cut shoots of 16 woody species measured in the controlled conditions of a growth chamber. Two distinct patterns of night-time water use associated with leaf nitrogen concentration ([N]) were observed: shoots with high NWU were characterised by significantly ($P < 0.05$) lower [N], whereas shoots with highest INT had greater [N]. The species from sub-family *Betuloideae* demonstrated strong positive relationship between INT and leaf net photosynthesis ($R^2 = 0.99$; $P < 0.01$) and dark respiration ($R^2 = 0.90$; $P < 0.05$), suggesting that INT associates with variables that can support intensive growth. Our results suggest that high NWU could potentially compensate limited nitrogen uptake in species able to grow in nutrient-poor habitats. Furthermore, night-time stomatal regulation mechanisms may differ between species according to their [N] and this may explain the contradictory results between previous studies.

P127

Novel Grapevine miRNAs Regulated by Drought Stress

Chiara Pagliarani, Diana Alberto, Manuela Ferrero, Andrea Schubert
University of Turin Dept Agricultural, Forestry and Food Sciences, Grugliasco, Italy

Plant microRNAs (miRNAs) play a pivotal role in stress responses. We investigated the effects of drought stress on miRNA abundance in leaf and root of *Vitis vinifera* Cabernet Sauvignon (CS), of the M4 rootstock (*Vitis vinifera* X *Vitis berlandieri*), and of their auto- and reciprocal grafts.

Plants were droughted for 12 days. Leaf stomatal conductance and water potential were monitored, in order to sample at $g_s < 0.05 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and $\Psi_{\text{leaf}} \sim -1.4 \text{ MPa}$.

Low MW RNA was extracted in triplicate from stressed and control plants, to assess expression of 12 novel putative miRNAs by qRT-PCR.

Most of the putative miRNAs were unevenly expressed in leaf and root tissue, in particular n_191 and n_327. Drought stress significantly reduced expression of n_22 (targeting an ABC transporter), and of n_191 (targeting a protein kinase), while it increased expression of n_312 (no identified target). In the case n_312, drought affected expression in leaves only.

For n_191 and n_312, concentration was overall lower in CS than in M4 auto-grafted plants, and this was confirmed in reciprocal grafts. On the contrary, n_55 (targeting a protein kinase) and n_327 (targeting an SPL-like protein) showed no expression differences between genotypes in auto-grafted plants, but were more expressed respectively in M4 and CS shoots in grafted plants, in particular upon drought stress.

These results show that grape miRNAs are regulated by drought stress. Concentrations of miRNAs are affected by genotype and graft combination, opening the possibility that some may be mobile between stock and scion.

P129

Hormonal Profile and Antioxidant Defense System of *Brassica rapa* Plants during Drought and Recovery Period

Iva Pavlovic¹, Jutta Ludwig-Müller², Branka Salopek-Sondi¹

¹Ruder Boskovic Institute, Zagreb, Croatia, ²Institute of Botany, Dresden University of Technology, Dresden, Germany

Understanding the plant response and tolerance to drought presents important research issue. Drought often causes significant agricultural losses during lack of rainfall in hot and dry year periods. To overcome a problems caused by such unpleasant environment plants had developed defense system through hormonal and antioxidant responses. With aim to investigate response of *Brassica rapa* L. ssp. *pekinensis* to drought, three week old plants were subjected to 7 days of non watering period. One set of plants was harvested after 7 days of drought conditions, while other set was re-watered and harvested after 24 hours. Both sets of treated plants together with corresponding controls were subjected to biochemical and hormonal analysis. Activities of antioxidant enzymes (catalase, guaiacol peroxidase and ascorbate peroxidase) were altered upon drought and recovery period and were higher in comparison to the control. Levels of reduced GSH as well as the MDA content significantly increased during drought period and remain elevated upon recovery. Drought treatment also affected hormonal status of plants. ABA level was 2.6 times higher in drought stressed plants while plants after recovery period contained physiological levels of ABA so as the controls. Furthermore, both, drought and recovery plants accumulated higher level of conjugated auxin than corresponding controls with tendency of the decrease upon recovery period. Obtained results suggest the significance of measured parameters in *Brassica rapa* plant defense and tolerance to drought.

Key words: drought, glutathione, MDA, abscisic acid, auxin

P130

Sound Wave Improve Drought and Salt Tolerance in Rice Plant in Wavelength Specific Manner

Mi-Jeong Jeong, Soo-Chul Park, Seung Kon Lee, Taek Ryouon Kwon, Soo In Lee, Jin A Kim, Sung-Han Park

Rural Development Administration(RDA), Suwon, Gyeonggido, Republic of Korea

We investigated whether sound waves could improve salt tolerance in rice seedling. The rice seedlings were sound treated with 800 Hz for 1hr, and then treated with 0, 75, 150, and 225mM NaCl for 3 days to observe changes in physiological and morphological aspects. Sound treatment seedlings resulted in enhanced salt stress tolerance, mainly demonstrated by the sound treated seedlings exhibiting of increased root relative water contents (RWC), root length and weight, photochemical efficiency (ratio of variable to maximum fluorescence, F_v/F_m), and germination rate under salt stress condition. This demonstrates that a specific sound wave might be used, not only to alter gene expression in plant, but also to improve salt stress tolerance. In order to test the sound's effect on plant and its contribution in drought tolerance, plants were subjected to various sound frequencies for an hrs. After 24-hrs sound treatment, plants were exposed to drought for next five days. During the experiment it was observed that sound initiated physiological changes showing tolerance in plant. Sound frequency with ≥ 0.8 kHz enhanced relative water content, stomatal conductance and quantum yield of PSII (F_v/F_m ratio) in drought stress environment. Hydrogen peroxide (H_2O_2) production in sound treated plant was declined compared to control. *ThermaCAM* (Infra-red camera) a software which was used to analyze the plant images temperature showed that sound treated plant and leaf had less temperature (heat) compared to control. The physiological mechanism of sound frequencies induce tolerance in rice plants are discussed.

P131

Response of 1-Node-Cuttings of *Arundo donax* Clones to Salinity Stress Conditions

Maria Dolores Curt, Irene Barquero, Marina Sanz, Jesus Fernandez
Technical University of Madrid (UPM), Madrid, Spain

Arundo donax L, a perennial grass originated from the circum-Mediterranean-Asian area, has received much attention from researchers. On the one hand, it has been considered an invasive species in riparian ecosystems; on the other hand, it has been proposed as an energy crop for biomass production in marginal lands. From both fields of interest, a key issue is the species propagation. In the frame of the EU OPTIMA project (www.optimafp7.eu) a specific task was proposed to evaluate the potential of *Arundo* to be grown for biomass production under salinity stress conditions. In the present work, we investigated the response of stem cuttings of *A. donax* to NaCl concentration in order to assess the propagation capacity of 10 clones of different origin. One-node-cuttings were prepared and placed separately in water solutions of increasing NaCl concentrations. Five treatments from 0% (control) to 2% were tested with 10 replications. The growth stage of cuttings was weekly recorded. Five growth stages: tight bud, bud swell, bud break, shoot expansion and visible roots were identified and a quantitative method of evaluation was developed for clone comparison. In our experimental conditions, no rooting was noticed for treatments > 1%; however, cuttings progressed to the stage of bud break when subjected to more concentrated treatments. Regarding the influence of clone origin on propagation capacity, the index developed for this work showed that salt tolerance is clone-dependent. Two ecotypes from Greece stood out among the ten clones tested for their tolerance to the salinity stress conditions.

P132

Roles of Abscisicacid (ABA) Andethylene in Regulating Stomatal Conductance under Atmospheric and Soil Water Deficit

Guzel Kudoyarova¹, Dmitry Veselov¹, Guzel Sharipova¹, Alla Korobova¹, Ian Dodd², Lin Chen², Sally Wilkinson², William Davies²

¹*Institute of Biology, Ufa, Russia,* ²*Lancaster University, Lancaster, UK*

Although stomatal closure limits crop water loss during drought, carbon gain often decreases. In some published work, plants that maintain transpiration under mild drought show higher productivity (Collins et al., 2008). Effects of atmospheric drought on 15 wheat and barley cultivars were studied under fully irrigated field conditions. At tillering, increasing atmospheric VPD from 0.5 to 1.5 kPa caused stomatal closure of 6 cultivars, while the rest maintained their stomatal conductance. The possible involvement of ethylene and ABA in this contrasting stomatal behaviour was tested in hydroponics and sand culture, where the field stomatal responses were reproduced. Effects of inhibiting ethylene perception were studied by applying 1-MCP to wheat plants after withholding water. The loss of ethylene sensitivity resulted in faster and greater stomatal closure at similar soil water content due to greater foliar ABA accumulation, accompanied by higher RWC, but lower biomass accumulation. This effect was not detected in aged wheat leaves, where ethylene antagonized ABA-induced stomatal closure (Chen et al., 2013). MCP treatment also decreased stomatal conductance at increased VPD in wild type barley, while producing no effect on its ABA deficient mutant *Az34*. Wheat cultivars that had greater production of ethylene maintained higher transpiration during soil water deficit. The results suggest that ethylene maintains stomatal opening by preventing ABA accumulation and/or antagonising ABA-induced stomatal closure under water shortage. This hormonal interaction may be important for regulating stomatal conductance under both atmospheric and soil drought and have an important influence on yield.

P133

Nanoparticles and Plants - Friends or Foe?

Tomas Vanek, Radka Podlipna, Premysl Landa
Institute of Experimental Botany ASCR, Prague, Czech Republic

The use of nanoparticles (NPs) in commercial products and industrial applications has increased greatly in recent years although understanding of the interaction mechanisms at the molecular level between NPs and biological systems, is largely lacking.

Our work is focused to the study of effect of nanoparticles to the higher plant metabolisms, both in laboratory and real conditions to elucidate potential of phytoremediation methodology for removing NPs from environment. In laboratory conditions nanoparticles of TiO₂, ZnO₂, AlO₂, Fullerenes and Graphite fibers were tested using *Nicotiana tobacco* cells BY2 and *Arabidopsis thaliana* as a model systems.

Generally, all nanoparticles decreases plant cells viability - the most toxic effect was found for ZnO₂, where only 67% of starting viability was detected. All nanoparticles (with exception of graphite fibers) decreased the production of ethylene. Part of their unfavorable effects might be disturbance of defense pathways in tobacco cells, probably via disturbance of ion homeostasis. All nanoparticles exhibit negative effect on cell division and stimulate various stress responses, e.g. antioxidant system and ethylene formation, while microarray data (1) confirm stimulation of antioxidant system as well as general stress response and down-regulation of genes related to cell division.

This work was supported by LH11047 and LD 14100 projects.

1) Landa, P., Vaňková, R., Andrlová, J., Hodek, J. Maršík, P., Štorchová, H., White, J. C. Vaněk, T. *Journal of Hazardous Materials*. 241, (2012), p. 55-62

P134

***Arundo donax* Vs Drought Stress: The Power of Drought Tolerance at Early Stages**

Romero-Munar Antònia, Achir Chahira, Baraza Elena, Cifre Josep, Gulías Javier
University of Balearic Islands (UIB), Palma de Mallorca, Balearic Islands, Spain

Giant reed (*Arundo donax* L.) is a perennial rhizomatous grass that is considered an interesting crop for bioenergy production under Mediterranean conditions. In a climate change scenario, it is interesting to study the response of this species to drought at early stages is a key point to evaluate its viability as a bioenergy crop in large areas of the Mediterranean Basin. The objective of this study was to evaluate giant reed response to drought stress at early stages, determining the effects of limited soil water availability on plant growth, gas exchange and plant water use efficiency (WUE). Plantlets of a commercial clone were grown under greenhouse conditions at Mallorca, West Mediterranean Basin. After 40 days under soil field capacity, two water treatments were imposed: well-watered (100% of field capacity) and progressive drought during 62 days. Soil water content, leaf elongation and appearance rates, plant water consumption and gas exchange parameters were measured along the experiment. Total plant biomass, leaf water and osmotic potential were determined at the end of the experiment to estimate plant WUE.

The main conclusions of this work are that plant growth and leaf gas exchange parameters were significantly affected by soil water availability, but only when this was below 40%. The reduction of plant leaf area under water-limited conditions was mainly due to a reduction of both the number of shoots and leaf size. The decrease of osmotic potential under progressive drought suggests that osmotic adjustment may play a role in giant reed response to drought.

P135

Through the «Omics» to the Physiological Function of Grape ASR in Sugar Metabolism, Sugar Transport and Sugar Signaling

Jonathan Parrilla¹, Anna Medici¹, Cécile Gaillard¹, Mickaël Maucourt², Thomas Schneider³, Jeremy Verbeke¹, Bernd Roschitzki⁴, Maryse Laloï¹, Dominique Rolin², Enrico Martinoia³, Rossitza Atanassova¹

¹Université de Poitiers, UMR CNRS 7267 Écologie & Biologie des Interactions, Équipe Physiologie moléculaire du transport de sucres, Poitiers, France, ²Université de Bordeaux Segalen et Plateforme Métabolome Fluxome, Centre INRA de Bordeaux, Bordeaux, France, ³Institute of Plant Biology, University of Zurich, Zurich, Switzerland, ⁴Functional Genomic Center Zurich, Federal Institute of Technology, University of Zurich, Zurich, Switzerland

Vitis vinifera hexose transporter VvHT1 was the first identified sugar carrier whose gene is regulated by its own substrate, the glucose. Our search for transcription factors acting on VvHT1 promoter resulted in the identification of the grape ASR (Abscisic acid, Stress and Ripening), VvMSA. RNAi suppression of VvMSA strongly inhibited VvHT1 gene expression and glucose absorption in grape cells. To better understand the complex relationship between VvHT1 and VvMSA, we created embryogenic and non-embryogenic grape cell suspensions, sharing the same genetic background, but displaying different growth rates, related to specific requirement for carbon sources. A global metabolomic approach by 1H-NMR was performed on both cells and culture media, providing evidence for two cell models with distinct sugar metabolisms. In this regard, wild type (WT), VvMSA-overexpressing and VvMSA-RNAi repressed cells were treated with glucose analogues, differentially triggering glucose sensing and signalling. The expression profiles of VvHT1 and marker genes argue in favour of at least two different glucose-signalling pathways involved in VvHT1 expression regulation, the hexokinase-dependent and the glycolysis-dependent pathways. The glycolysis-dependent pathway involves VvMSA as transcription regulator of VvHT1. To further elucidate the biological functions of ASRs, a quantitative proteomic analysis (iTRAQ) was performed comparing the nuclear proteins of WT and VvMSA-RNAi cells. Among the 1058 identified proteins, 14 candidates were differentially expressed between WT and VvMSA-silenced cells. The functional duality of ASRs as chaperones for direct protection and as transcriptional regulators was further discussed in relation to epigenetic regulation of gene expression.

P136

Oxidative Stress Associated with Rootstock-Scion Interactions

Patricia Irisarri Sarto¹, Ana Sobrino Pina¹, Piotr Binczycki², Helle Juel Martens²

¹Unidad de Fruticultura CITA-DGA, Zaragoza, Spain, ²University of Copenhagen, Faculty of Science, Department of Plant and Environmental Sciences, Copenhagen, Denmark

Exposing a plant to stress situations, such as grafting, generally triggers antioxidant defense systems. In fruit tree grafting, quince (*Cydonia oblonga*) is widely used as a rootstock for pear (*Pyrus communis*), but some economically important pear cultivars are incompatible with some available quince rootstocks. We show that specific antioxidant isoforms are involved in the compatibility processes. Our work is based on an *in vitro* callus graft system mimicking the events taking place in fruit tree grafts. Certain early reactions, like adhesion of the graft partners after deposition of cell wall material between the opposing cut surfaces, and the formation of new callus, are common wound responses that occur passively in grafts regardless of compatibility relationship between the graft partners. However, several days after grafting lower levels of antioxidant enzyme activities were measured at the graft interface in incompatible unions when compared with compatible and ungrafted tissue. This finding, together with live cell imaging of ROS-specific probes, ultrastructural mitochondrial changes, necrotic cells and DNA fragmentation related to apoptotic processes give indications that within incompatible rootstock/scion interfaces either the level of reactive oxygen species (ROS) is increased or a less efficient detoxification system is initiated here.

Keywords: ROS, antioxidant enzymes, graft compatibility, pear, quince, callus culture.

P137

Chl a Fluorescence and Photosynthetic Response of Chinese Cabbage to High Temperature in Field Studies Using Temperature Gradient Tunnels

Soonja Oh^{1,2}, Kyung Hwan Moon², In-Chang Son², Eun Young Song², Young Eel Moon³, Seok Chan Koh⁴

¹Environmental Research and Education Institute, Jeju, Republic of Korea, ²Agricultural Research Center for Climate Change, RDA, Jeju, Republic of Korea, ³Citrus Research Station, RDA, Jeju, Republic of Korea, ⁴Department of Biology, Jeju National University, Jeju, Republic of Korea

In order to gain insight into the physiological responses of plants to high temperature, the effects of temperature on Chinese cabbage (*Brassica campestris* subsp. *napus* var. *pekinensis* cv. Detong) were investigated through analyses of photosynthesis and fast chl a fluorescence transients, under 3 different temperatures in the temperature gradient tunnel. In the rosette stage, growth was greater at ambient +4°C and +7°C than at ambient temperature. Photosynthetic CO₂ fixation rates of cabbage grown under the different temperatures did not differ significantly. However, dark respiration was significantly higher in the cabbage that developed under ambient temperature relative to elevated temperature. The chl a fluorescence transient was also considerably affected by high temperature; the fluorescence yield F_J , F_I , and F_P decreased considerably at ambient +4°C and +7°C, with induction of F_K and decrease of F_V/F_O . The values of RC/CS, ABS/CS, TRo/CS, and ETo/CS decreased considerably, while Dlo/CS increased with increased growth temperature. The symptoms of soft-rot disease were observed in the inner part of the cabbage heads after 7, 9, and/or 10 weeks of cultivation at ambient +4°C and +7°C, but not in the cabbage heads growing at ambient temperature. These results show that Chinese cabbage could be negatively affected by high temperature under a future climate change scenario. Therefore, to maintain the high productivity and quality of Chinese cabbage, it may be necessary to develop new high temperature tolerant breeds or to markedly improve cropping systems.

Additional key words: Chinese cabbage, high temperature, photosynthetic CO₂ fixation rate, chl a fluorescence transient, temperature gradient tunnel.

P138

Winter PSII Photochemical Efficiency and Proteomics of the Needles from Korean Fir (*Abies koreana* W.) in Subalpine Zone of Mt. Halla, Korea

Soonja Oh^{1,2}, Seok Chan Koh³

¹Environmental Research and Education Institute, Jeju, Republic of Korea, ²Agricultural Research Center for Climate Change, RDA, Jeju, Republic of Korea, ³Department of Biology, Jeju National University, Jeju, Republic of Korea

Korean fir (*Abies koreana* W.) is an endemic tree species that is facing population declines in Korea. It was recorded as being in the endangered category in the IUCN Red list of threatened species. However, few studies have examined photosynthesis, including PSII photochemical efficiency, and proteomics of Korean fir needles. In this study, we used chlorophyll fluorescence transients analysis and 2-DE followed by MALDI TOF/TOFTMMS/MS analysis and Mascot analysis to visualize the specific protein profiles in the acclimation to extreme low temperature in winter. Intrinsic PSII efficiency (Fv/Fm) was at maximal levels in the summer, then decreased in the fall and remained below 0.2 in the winter. The low levels of intrinsic PS II efficiencies in the winter were accompanied by the strongest quenching of Fm and Fo. From the 2-DE protein profiles of fir needles, the 226 protein spots were detected, and most of them were significantly accumulated during the winter season. Among these protein spots, 31 protein spots were identified by MALDI TOF/TOFTMMS/MS analysis, and 13 of protein spots matched proteins associated with biotic and abiotic stresses found in other plants. Superoxide dismutase and peroxidase were accumulated at high levels in winter, suggesting that fir needles have defense mechanisms against cold/freezing and/or high light in winter. These results are consistent with down-regulation of light-harvesting efficiency involving retention of high zeaxanthin levels, and locked-in photo-protective thermal dissipation from low chlorophyll fluorescence emission in mid-winter in our previous study.

Additional key words: Korean fir, intrinsic PS II efficiency (Fv/Fm), 2-DE protein profile, superoxide dismutase, peroxidase, cold/freeze stress

P139

Effects of Heavy Ion Irradiation on Growth, Anatomy and Photosynthetic Efficiency of Mung Bean Plants

Carmen Arena¹, Chiara Romeo Di Tuosto¹, Bruno Hay Mele¹, Pia Rosa Cataletto¹, Veronica De Micco²

¹Department of Biology, University of Naples Federico II, Naples, Italy, ²Department of Agricultural and Food Sciences, University of Naples Federico II, Portici, Naples, Italy

Heavy ions may affect plants at molecular, morpho-anatomical and physiological levels. They are more effective than low-LET radiation in inducing changes on physiology and development of plants during various phases of their life cycle. In this work, dry seeds of the species *Dolichos melanophthalmus* DC. (mung dwarf variety) were exposed to Ti-ion and C-ion at two doses (1 Gy and 10 Gy) to assess the effects on: a) growth, by monitoring germination rate, seedling development, plant height, leaflets, pods and seeds number, as well as functional leaf traits and b) the integrity of photosynthetic apparatus, by determining photosynthetic pigment composition and photochemistry. Germination rate of controls was higher than irradiated seeds; among treatments C-ion irradiated seeds showed the lowest values. Survived seedlings from irradiated seeds showed differences in hypocotyls and radicles length compared to control; moreover, quantitative anatomy suggested different starch metabolism after irradiation. Among treatments, C-ion 1 Gy irradiated seeds produced plants showing the lowest number of leaves, pods and seeds. Photochemistry and functional leaf traits did not vary among samples, but total chlorophyll and carotenoid content was increased significantly in irradiated compared to not-irradiated plants. Overall results showed that all plants from irradiated seeds reached the reproductive stage completing successfully the seed-to seed cycle, thus suggesting the high radioresistance of this species. This property indicates *D. melanophthalmus* as a good candidate to growth on Bioregenerative Life Support Systems (BLSSs) for sustaining human nutritional needs in the Space.

P140

The Role of Different Doses of X-rays on *Solanum lycopersicum* L. Plants: Effects on DNA and Photochemical Activity

Carmen Arena¹, Veronica De Micco², Pia Rosa Cataletto¹, Bruno Hay Mele¹, Ermenegilda Vitale¹,
Valentina Morra¹, Sergio Esposito¹, Mimmo Turano¹

¹Department of Biology, University of Naples Federico II, Naples, Italy, ²Department of Agricultural and Food Sciences, University of Naples Federico II, Portici, Naples, Italy

The ability of plants to provide a source of food and to recycle carbon dioxide into oxygen is important for long duration manned space missions. However, plant survival in space is constrained by several factors including ionizing radiation which can induce different effects on growth, metabolism and reproduction. The effects of ionizing radiation on plants vary according to the type of radiation, dose and plant features such as: species, developmental stage and genetic traits. In general, detrimental effects increase with dose, whereas low doses may have positive outcomes. This study explores the effect of different X-rays doses (0.3, 10, 20, 50, 100 Gy) on growth, photosynthesis and leaf anatomy. Specifically, photochemical efficiency and photosynthetic pigment content were analysed. Moreover, the relationship between phenotypic changes and radiation-induced DNA mutations were explored. DNA alterations were detected through Random Amplified Polymorphic DNA (RAPD). Irradiation treatments and analyses were carried out on leaves of *Solanum lycopersicum* L. 'Microtom', during the reproductive phase. Photochemical efficiency decreased significantly at high doses, but no differences were detected for pigment content at different doses. In irradiated plants, RAPD profiles showed the presence of polymorphic bands which are not observed in not-irradiated controls. More specifically, a dendrogram based on Nei Li genetic similarity coefficients shows two clusters: one containing not-irradiated plants, the other the irradiated samples. Overall results suggest that X-rays have a mutagenic effect on tomato plants not related to negative effects on photosynthesis. Gained knowledge will help scientists to identify plants able to endure space conditions.

P141

Expression of Some Stress-Induced Proteins during Desiccation and after Rehydration of Sun- and Shade-Adapted Resurrection Plant *Haberlea rhodopensis*

Gergana Mihailova¹, Éva Sárvári², Ádám Solti², Éva Hunyadi-Gulyás³, Katya Georgieva¹

¹Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria,

²Department of Plant Physiology and Molecular Plant Biology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary, ³Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

The changes in some HSPs and dehydrins during dehydration and after rehydration of the homoiochlorophyllous resurrection plants *Haberlea rhodopensis* were investigated. Plants growing at low ($25 \mu\text{mol m}^{-2} \text{s}^{-1}$) or high ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) light intensities were used. Well-watered plants and plants dehydrated to different extent in natural conditions were studied. Rehydration of plants desiccated to air-dry state was carried out in laboratory conditions.

The results showed that the expression of HSPs proteins differed significantly during desiccation of the sun- and shade-adapted plants. The immunoblot analysis after 1D SDS-PAGE revealed the presence of sHSPs proteins only in severely dehydrated (20% RWC) and desiccated (8% RWC) shade plants, whereas in sun plants the sHSPs bands appeared in control, dehydrated and rehydrated stages, but the expression pattern between them was quantitatively and qualitatively different. A HSP70-like protein, identified by mass spectrometry, however, accumulated more strongly in severely dehydrated and desiccated shade-adapted plants.

The results from the Western blot using antibodies raised against dehydrins showed a similar expression pattern in sun and shade plants. Twelve cross reacting polypeptide bands differently represented at different stage of dehydration and after rehydration were found. The proteins were constitutively expressed in control sun and shade plants. Similarly to the HSPs, the dehydrins were more expressed at 20% and 8% RWC.

ACKNOWLEDGEMENTS

This work was supported by the grant № BG051PO001-3.3.06-0025, financed by the European Social Fund and Operational Programme Human Resources Development (2007–2013) and co-financed by Bulgarian Ministry of Education and Science.

P142

Crosstalk between Jasmonates and Abscisic Acid in Roots of Arabidopsis under Water Stress Conditions

Carlos de Ollas, Vicent Arbona, Aurelio Gómez-Cadenas

Departamento de Ciencias Agrarias y del Medio Natural, Universitat Jaume I, Castelló de la Plana, Spain

Phytohormones are central players in sensing and signaling numerous environmental conditions like drought. In this work, hormone profiling together with gene expression of key enzymes involved in abscisic acid (ABA) and jasmonic acid (JA) biosynthesis was studied in roots of Arabidopsis plants under water stress conditions. JA concentration transiently increased after stress imposition whereas progressive and concomitant ABA and Jasmonoyl Isoleucine (JA-Ile) accumulations were observed. Molecular data suggest that, at least, part of the hormonal regulation takes place at the biosynthetic level. These observations also point to a possible involvement of JA on ABA biosynthesis under stress. To test this hypothesis, mutants impaired in JA-biosynthesis and in JA-dependent signaling were employed. Results showed that the early JA accumulation leading to JA-Ile build up was necessary for the subsequent ABA increase in roots under stress whereas the opposite could not be stated. Signal transduction between water stress-induced JA-Ile accumulation and COI1 is necessary for a full induction of the ABA biosynthesis pathway and subsequent hormone accumulation in roots of Arabidopsis plants. The present work adds a new level of interaction between JA and ABA at the biosynthetic level that together with the previously described interaction between their signal transduction cascades would allow plants to fine-tune specific responses to different stimuli.

P143

Proteome Analysis of Cold Response in Spring and Winter Wheat (*Triticum aestivum*) Crowns Reveals Similarities in Stress Adaptation and Differences in Regulatory Processes between the Growth Habits

Klára Kosová¹, Pavel Vítámvás¹, Sébastien Planchon², Jenny Renaut², Radomíra Vanková³, Ilja Tom Prášil¹

¹*Crop Research Institute, Prague 6 - Ruzyne, Czech Republic,* ²*Centre de Recherche Public Gabriel Lippmann, Belvaux, Luxembourg,* ³*Institute of Experimental Botany, Academy of Science, Prague 6, Czech Republic*

A proteomic response to cold (4 °C) treatment has been studied in crowns of frost-tolerant winter wheat cultivar Samanta and frost-sensitive spring wheat cultivar Sandra after short-term (3 days) and long-term (21 days) cold treatments. Densitometric analysis of two-dimensional differential gel electrophoresis (2D-DIGE) gels has resulted in detection of 386 differentially abundant protein spots revealing at least 2-fold change between experimental variants. Of these, 58 representative protein spots have been selected for MALDI-TOF/TOF identification and 36 proteins have been identified. The identified proteins revealing an increased relative abundance upon cold in both growth habits include proteins involved in carbohydrate catabolism (glycolysis enzymes), redox metabolism (thioredoxin-dependent peroxidase), chaperones as well as defence-related proteins (protein revealing similarity to thaumatin). Proteins exhibiting a cold-induced increase in winter cultivar only include proteins involved in regulation of stress response and development (germin E, lectin VER2) while proteins showing a cold-induced increase in spring cultivar only include proteins involved in restoration of cell division and plant growth (eIF5A2, glycine-rich RNA-binding protein, adenine phosphoribosyltransferase). The obtained results provide new insights in cold acclimation in spring and winter wheat at proteome level and enrich our previous work aimed at phytohormone dynamics in the same plant material.

P144

Autophagy during Nutritional Stress in Arabidopsis and Barley

Liliana Avila-Ospina, Celine Masclaux-Daubresse

Institut Jean-Pierre Bourgin, INRA Centre de Versailles-Grignon, Versailles, France

As non-moving organisms, plants rely on physiological responses in order to survive difficult environmental conditions. A response to these stressful situations is autophagy, a process whereby cytoplasmic components are sequestered into a structure called autophagosome and delivered to the vacuole for breakdown. Autophagy is associated to lifespan and stress responses. Recent findings evidence that autophagy plays an important role in nitrogen remobilization in *Arabidopsis*.

In order to improve crop production, it is necessary to increase the capacity of plants to recycle nitrogen effectively from source organs to sink tissues. This work describes the autophagy system in barley and examines their dynamics during leaf senescence and nutrient deprivation.

P145

Small Rubber Particle Proteins Facilitate Rubber Biosynthesis and Play a Role in Abiotic Stress Response

Natalie Laibach¹, Andrea Hillebrand², Dirk Prüfer^{1,2}, Christian Christian Schulze Gronover¹
¹Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Aachen, Germany, ²Institute for Plant Biology and Biotechnology (IBBP), Westphalian Wilhelm's University Muenster, Muenster, Germany

Natural rubber is a poly(cis-1,4-isoprene) which is compartmentalized in rubber particles inside of laticifers. While the biosynthesis of rubber is proposed to be catalyzed by cis-prenyltransferases, also other proteins, called small rubber particle proteins (SRPPs), influence rubber production in a positive manner. In *T. brevicorniculatum* five SRPP homologs have been identified (TbSRPP1-5) which are located on the rubber particle surface. Their positive influence on rubber particle stability is assumed to be the cause for their supporting effect on rubber biosynthesis. Interestingly, TbSRPPs are, though predominantly, not exclusively expressed in the latex and share sequence homologies with stress related proteins (SRPs). Since their biological function remains unclear, we characterized TbSRPPs towards their behavior under abiotic stress. We could observe that TbSRPPs were highly inducible by several abiotic stresses. Furthermore, *Arabidopsis thaliana* plants expressing TbSRPP2&3 show a more drought and salt tolerant phenotype than wild-type plants. As *Arabidopsis* possesses three endogenous homologs of TbSRPPs (AtSRP1-3) which are also inducible by abiotic stress they were therefore included in our investigations.

P146

Investigation on the Synthesis and Role of Salicylic Acid in Wheat Varieties with Different Levels of Cadmium Tolerance

Magda Pál, Viktória Kovács, Orsolya Kinga Gondor, Tibor Janda, Gabriella Szalai
Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary

The aims of the study were to reveal how cadmium influences salicylic acid (SA) synthesis, and to find possible connections between SA content and cadmium tolerance and between SA and certain protective compounds during cadmium stress in wheat. Wheat genotypes with different endogenous SA contents were screened to determine the level of cadmium tolerance and to detect how changes in SA synthesis, antioxidants and the heavy metal detoxification system are related to each other. Although exposure to cadmium induced SA synthesis, there was no direct relationship between the SA levels and cadmium tolerance. It is suggested that the phenyl-propanoid synthesis pathway responsible for the accumulation of SA induced by cadmium. The synthesis of protective compounds was induced by cadmium to varying extents in wheat genotypes with different levels of tolerance; the roots and leaves also responded differently to cadmium stress. The higher tolerance could be explained by the greater induction of root phytochelatin synthase, resulting in a high level of phytochelatin, which was related with changes in the glutathione content, the glutathione redox state and the glutathione reductase activity in the roots.

Acknowledgements

This work was supported by a grant from the Hungarian National Scientific Research Foundation (OTKA PD83840), which is gratefully acknowledged. Magda Pál is a grantee of the János Bolyai Scholarship.

P147

The Heat Shock Factor HSFA4A Confers Salt Tolerance and Is Regulated by Oxidative Stress and the MAP Kinases, MPK3 and MPK6

Imma Pérez Salamó, László Szabados, Csaba Papdi, Laura Zsigmond, Gábor Rigó
Biological Research Center, Szeged, Hungary

Plants are exposed to constantly changing environments and often have to survive in adverse conditions. To identify regulatory factors involved in stress tolerance, a novel screening strategy was developed in our laboratory. Arabidopsis cell suspension cultures were transformed with the COS cDNA library (Papdi et al., 2008), and screened for salt tolerance. The heat shock transcription factor A4A (HSFA4A) was identified as one of the factors which were able to confer salt tolerance of the transformed calli. We showed that estradiol-inducible overexpression of HSFA4A in Arabidopsis plants confers high level of salt tolerance, as well as reduced sensitivity to anoxia and oxidative agents. Consistently, the *hsfa4a* T-DNA insertion mutant is hypersensitive to salt stress. HSFA4A overexpression decreases, whereas the *hsfa4a* mutation elevates hydrogen peroxide accumulation and lipid peroxidation. Gene expression analysis indicates that HSFA4A co-ordinately regulates the expression of a set of genes involved in stress responses. HSFA4A shows dimerization in yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays, which is reduced by alanine replacement of three conserved cysteine residues. Moreover, HSFA4A interacts with mitogen-activated protein kinases MPK3 and MPK6 in yeast and plant cells. HSFA4A is phosphorylated by MPK3 and MPK6 *in vitro* primarily on Ser309, which is required for transcriptional activation of the HSP17.6A target in a transient expression system. Our results suggest that HSFA4A could act as a downstream regulator in MPK3/6-dependent stress response pathways.

P148

Protective Effect of Different Forms of Salicylic Acid against Cd Stress in Young Maize Plants

Orsolya Kinga Gondor, Magda Pál, Tibor Janda, Gabriella Szalai
Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary

Salicylic acid (SA) has been known as a signal molecule in the induction of defence mechanisms in plants for a long time. SA is also a promising compound for the reduction of abiotic stress sensitivity in plants, since several methods of application (soaking seeds in SA prior to sowing, adding SA to the hydroponic solution, irrigating or spraying with SA solution) have been shown to protect various plant species against abiotic stress factors. The aim of our work was to study the protection of different SA forms (SA and its sodium salt, NaSA) against Cd stress in young maize plants. Part of seven-day-old maize plants were pre-treated either with 0.5 mM SA or NaSA for 1 day. After it plants were treated with Cd or Cd was added together with SA or NaSA to part of the plants. The Cd content of the treated plants were lower than the untreated ones. The SA or NaSA pre-treatments did not cause oxidative stress themselves but decreased its severity after Cd treatment. The root viability of NaSA treated plants was better than the SA treated ones during Cd stress. The viability of leaves was also better in the case of NaSA pre-treatment or treatment than the SA treated ones. Changes in phytochelatin content, phytochelatin synthase and antioxidant enzyme activities will also be discussed. It can be concluded that the different forms of SA did not provide the same level of protection for plants against Cd stress.

P149

Defining Physiological Thresholds under Water Deficit for Improved Plant-Based Irrigation Scheduling in Cotton

Daryl Chastain², John Snider¹, Guy Collins¹, Calvin Perry³, Seth Byrd²

¹University of Georgia, Tifton, GA, USA, ²University of Georgia, Athens, GA, USA, ³Stripling Irrigation Research Park, Camilla, GA, USA

While traditional water balance approaches to irrigation scheduling have been successful in obtaining high crop yields, there is evidence that suitable plant-based irrigation thresholds could provide a means to conserve water resources, while maintaining profitable yields; however, the most appropriate measure of crop water status for irrigation scheduling has been heavily debated. Based on photosynthetic responses to predawn Ψ_1 during the 2012 growing season, cotton grown in the southeastern USA (Georgia) in 2013 was maintained under dryland conditions, irrigated according to a conventional water balance approach, or irrigated using three distinct irrigation thresholds based on predawn water potential (-0.5, -0.7, -0.9 MPa). In addition, canopy temperature was monitored throughout the growing season for use in modifying the crop water stress index (CWSI) for cotton grown under low vapor pressure deficit. Our results showed that 1) using predawn Ψ_1 as a means of scheduling irrigation decreased water usage 7% (-0.5 MPa threshold) to 21% (-0.9 MPa threshold) relative to the water balance approach for the 2013 growing season, 2) canopy temperatures show potential for usage in scheduling irrigation, where all treatments exhibited identical seasonal CWSI values, seedcotton yields, and lint yield, 3) current irrigation practices may potentially be modified with the appropriate plant-based water deficit stress thresholds to allow for greater water savings, without yield losses.

P150

Field-Grown *Gossypium hirsutum* Plants Exhibit Seasonal Variation in Heat Tolerance without Exposure to Heat Stress or Water Deficit Conditions

John Snider¹, Daryl Chastain², Guy Collins¹

¹University of Georgia, Tifton, GA, USA, ²University of Georgia, Athens, GA, USA

Fast fluorescence (OJIP analysis) is used to fully characterize photochemical sensitivity to environmental stresses for many plant species, but its utilization for characterizing thermotolerance acclimation to abiotic stress in field-grown plants is relatively unexplored. To this end, the temperature responses of maximum quantum yield of photosystem II (F_v/F_m), variable fluorescence (F_v/F_0), quantum yield of electron transport (ϕ_{E_0}), and efficiency of PSI electron acceptor reduction (REo/ABS) were characterized from the OJIP transient for leaves of three *Gossypium hirsutum* cultivars (DP 1252 B2RF; PHY 339 WRF, and PHY 499 WRF), at three sample times during the growing season (June 21, July 2, and July 18, 2013), and under three irrigation regimes. The temperature at which each parameter declined 15% from the optimum (T_{15}) served as the high temperature threshold for all parameters. T_{15} varied with sample date (highest on July 2 for all parameters), being 2°C (F_v/F_0) to 5.5°C (ϕ_{E_0}) higher on July 2 relative to June 21, despite optimal temperature conditions and plant water status (predawn water potential) on all sample dates. T_{15} values, assessed using different fluorescence-based parameters, were highly correlated, but differed in magnitude, where F_v/F_0 and ϕ_{E_0} were more sensitive to high temperature than either F_v/F_m or REo/ABS. We conclude that 1) seasonal variation in thermotolerance may be influenced by plant age in addition to the environmental acclimation responses reported previously and 2) that thermotolerance is clearly dependent upon the chlorophyll fluorescence-based parameter evaluated. Both factors should be carefully considered in studies addressing thermotolerance acclimation in field-grown plants.

P151

Effects of NaCl Salinity on Growth and Ion Content of Nine Greek Olive Varieties

A. Assimakopoulou¹, I. Salmas¹, K. Nifakos¹, P. Kalogeropoulos¹, P.A. Roussos², G. Kostelenos³

¹TEI of Peloponnese, Department of Agricultural Technology, 241 00 Antikalamos, Messinia, Greece,

²Agricultural University of Athens, Laboratory of Pomology, Iera Odos 75, Athens 118 55, Greece,

³Kostelenos Nurseries, 180 20 Poros, Troizina, Greece

The purpose of our work was to evaluate nine Greek olive (*Olea europea*, L.) varieties for tolerance to NaCl salinity, most of which have never been evaluated, not even listed in the national catalogue of varieties, or considered to be extinct. One-year old olive (*Olea europaea* L.) trees of the varieties Aetonicholia, Arvanitolia Serron (Arvanitolia), Atsicholou, Koroneiki, Lefkolia Serron (Lefkolia), Pierias, Petrolia Serron (Petrolia), Smertolia and Chrysophylli were subjected to: 0 (E0), 50 (E50), 100 (E100) and 200 (E200) mM NaCl in hydroponics, for six months. Based on plant growth results of every variety in E50, E100 and E200 expressed as a percentage change of its control (E0), Arvanitolia, followed by Petrolia and Lefkolia, in E50, did not show significant total fresh weight decrease whereas Aetonicholia presented the greatest. In E100, Arvanitolia presented no significant decrease either and in E200 Arvanitolia showed a 17% decrease whereas Aetonicholia showed 52%. In all NaCl treatments, Arvanitolia presented the lowest leaf Na concentration and Koroneiki the highest. In contrast, root Na in E50 was highest in Arvanitolia, Atsicholou, Petrolia and Lefkolia. With regard to Cl, Arvanitolia presented low leaf Cl concentration whereas Koroneiki and Atsicholou the highest one. Root Cl level was the greatest in Lefkolia and Arvanitolia. Arvanitolia also presented the highest leaf K but the lowest root K level in all NaCl treatments. Based on our results, it seems that Arvanitolia, Petrolia and Lefkolia tolerate better NaCl salinity due to their capacity to retain Na and Cl ions in their roots.

P152

Growth, Ion Uptake and Yield Responses of Three Greek Small-Sized Tomato (*Lycopersicon esculentum* L.) Cultivars and Four Hybrids of Cherry Tomato under NaCl Salinity Stress

A. Assimakopoulou, K. Nifakos, I. Salmas, P. Kalogeropoulos

TEI of Peloponnese, Department of Agricultural Technology, Antikalamos, 241 00 Messinia, Greece

The effect of three NaCl treatments (0 (E0), 75 (E75) and 150 (E150) mM NaCl) on the growth, yield and ion uptake of three Greek traditional small-sized tomato cultivars (two landraces of Santorini tomato - "Santorini Authentic" and "Santorini Kaisia"- and the cultivar "Chios"), and four hybrids of cherry tomato (Cherelino F1, Scintilla F1, Delicassi F1 and Zucchero F1), was recorded. Plants were grown in plastic pots of 12,0 L of loamy soil and perlite mixture (3:1, v/v), in a glasshouse experiment, for four weeks, in the spring. According to plant growth results of every variety in E75 and E150 expressed as a percentage change of its control (E0), Santorini Authentic in E75 presented the smallest reduction of the total biomass production (total plant FW+fruit FW) as well as the smallest upper plant part FW reduction whereas in E150 there were no significant differences among the seven genotypes, respectively. Based on plant growth and Na, Cl and K concentration results, the greater NaCl salinity tolerance of Santorini Authentic might be due to the higher Na and Cl retention by the roots in combination with the non significant decrease of leaf K. However, in all NaCl treatments, the ratios K/Na and Ca/Na, which are often used as predictive salinity screening parameters, were not differentiated among the genotypes tested. With regard to the commercial total fruit FW produced, Zucchero in E75, followed by Santorini Kaisia and Chios, presented the lowest reduction compared to Cherelino and Delicassi in both NaCl treatments.

P153

The Protective Role of Cytokinins against Lead Stress in the Green Alga *Scenedesmus obliquus*

Andrzej Bajguz, Alicja Piotrowska-Niczyporuk, Monika Bralska
University of Białystok, Institute of Biology, Białystok, Poland

Heavy metals can cause adverse effects on the growth, cell division, photosynthesis, and destruction of primary metabolites in algae. Moreover, lead is strongly phytotoxic, partly because of the generation of reactive oxygen species. Algae have evolved enzymatic (catalase, ascorbate peroxidase, glutathione reductase, superoxide dismutase) and non-enzymatic (ascorbate, glutathione) antioxidant mechanism to prevent the oxidative stress. Therefore, the search for phytohormones mediating stress tolerance is an important step towards the better understanding how algae respond to pollutants.

The present study was undertaken to test the influence of exogenously applied cytokinins (benzyladenine, BA; kinetin, Kin; trans-zeatin, *tZ*) (10^{-12} - 10^{-5} M) upon the growth and metabolism of *Scenedesmus obliquus* (Turpin) Kützing (Chlorophyceae) (SAG Strain No.: 276-6) exposed to lead stress (10^{-8} - 5×10^{-4} M). Obtained results indicate that exogenously applied phytohormones can reduce the phytotoxicity of lead. The treatment with cytokinins can alleviate stress symptoms by inhibiting lead biosorption, restoring algal growth and primary metabolite level of protein, sugar and chlorophylls. The stimulating effect of cytokinins during stressful conditions was arranged: *tZ* > Kin > BA. Moreover, these phytohormones enhance antioxidant activities in cells growing under lead stress. Increased activity of antioxidant system in response to cytokinins reduced oxidative stress expressed by lipid peroxidation and hydrogen peroxide level in algae. It suggests that cytokinins play an important role in *Scenedesmus obliquus* response to abiotic stressor and algal adaptation ability to metal contamination of environment.

This project has been financed from the funds of the National Science Centre allocated on the basis of the decision number DEC-2012/05/B/NZ8/00958.

P154

Effects of Low Temperature and Drought on Rice with Different Levels of Drought Tolerance

Magda Pál¹, Viktória Kovács¹, Gabriella Szalai¹, Vilmos Soós¹, Xiaosong Ma², Hongyan Liu², Hanwei Mei², Tibor Janda¹

¹*Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary,* ²*Shanghai Agrobiological Gene Center, Shanghai Academy of Agricultural Sciences, Shanghai, China*

Salicylic acid is a well-known signal molecule, which may provide protection under biotic or abiotic stress conditions. Leaves of rice plants (*Oryza sativa* L.) are characterised by a very high salicylic acid content, the exact physiological role of which, however, is still poorly understood. The aims of the present work were: 1. to reveal the relationship between the level of drought resistance in rice and the basal level of SA and related compounds; 2. to characterize changes in the biosynthesis and metabolism of salicylic acid and polyamines under drought and low temperature conditions; 3. to investigate the correlation between stress-related compounds in order to find connections between various stress-related signalling molecules. The results indicate that although salicylic acid and related compounds are important for the modulation of the redox balance and may play an antioxidative role, the basal endogenous content only has special importance in certain plant species under specific developmental or environmental conditions. The salicylic acid contents in rice leaves were not substantially affected by the stress conditions applied; however, other stress-related compounds such as polyamines exhibited marked, stress-specific responses. These data showed that there is no direct link between the changes induced in polyamines by abiotic stress and the salicylic acid metabolism in rice. The data also suggest that salicylic acid synthesis via ortho-hydroxy cinnamic acid may play a more decisive role in rice than the chorismate-isochorismate- salicylic acid pathway.

P155

Role of Bacterial-Type PEPC in Stress Tolerance of *Arabidopsis thaliana*

Alfonso J. Sánchez, José A. Monreal, Isabel Ruiz-Ballesta, Cristina Echevarría, Ana-Belén Feria
Universidad de Sevilla, Sevilla, Andalucía, Spain

Phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) fulfills a wide range of crucial non-photosynthetic functions, particularly with regard to the anaplerotic replenishment of the tricarboxylic acid cycle intermediates consumed during biosynthesis. In C_3 plants, PEPC is widely expressed across most organs. In *Arabidopsis thaliana*, the PEPC gene family is composed of four genes. Based on sequence analysis it has been deduced that genes *Atppc1*, *Atppc2* and *Atppc3* encode plant-type PEPCs, whereas gene *Atppc4* encodes a bacterial-type PEPC. PEPC genes display differential expression throughout *Arabidopsis* organs¹ and in response to environmental stress. *Atppc3* is the only one gene expressed exclusively in roots, whereas *Atppc1* and *Atppc2* are ubiquitous. *Atppc4* transcripts are found in siliques, flowers and roots, nonetheless in very low levels. Our group previously reported² that absence of the *Atppc3* gene affects root development and improves plant survival under saline stress conditions. In the present study we have observed that expression of the bacterial-type PEPC *Atppc4* is concomitant with expression of the plant-type *Atppc3* in response to stress. Moreover, this phenomenon is absent in the *Atppc3*⁻ mutant. These results suggest a combined function of the bacterial- and the plant-type PEPC enzymes, with regard to the adaptation of plants to environmental changes and also indicate that PEPC comprises an important role in roots, where both enzymes are expressed and the anaplerotic pathway is crucial.

1 Sánchez R. (2006), *Planta*, 223. 901-909.

2 Cerrejón A. (2013). *XIII Congresso Luso-Espanhol de Fisiologia Vegetal S7/P30*.

Acknowledgments: Funding by project nº AGL2012-35708 from 'MEC' and Group BIO-298".

P156

Photosynthetic and Biochemical Responses to Soil Water Deficit and Re-Watering of Two “Long Storage” Tomato Landraces

Gianpiero Guida^{1,2}, Pasquale Giorio¹, Rossella Albrizio¹, Marco Oliva¹, Mohamed Houssemeddine Sellami¹, Roberta Buonomo¹, Anna De Maio², Carmen Arena², Stefania Grillo³, Paolo Iovieno³, Carmela Mistretta¹

¹National Research Council of Italy, Institute for Agricultural and Forestry Systems in the Mediterranean (CNR-ISAFO), Ercolano (NA), Italy, ²Department of Biology, University of Naples Federico II, Naples, Italy, ³National Research Council of Italy, Institute of Biosciences and Bioresources (CNR-IBBR), Portici, Italy

Long-storage tomato landraces are niche crops traditionally cultivated in southern Italy for drought tolerance and high nutritional and organoleptic quality of fruits. Nowadays, there is a growing interest in these crops as for exploiting biodiversity and the “Made in Italy” food production. We studied the physiological and biochemical performances of two landraces in response to soil water deficiency and re-watering. The potted-plant experiments were carried out in greenhouse during 2012 and 2013.

During the first year, an intense water stress determined a decrease of both stomatal conductance (g_s) and net CO_2 assimilation (A) along with an increase in proline and ABA content, without any effect on photochemistry. In the second year, a more severe water stress led to a greater decline of both g_s and A , and a reduction in photochemistry. During the re-watering, the full recovery of gas exchanges and photochemistry, indicated the occurrence of efficient stomatal control and photochemical regulation, rather than impairment of photosynthetic apparatus. The activity of poly ADP-ribose polymerase (PARP), a key regulator of the energy homeostasis during stress conditions, showed during the first cycle of stress an evident increase, followed by a decrease. Re-watering, instead, produced a new non-expected increase of activity, which returned to control values during the second cycle of water stress. The two tomato landraces succeeded to cope with water stress by a useful synergy of biochemical and physiological regulatory mechanisms. This work was financially supported by “CISIA for Made in Italy” Project of the National Research Council of Italy.

P157

Responses of Cultivated (*Nicotiana tabacum*) and Wild Type (*Nicotiana glauca*) Tobacco Plants to Cadmium Toxicity

Maria Christofaki¹, George Konsolakis¹, Kostas Paschalidis¹, Maria Eirini Christoulaki¹, David Aldred², Konstantinos Loulakakis¹

¹Technological Educational Institute of Crete, Heraklion, Crete, Greece, ²Cranfield University, Bedfordshire, UK

One of the most hazardous heavy metals is cadmium (Cd) that accumulates in soil and water, causing a variety of morphological and biochemical dysfunctions in living organisms. Several plant species are able to accumulate and withstand large quantities of Cd in their tissues, without dramatic alterations in their growth, usually observed in plants. In an effort to better understand the responses of plants to Cd stress, a comparative study was held between two solanaceous species, the annual cultivated *Nicotiana tabacum* and the perennial wild-type *Nicotiana glauca*, plants with promising properties for phytoremediation. Plants of both species were grown in the presence of different concentrations of Cd, in pots containing artificial substrate for 6 weeks. The accumulation and distribution of the metal in various plants tissues (lower and upper leaves, lower and upper shoots, roots) were determined, as well as several morphological and physiological parameters of the plants were followed. Both plant species accumulated Cd in a dose dependent manner. *N. tabacum* accumulated more in all the above ground tissues than *N. glauca*. The examined species responded well under Cd stress, however, visual phytotoxicity symptoms and stunted growth were observed, especially at the highest treatment. In addition, photosynthetic apparatus was negatively affected, especially by Cd excess. The net photosynthesis rate reduction was in direct relation with the reduction observed in chlorophyll content, stomatal conductance, transpiration rate, photochemical efficiency of PSII. Our results indicate mechanisms enabled by both plant species to cope with Cd-stress, thus opening new horizons for phytoremediation.

P158

Morphophysiological and Cytogenetic Response Mechanisms of Various Species of Wheat to Drought and Salt Stresses

Nina Terletsкая, Nina Khailenko, Leonid Mamonov
Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan

Any stress can put a plant body from a state of dynamic equilibrium and run complex mechanism of complex responses, the main task - to preserve the viability of the plant and to adapt to changing conditions.

Analyzed the physiological responses and cytogenetic characteristics of different species of wheat under salt and osmotic stress.

With a total of nonspecific phenomenon on slowing and stopping the growth processes under the influence of drought and salinity revealed specific and varietal specificity of the influence of stressors on the morphological structure of cereal, which makes it possible to diagnose stress-tolerance of sorts and species in adverse conditions.

On the background of nonspecific cytological reactions vegetative and generative cells of cereals in drought and salinity, demonstrated a variety of specific changes in osmotic characteristics and disorders chromosome apparatus, depending on the intensity of exposure to the stressor and genotypic features of the self-regulation of study forms. A genotype specificity expressed as intensity and speed manifestations plasmolysis indicates that this feature may serve as the basis for selection and cytological characteristics tolerant forms at mature pollen grains.

It is shown that the wild relatives of wheat can be promising as sources of various characteristics of drought and salt tolerance for involvement in breeding programs to develop new sorts of wheat (*T. aestivum* L.). The drought- and salt-tolerant species, as *T. dicoccum* Shuebl. can be successfully used in crosses with cultivated varieties of wheat breeding for resistance to osmotic and salt stress.

P159

Impact of Hypoxia and CO₂-Media on Free-Radical Oxidation in Cell Compartments of Pea and Soy Plants

Antonina Ershova, Olga Berdnikova
Voronezh State Pedagogical University, Voronezh, Russia

Exploration results of rate and mechanisms of ROS formation in plants under hypoxic stress are controversial. Rate of free-radical processes in mitochondria, chloroplasts, and cytoplasm of pea and soy seedlings during hypoxia and CO₂-media (3-24h) were studied. Hydrogen peroxide content was determined enzymatically, superoxide anion-radicals, lyxoxigenase (LOX) and superoxide dismutase (SOD) enzyme activity - spectrophotometrically, free-radical oxidation by chemiluminescence method. It was shown that in studied compartments of intolerant pea seedlings the rate of free-radical oxidation under hypoxia was increasing and to the end was 1.5-2.5-fold higher to aerated plants. In moderately tolerant soy this process was observed during first hours and in cytoplasm only. Accumulation of all types of ROS was noted in mitochondria and chloroplasts of pea seedlings and to lesser extent in soy. Presence of mitochondrial LOX in cells of pea and soy seedlings was proved electrophoretically. After 3-6h of hypoxia an increase of mitochondrial LOX activity was detected. Chloroplast and cytoplasm LOX was increasing under such conditions in pea cells only. It was shown that antioxidant enzyme of SOD was significantly increasing under hypoxia in all cell compartments in moderately tolerant soy seedlings but in pea this was the case in mitochondria. The data proves the correlation between plant tolerance to hypoxia and rate of free-radical processes in cell compartments. Important role of lyxoxigenase path during ROS formation in plant mitochondria under oxygen deficit was shown. It was revealed that CO₂-media increased hypoxia effect on ROS formation and activity of LOX and SOD.

P160

Photochemical and Biochemical Response of Sugarcane to Progressive Drought and Recovery

Lauricio Endres, Romel Duarte Vilela, Joao Antonio Carvalho Silva, Alisson Almeida Santos
Universidade Federal de Alagoas, Maceio/Alaogas, Brazil

The objective of the study was to investigate the physiological mechanism responses of different varieties of sugarcane to drought stress. Two varieties, RB855536 (more sensitive) and RB867515 (more tolerant) were submitted to progressive drought conditions (2, 4, 6 and 8 days) and recovered from stress by rewatering at each time point in a green house experiment. Drought induced a decrease of water and osmotic potential in the leaves, but they recovered to normal level after rewatering. There was a large decrease of maximum quantum efficiency of photosystem II with water deficit in both varieties, but RB867515 recovered faster after rewatering. Drought stress induced a decrease in leaf starch and sucrose and increase of leaf glucose and fructose in both varieties. The activity of leaf catalase, ascorbate peroxidase and superoxide dismutase activity increased more in RB867515 than in RB855536 under drought conditions. The results suggest that RB867515 is more tolerant to drought conditions because it has a more efficient antioxidant system which results in lower photoinhibition and better photosynthetic activity during drought stress.

P161

Interactive Effects of Drought and Shade on Functional Traits of Cork Oak and Recovery after Re-Watering

Ermelinda Silva, José Moutinho-Pereira, Helena Ferreira, Berta Gonçalves, Eunice Bacelar, Carlos Correia

CITAB, Universidade de Trás-os-Montes e Alto Douro, UTAD, Vila Real, Portugal

Quercus suber L. (cork oak) is a sclerophyllous evergreen tree of great importance in the Western Mediterranean region. Summer drought associated with high light intensity and elevated temperatures are the main factors limiting the distribution and biomass production of plants in these environments. The effects of water stress on Mediterranean evergreen species have been well-documented. However, their combined constraints to drought and shade and the underlying mechanisms of recovery after rehydration are relatively unknown. The present study attempts to describe physiological alterations of two year old cork oak plants exposed to two light treatments (100 and 10% of ambient light) and two contrasting water regimes (well-watered and plants with $\frac{1}{2}$ of the water applied to control plants) during 4 months and a subsequent 7 days period of re-watering. Shade and low water availability induced significant differences, predominantly negative, for most variables. Moreover, drought effects were more severe under shade conditions, in association with lower phenotypic plasticity in response to light for physiological (photosynthesis and water status), morphological (leaf area, leaf area ratio, specific leaf area) and anatomical (leaf tissues thickness) variables, although their higher metabolic plasticity (phenols, soluble sugars and soluble proteins). Upon re-watering plants under full light conditions recovered the physiological performance, while shaded plants do not fully recovered, in association to a decrease in metabolic and physiological plasticity. These results may have important implications for the restoration and conservation of oak woodlands under climate change scenarios.

P162

Addressing Molecular and Cellular Response Mechanisms Induced by Salt Stress in Two Contrasting Salt Sensitivity Varieties of Italian Rice (*Oryza sativa* ssp. Japonica)

Elide Formentin, [Cristina Sudiro](#), Elisabetta Barizza, Michela Zottini, Fiorella Lo Schiavo
University of Padova, Padova, Italy

Soil salinity affects crop production on over a quarter of all agricultural land on Earth, in particular in coastal areas. Italy is the largest rice-producing country in Europe and high quality Italian rice varieties are cultivated in the saline soil of the Delta of the Po river. In the frame of a research project of national interest (RISINNOVA), salinity response was analysed in two Italian rice varieties (Baldo and Vialone Nano) selected for contrasting salt sensitivity.

Among components involved in the signaling pathway induced by salt, the role of nitric oxide was investigated both in cultured cells and in plant roots. The results of this analysis unveil a different kinetics in NO production between the two cultivars, showing a more rapid NO generation in the Baldo tolerant cultivar, compared with Vialone Nano.

At molecular level, an RNA sequencing analysis, aimed to compare the transcriptional profile of the two cultivars, was performed. The results highlight different responses. In the more sensitive cultivar Vialone Nano, a clear down-regulation of genes involved in chlorophyll biosynthesis along with leaf chlorosis is observed, indicating an early induction of senescence events. Moreover, the up-regulation of heat-shock protein coding genes indicates that Na⁺ reached toxic concentrations in V. Nano leaves leading to a massive protein misfolding. This result is confirmed by ionic content measurements. Baldo leaves, instead, respond to salt stress putting in place the activation of specific responses to water deficit in coordination with ROS detoxification pathways, so resulting in being more successfully.

P163

Signaling Components in the Cold Stress Response of Barley

Zsuzsa Marozsán-Tóth, Ildikó Vashegyi, Gábor Galiba, Balázs Tóth
Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary

As a consequence of climate change, occurrence of erratic weather conditions is increased generating temperature, drought or osmotic stress for cereals and other cultivated plants causing significant loss of production and decrease of yield. This phenomenon intensified the need for enhanced abiotic stress tolerance in cereals. To improve the adaptation ability of the crop plants, it is very important to understand how these plants are able to percept environmental signals and how these signals are converted to molecular response in the plants. Thus, the investigation of the major signal transduction mechanisms and identification of signaling components involved in abiotic stress tolerance is very important not only in model organisms, but in cultivated plants as well. In the present work components of the phospholipid signaling were identified and investigated in barley and their influence on the cold response was also studied. In details, a phosphatidylinositol transfer protein and a phosphatidylinositol 4-kinase were identified in barley using homology search and their biochemical and cell biological characterization was performed. Moreover, the role of this signaling pathway in the cold regulation of the CBF transcription factors was also studied.

P164

Poplar for the Phytoextraction of Boron from Contaminated Soil and Water

Kubilay Yildirim¹, Dilek Kaya Ozdogan², Teoman Kahraman³

¹*Gaziosmanpasa University, Tokat, Turkey*, ²*Soil, Fertilizer and Water Resources Central Research Institute, Ankara, Turkey*, ³*Poplar and Fast Growing Forest Trees Research Institute, Izmit, Turkey*

Boron pollution is one of the most serious environmental problems affecting more than 20% of agricultural area in Turkey. The present study investigated the capacity of some populus species (*Populus alba*, *Populus nigra* and *hybrid poplar*) for the phytomanagement of boron. For this purpose, we established pot trial including one year old poplar seedlings. Then boron treatment was applied within the irrigation water to these seedlings over the course of a growing season. The total concentration of boron was measured in the roots, stems, green leaves, fallen leaves, and the corresponding soil. Our results indicated that all poplar species could resist maximum 30 mg/kg boron contamination in the soil. At this critical level poplar leaves had an average B concentration of 1050 mg/kg, while the stems contained 120 mg/kg B. Leaf B concentrations increased linearly with leaf age and the fallen leaves had higher boron concentrations. Boron has an important usage in wood industry as a fire retardant. Therefore, the wood material obtained from the boron treated seedling were subjected to a burning test in the study. The results of this test indicated that the wood that is obtained from the boron treated seedlings were 18% resistant to fire compared to control materials. The study revealed that the poplar is an excellent species for phytoextraction of boron from the contaminated sites. Harvesting alternate rows of trees on contaminated soils or river sites would remove boron and produce high quality wood material that is resistant to fire.

P165

The Chloroplast Antioxidant System - Priming the System for Cold?

Jörn van Buer, Margarete Baier
Freie Universität Berlin, Berlin, Berlin, Germany

Priming is a process mostly described in the pathogen defense of plants. A local event like the recognition of a pathogen associated pattern leads to a global defense response. In contrast to the pathogen defense we define priming in a time dependent manner. A priming stimulus is followed by a period at normal growth conditions. Afterwards the plants are triggered by facing the same stress stimulus again: here chilling stress.

Chilling stress leads to an over reduction of the plastoquinone pool. This causes higher production of reactive oxygen species (=ROS). ROS are not only toxic, but can act as signal molecules. The plastid ROS and its redox state is controlled by the chloroplasts antioxidant system.

The comparison of primed and unprimed Arabidopsis plants directly after triggering revealed a dramatic increase of ROS marker genes by qRT-PCR in unprimed plants. This indicates that one day of chilling stress is enough to alter the overall ROS buffer capacity in a plant. The cold regulated gene Cor15A shows no difference when comparing the expression level of primed and unprimed plants after the triggering.

The stromal ascorbate peroxidase exhibit an increase in transcript upon chilling stress after triggering. This effect can be observed independently from priming. In contrast, its thylakoid bound homologue significantly increases the abundance of transcript after triggering when plants were primed before. This increased induction of the tAPx could ensure that the overall burden of H₂O₂ stays low in primed plants.

P166

A Hydrogen Peroxide Signal is Required for the Enhancement of Invertase Activity in Roots of Osmotically Stressed Wheat Plants

Helga Königshofer, Hansgeorg Löppert

University of Natural Resources and Life Sciences, Institute of Botany, Vienna, Austria

Invertases are particularly important enzymes in sink organs since they exert a marked influence on carbon partitioning and contribute considerably to sink strength. In recent years, evidence has been obtained that invertases respond to a variety of abiotic and biotic stress-related stimuli. In this study, we monitored the activity of invertases in the root tips (containing the cell-division zone and the elongating zone) of the drought tolerant wheat cultivar *Triticum aestivum* L. cv. Josef in the course of osmotic stress applied by a solution with 20% PEG 6000 ($\psi = -0,689$ MPa). After 4 hours of stress imposition, the activities of vacuolar and cell wall bound invertases started to increase in the root tips and were about 3-4-fold higher than in control plants after 24 hours. In parallel, glucose and fructose accumulated to a similar extent in significant osmotically active amounts.

The enhancement of invertase activities was preceded by a transient rise in hydrogen peroxide (H_2O_2) production 1-2 hours after the application of PEG. Inhibition of the H_2O_2 outburst by diphenylene iodonium (DPI) caused a strong suppression of both the stress-induced increase in invertase activities and the rise in hexose content. As a consequence, osmotic adjustment of the root tips under stress was markedly impaired in the presence of DPI. Thus, the generation of a H_2O_2 signal seems to be a prerequisite for the stimulation of invertases and the accumulation of osmotically active hexoses in root tips of wheat plants under water deficit.

P167

Biochemical Analysis of Tomato Mutants Grown in Nutrient Solution with Different pH and Submitted to Cadmium

Georgia Bertoni Pompeu¹, Priscila Lupino Gratao², Ricardo Antunes de Azevedo¹

¹Universidade de São Paulo / ESALQ, Piracicaba, Sao Paulo, Brazil, ²FCAV/UNESP, Jaboticabal, Sao Paulo, Brazil

Cadmium (Cd) is a heavy metal considered one of the most toxic pollutants for animals and plants, even at low concentrations. Tomato mutants Micro-Tom (MT) and Sitiens (Sit) are considered excellent models of biochemical studies due to the small size, short life cycle and fruit production. The main aim of this work was to evaluate the influence of different pH on the plant defense to Cd stress. Cd absorption, dry weight, lipid peroxidation and hydrogen peroxide content were evaluated in MT and Sit plants grown in hydroponic solution containing different pH (5, 6 or 7) and 50 μM CdCl_2 for 48 h during pre-flowering. Roots and leaves of MT grown at pH 7 exhibited the highest Cd absorption, while Sit exhibited the highest Cd absorption at pH 6. Roots and leaves of MT and Sit exhibited higher dry weight when grown at pH 5 and 6, respectively, in the presence of metal. On the other hand, lipid peroxidation was not altered by the different pHs tested, although the presence of Cd caused induction of peroxidation in both mutants. It was observed that pH, presence of Cd and cultivars influenced H_2O_2 production in roots and leaves of MT and Sit. (funded by FAPESP/Brazil)

P168

Cold-Induced Expression of Dehydrins in Rosaceae Family

Perttu Haimi, Inga Stepulaitiene, Danas Baniulis, Gražina Staniene, Jurate Brone Šikšnianiene, Rytis Rugienius

Institution of Horticulture, Research Centre for Agriculture and Forestry, Babtai, Kaunas region, Lithuania

Drought, salinity, heat and freezing are abiotic stress factors that cause dehydration in plants. The accumulation of Late Embryogenesis Abundant (LEA) proteins is one of the main mechanisms against water stress. Dehydrins, or group 2 LEA proteins, are an ubiquitous group of intrinsically disordered proteins with a wide range of molecular masses, which are found throughout the plant kingdom. Dehydrins are functionally diverse, and their protective effects arise from their interactions with membranes and proteins, stabilizing these structures and preventing aggregation [1]. Dehydrins are often identified based on the conserved lysine-rich sequence, called the K-segment. Plants of Rosaceae family, such as strawberry, apple, sweet and sour cherry, are often damaged in winter at temperate regions due to the lack of acclimation and cold hardiness. However, there are limited data on dehydrin proteins characteristic to plants of the Rosaceae family. We studied the accumulation of dehydrin-like proteins of in vitro cold-acclimated microshoots of pear, apple and strawberry by immunoblot analysis using polyclonal antibodies against the K-segment [2]. The analysis revealed a species and genotype -specific accumulation pattern of dehydrins. Further, the protein identity was assessed using samples of apple, the model species of the Rosaceae family with available genome information. Proteins were separated and detected using 2D difference gel electrophoresis (DIGE) and the anti-dehydrin antibody immunoreactive proteins were digested by trypsin and analyzed using nano-LC/MS technique.

[1] Sun X, et al. (2013) .Plant Cell, 25: 38-55

[2] Close TJ, et al. (1993) Plant Mol. Biol., 23: 279-286.

P169

Growing Plants in the Stressful Space Environment Starts from the Optimisation of Cultivation in Soilless Systems

Veronica De Micco, Maria Giordano, Roberta Paradiso, Stefania De Pascale, Giovanna Aronne
University of Naples Federico II, Department of Agricultural and Food Sciences, Portici, Naples, Italy

The permanence of humans in extraterrestrial environments relies on the possibility to grow plants in closed ecological systems. The Space represents a stressful environment where plant development is constrained by factors normally not occurring on Earth (e.g. high levels of ionising radiation) and others acting at different levels (e.g. reduced gravity). The intensity by which these factors are perceived as stressors depends on many conditions, not least the plant's physiological and nutritional status. Consequently, the success of plant adaptation to Space depends on the possibility to optimise plant cultivation in Bioregenerative Life Support Systems (BLSSs), whose concept is based on hydroponic systems, being soilless cultivation a way to minimise environmental stresses.

Here, we report the results of cultivation trials conducted on soybean [*Glycine max* (L.) Merr.] within the research program MELiSSA (Micro-Ecological Life Support System Alternative) of the European Space Agency (ESA). The final goal is to optimise cultivar selection and cultivation protocols in hydroponics in order to maximise plant growth efficiency, thus reducing plant sensitivity to space stressors. The response of plants to different hydroponic systems, sources of nitrogen and presence/absence of specific symbiotic microbes in the rhizosphere is analysed. The first results in soilless cultivation show that the appropriate cultivar choice and the tough modulation of cultivation parameters allow obtaining plant growth performances improved if compared to open field, thus confirming soybean as a good candidate for cultivation in BLSSs.

P170

Effect of X-Rays on Plant Growth and Fruit Development in Tomato Plants Irradiated at Two Reproductive Phases

Veronica De Micco¹, Maria Giordano¹, Pasquale Scognamiglio¹, Roberta Paradiso¹, Giovanna Aronne¹, Stefania De Pascale¹, Carmen Arena²

¹Department of Agricultural and Food Sciences, University of Naples Federico II, Portici, Naples, Italy,

²Department of Biology, University of Naples Federico II, Naples, Italy

After the Chernobyl accident, the study of pollutant radiation effects in ecosystems has been widely considered and has aroused a renewed interest after the Fukushima disaster. Ionizing radiation influences growth and reproduction of plants depending not only on the radiation quality/quantity, but also on other factors inherent to the plant, including its developmental stage at the time of irradiation. The aim of this study was to evaluate the effect of different X-rays doses (0.3 - 100 Gy) on plants of *Solanum lycopersicum* L. 'Microtom' irradiated at two phases of the reproductive cycle: when at least one flower was blossomed (reproductive phase 1 - RP1) and during fruit ripening (RP2). The consequence of irradiation was assessed through the analysis of morphological development, leaf anatomical features, photochemistry and leaf gas exchanges, flowering phenology, fruit growth, anatomical and chemical fruit traits. Light and epi-fluorescence microscopy observations on ripe fruits were performed to detect possible radioprotective strategies based on mechanical or biochemical defences. The overall results showed that adult plants can maintain leaf integrity and high photosynthetic efficiency, while the reproductive cycle of the RP1 plants can be interrupted at high doses of irradiation, thus preventing fruit formation. The exposure of plants to increasing doses of X-rays during fruit ripening (RP2) primes a progressive increase in the amount of phenolic compounds, especially in sub-epidermal cell layers which are also more numerous than in not irradiated control fruits. Such compounds, having antioxidant properties, can act as natural screens against radiation.

P171

UV-Visible-NIR Spectral Reflectance of Tomato Leaves in Response to Salinity

Esther Leon-Victoria¹, Maria Belen Garcia del Moral², Luis F. Garcia del Moral¹

¹Universidad de Granada, Granada, Spain, ²Universidad de Almeria, Almeria, Spain

Three commercial tomato cultivars were subjected to three treatments of moderate salinity (0, 50 y 100 mM NaCl) with 9 replications. At the beginning of flowering, 3 measures of spectral reflectance were taken on the adaxial face of three mature leaves by using a portable spectral radiometer (FieldSpec Pro JRA110080, ASD) with a reflectance range of 350-2500 nm. Results showed that the spectral signature obtained was different for each salinity treatment. Thus, for the UV and visible spectra (350-700 nm) salinity induces higher reflectance, indicating loss of chlorophylls, carotenes and xanthophylls due to the accumulation of NaCl, one of the typical effects of salinity in sensitive plants. Moreover, lower reflectance (and therefore higher absorption) was observed for the control treatment between 750 and 1.300 nm (near-infrared spectrum). On the contrary, the reflectance was higher for the control treatment in the band between 1.400 and 2.500 nm (mid-infrared spectrum). In special, the higher reflectance found for salinity treatments at 980 nm (absorption by RuBisCO), seems to indicate a direct damage caused by salt to the photosynthetic enzymes, damage being more pronounced for higher salt concentration (100 mM) as compared to 50 mM. A similar effect was observed for the lipid content in the 1.150 nm region (indicating possible damage to membrane phospholipids), whereas reflectance was lower for salinity treatments at 1.450 nm and 1.925 nm, regions in which absorbs water and soluble carbohydrates, indicating a higher succulence and osmorregulation under salinity as compared with controls.

P172

Overexpression of OsDWA1, Arabidopsis DWD Homologue Gene, Improves Drought Stress Tolerance in Rice

Seok-Jun Moon, Seon-A Jeon, Beom Gi Kim, Dool Yi Kim, In Sun Yoon, Myung Ok Byun
National Academy of Agricultural Science, Suwon, Republic of Korea

In this experiment, we isolated the rice DWA gene, Arabidopsis DWD homologue gene which was well known as a substrate receptor for cullin-RING ubiquitin ligase 4 complexes. This gene encodes a protein of 875 amino acids, and has several of repeated WD40 domain in N-terminal and a katanin conserved domain in C-terminal, which was known to function as a microtubule severing enzyme. And also, OsDWA1 gene shows homology to Arabidopsis DWA genes which already reported to take part the ABA signal transduction pathway.

To predict the possible roles of OsDWA1 gene in abiotic stress response, we performed the qRT-PCR. Expression of OsDWA1 was induced by ABA treatment; however, was not significantly response to mannitol, NaCl and cold treatment. To determine its biological function in rice, we developed the OsDWA1-overexpressing transgenic rice plants by Agrobacterium-mediated transformation. Some of transgenic rice plants were selected, and we checked the expression of OsDWA1 gene by northern blot analysis. OsDWA1-overexpressing transgenic rice plants (T1 and T2) showed stronger tolerance to drought stress than non-transformed control plants. Interestingly, these transgenic plants showed ABA-sensitive phenotype in root growth assay. Collectively, these results indicate that expression of OsDWA1 gene in rice acts positively to counteract drought tolerance.

P173

Heavy Metal Tolerance in *Silene dioica*

Radim Cegan, Vojtech Hudzieczek, Eva Nevrtalova, Jiri Baloun, Boris Vyskot, Roman Hobza
Institute of Biophysics ASCR, v.v.i., Brno, Czech Republic

Plants growing in metal polluted sites evolved various mechanisms to survive abiotic stress. These plants exhibit altered expression of various stress responsive and metal homeostatis genes. *Silene dioica* represents one of the first studied plants with adaptation to increased heavy metal soil concentration, namely copper and cadmium. This dioecious plant belongs to the section *Elisanthe*, family *Caryophyllaceae* and possesses sex chromosomes with human-like sex determination system. Although *S. dioica* is a classical model of heavy metal resistance only limited data have been published about molecular mechanisms of this adaptation. To study these processes we have selected several ecotypes growing on copper mines heaps in Slovakia where concentration of copper is higher than usual. We performed copper accumulation and tolerance tests and we chose the best candidates for further analysis. For better characterisation we established the reference transcriptome by massive parallel sequencing of polyA selected and normalized cDNA libraries from *S. dioica* male and female individual. The libraries were generated from single pool of transcripts, combining RNA from roots, leaves, buds and flowers for each sex. To find out candidate genes responsible for heavy metal tolerance we applied transcriptome sequencing (RNA-Seq) of treated and untreated plants (roots and leaves) to identify which genes are regulated by copper stress and for supporting the reference assembly.

This work was supported by the Czech Science Agency (grant nos. 13-34962P and P501/12/G090).

P174

Is the Systemic Acquired Acclimation (SAA) a Photoprotective Mechanism against Light Stress in Common Bean Plants?

Luca Vitale¹, Gianpiero Guida^{1,2}, Pia Rosa Cataletto², Carmela Mistretta¹, Anna De Maio², Pasquale Giorio¹, Rossella Albrizio¹, Vincenzo Magliulo¹, Carmen Arena²
¹CNR-ISAFOM, Naples, Italy, ²University of Naples Federico II-Department of Biology, Naples, Italy

Long-term exposure to high light (HL) increases reactive oxygen species (ROS) production causing damages to DNA, proteins, and lipids, as well as to components of the photosynthetic apparatus. Plants have evolved different mechanisms to enhance the dissipation of the excess light energy under HL. The Systemic Acquired Acclimation (SAA) has been demonstrated to be an effective mean in *Arabidopsis* against oxidative damage. In this species, a systemic signal is rapidly transmitted from high-lighted exposed to distal shaded leaves, resulting in improved tolerance to oxidative stress. This work aimed to evaluate the possible role of SAA in photoprotection in common bean plants. To test this hypothesis, upper leaves of bean were exposed for 2h to high light (HL), whereas the below leaves were shaded (LL). After, LL leaves were exposed to high light (LL→HL). In HL, LL and LL→HL leaves, gas exchange and chlorophyll a fluorescence measurements were performed to assess the functionality of photosynthetic apparatus, whereas poly(ADP-ribose) polymerase (PARP) expression and activity, total antioxidant capacity and H₂O₂ content were monitored to evaluate the incidence of oxidative stress. The overall results were pondered to estimate the potential for photoprotection in bean plants due to the possible occurrence of SAA mechanism.

P175

Role of Peroxynitrite in the Responses Induced by Heat Stress in Tobacco BY-2 Cultured Cells

Massimo Malerba¹, Raffaella Cerana²

¹Università Milano-Bicocca, Department of Biotechnology and Biosciences, Milano, Italy, ²Università Milano-Bicocca, Department of Earth and Environmental Sciences, Milano, Italy

Temperatures above the normal optimum are sensed as heat stress (HS) by all living organisms and represent one of the major environmental challenges for plants. Our previous results show that in tobacco BY-2 cultured cells HS (5 min at 50°C) induces a set of stress responses (e.g. cell death, cytoplasmic shrinkage, nitric oxide (NO) and H₂O₂ production, release of cytochrome c from mitochondria). The role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the regulation of many plant physiological processes, programmed cell death included, is well established. On the contrary, the physiological role of peroxynitrite (ONOO⁻), the RNS species formed when NO reacts with the superoxide anion (O₂⁻), is less well known in plants. Peroxynitrite is capable to react with lipids, nucleic acids and proteins, triggering defence responses in animals and plants. In this work the role of peroxynitrite on some of the stress responses induced by HS in tobacco BY-2 cultured cells has been investigated by measuring both in the presence and in the absence of 2,6,8-trihydroxypurine (urate), a specific scavenger of peroxynitrite, the following parameters:

- cell death
- cytoplasmic shrinkage
- lipid peroxidation
- production of RNS and ROS
- activity of caspase-3-like proteases
- release of cytochrome c from mitochondria
- accumulation of molecular chaperones Hsp 90 in the mitochondria and Hsp70 Binding Protein (BiP) in the endoplasmic reticulum, and of 14-3-3 proteins in the cytosol.

P176

Influence of Long-Term Cold Priming on the Chloroplast Antioxidant System and Memory Development in *Arabidopsis thaliana* Accessions

Jelena Cvetkovic, Margarete Baier
Freie Univesitat Berlin, Berlin, Germany

Unpredictable temperature drops, early arrival of winter or late frost in summer often increased generation of Reactive Oxygen Species (ROS) inside chloroplast. Plants have to provide specific mechanisms to protect themselves against uncontrolled abiotic stresses. Chloroplasts are places where ROS, as key players in plant cell signaling, keep active oxygen under control.

We hypothesized that transformation of chloroplast antioxidant system acts as a medium term stress memory and that storing and recalling information from the past could benefit plants. Long cold priming (14 days at 4°C) impacts on the ascorbate-glutathione and water-water cycles. The reduced and oxidized forms of ascorbate and glutathione and their ratios are important for chloroplast-nucleus gene regulation. We observed two different types of priming effects on the total ascorbate pool in *Arabidopsis thaliana* accessions; some keep the ascorbate level constantly high and in others the total ascorbate level decreases after cold stress. Measuring the transcript abundance level of genes encoding in chloroplast antioxidant enzymes, the second stimulus (1 day at 4°C) gives indications on existing memory. Transcript data show that priming affects the response of second cold stimuli. Eco-physiological data together with metabolite analysis respond to the second stimulus. Fitness data, such as seed yield, show benefits and the potential costs of cold challenge in natural *Arabidopsis thaliana* accessions. Higher seed production or plants with increased seed biomass are fitness promotional strategies.

P177

Root Hydraulic Conductance and Aquaporin Gene Expression in Prunus Rootstocks under Waterlogging Stress

Paula Pimentel¹, Rubén Almada¹, Ariel Salvatierra¹, Boris Sagredo^{2,1}, Manuel Pinto^{3,1}

¹*Centro de Estudios Avanzados en Fruticultura, Rengo, Chile,* ²*Instituto de Investigaciones Agropecuarias, CRI Rayentué, Rengo, Chile,* ³*Instituto de Investigaciones Agropecuarias, CRI La Platina, Santiago, Chile*

In heavy soils, poor aeration in the rhizosphere is an important problem for the cultivation of Prunus species. In these soils, heavy rain or excessive irrigation can cause waterlogging and root hypoxia. In fruit trees, the tolerance to oxygen deficiency in roots is mediated by the characteristics of the rootstock. Prunus rootstocks are classified as hypoxia-sensitive, although differences among genotypes have been reported. An early physiological response to soil flooding is reduction of root permeability. Aquaporins play a key role in root water uptake capacity, which would provide a molecular basis for fast and reversible regulation of transmembrane water transport. This work assessed root hydraulic conductance, physiological parameters and expression patterns of aquaporin genes of Plasma Membrane Intrinsic Protein (PIPs) subfamily in two Prunus rootstocks with contrasting response to waterlogging. Root hydraulic conductance and gas exchange parameters diminished during root hypoxia treatment in both genotypes studied. However, tolerant rootstock showed higher levels of root hydraulic conductance than the sensitive one. qPCR analyses evidenced a drop in the transcript levels of PIPs evaluated in hypoxic samples of both genotypes analyzed. Interestingly, some PIPs genes showed an increment in their transcript levels in tolerant-genotype after 9 days of hypoxia treatment, reaching in some cases, higher transcript levels than control plants. These facts show a relation between root hydraulic conductance and aquaporin transcript levels in hypoxic treated rootstocks which is genotype dependent.

Acknowledgments: This work was funded by grants from FONDECYT N°11110080 and N°11110079, CEAF_R0811001, CONICYT. Plants were provided by Agromillora Sur S.A.

P178

The Salt-Responsive Transcriptome of *Solanum lycopersium* cv MicroTom and the Modification of the Phytohormone Pattern

Nieves Fernandez-Garcia, Enas Bardisi, Jose Salvador Rubio-Asensio, Enrique Olmos
CEBAS-CSIC, Campus Universitario de Espinardo, Edificio 25, 30100, Murcia, Spain

Tomato, one of the most important food crops worldwide, is frequently cultivated in regions with adverse conditions like poor-watered and saline soils (i.e. Mediterranean basin). High sodium concentrations are toxic to many cellular processes and adaptation to high saline concentrations implicates modifications in plant metabolism, cellular structure and gen expression. Moreover, it is known that phytohormones are regulating the signalling and the response to different abiotic stresses. In the present work, we profit from the affymetrix microarray chip developed for tomato to characterize the transcriptome of salt-stressed tomato plants. We have also quantified the concentration of the following phytohormones: gibberellins, abscisic acid, salicylic acid, jasmonic acid, cytokinins and indolacetic acid using LC-MS/MS analysis.

Plants were grown hydroponically and salt treatment was applied for one week (150 mM of NaCl). Actually, we have developed a microarray analysis for MicroTom roots. Setting a minimum threshold of 2-fold differential expression ($FDR > 0.05$), 258 probes (2,53%) were up-regulated and 154 probes were down-regulated (1,51%). GO terms analysis demonstrated the over-representation of GO terms implicates in phytohormone metabolism such as ethylene, jasmonic acid or gibberellins.

The phytohormone analysis showed that ABA, jasmonic acid, salicylic acid and indolacetic acid concentrations were significantly increased in salt-stressed plants. Interestingly, the concentration of the bioactive gibberellin GA_4 was significantly increased but the concentration of the bioactive gibberellin GA_1 was drastically reduced in salt stressed plants.

P179

The Subcellular Location of Arabinogalactan Proteins (AGPs) and the Effect of Yariv Reagent in the Disruption of AGPs in Tobacco (BY-2) Cell Cultures and in a Cell Line Adapted to High Salinity

Enrique Olmos, Enas Bardisi, Jesus Garcia de la Garma, Jose Salvador Rubio-Asensio, Nieves Fernandez-Garcia

CEBAS-CSIC, Campus Universitario de Espinardo, Edificio 25, 30100, Murcia, Spain

Arabinogalactan proteins (AGPs) are widely distributed in plant kingdom. They typically consist of approximately 10% protein and 90% carbohydrate. AGPs have been located in the plasma membrane, tonoplast, cytoplasm, cell-wall and in the media of cell cultures. Yariv reagent is a synthetic probe that binds and aggregates AGPs. For these reasons, Yariv reagent has been frequently used for AGPs purification and quantification.

Previous studies have determined that AGPs are highly accumulated in the culture medium of salt-adapted tobacco BY-2 cells but are significantly reduced in the plasma membrane. In this work, to determinate the subcellular AGPs distribution and the environmental response of plant cell, we focused on the analysis of AGPs distribution using monoclonal antibodies against AGPs (JIM13, JIM15 and LM2). To evaluate the effect of Yariv reagent, control and salt-adapted cell were treated with 100 μ M of Yariv during 24 hours. Subcellular distribution was analysed by electron microscopy and laser confocal microscopy. Firstly, our results confirm that AGPs are highly accumulated in culture media of salt-adapted cells and are significantly reduced in the plasma membrane, tonoplast and cytoplasm. Secondly, Yariv reagent induces cell death in control cell cultures but not in salt-adapted cells. Thirdly, we have observed that Yariv reagent is accumulated at different points in the external face of the plasma membrane and it was confirmed by immunolocalization of AGPs (JIM13 antibody). However, no effect was observed in salt-adapted cells.

Acknowledgements

This work was supported by the project BFU-2009-06499/AGR, from the Spanish Ministry of Education and Science (MEC-CICYT).

P180

The Redox Sensitive β -Amylase 1 (BAM1) as a Possible Link between Starch Degradation and Proline Biosynthesis in Arabidopsis under Mild Osmotic Stress

Martina Zanella^{1,2}, Gian Luca Borghi¹, Diana Pazmino², Claudia Pirone¹, Alex Costa³, Paolo Trost¹, Diana Santelia², Francesca Sparla¹

¹Department of Pharmacy and Biotechnology FaBiT, Bologna, Italy, ²Institute of Plant Biology, Zurich, Switzerland, ³Department of Bioscience, Milano, Italy

Starch is a highly compact polymer made up of glucose monomers. The organized structure of starch granules reflects their function: to store energy without altering the osmotic balance.

Under physiological conditions, primary starch degradation occurs in the chloroplast stroma during the night and requires the activity of β -amylase 3 (BAM3) that releases maltose units from the polyglucan chains. Through MEX1 (Maltose EXporter 1), maltose is exported into the cytoplasm where it is further metabolized. Among the different Arabidopsis β -amylase isoforms (nine in total), BAM1 is the only one to be redox sensitive, and it was suggested to be involved in diurnal starch degradation in response to osmotic stress. Quantitative PCR confirmed this hypothesis, showing a simultaneous decrease of BAM3, and increase of BAM1 transcript levels, in response to 150 mM mannitol treatments. In order to study the fate of the carbon skeletons derived from starch degradation during osmotic stress, *bam1* and *bam3* mutants were hydroponically grown and their response compared to WT plants. Our findings strongly suggest that under osmotic stress, starch is mainly degraded during the day by BAM1 and that about 60% of starch-derived carbon skeletons are converted into proline. Given the lower level of lipid peroxidation observed in WT and *bam3* plants compared to *bam1* mutant under same conditions, starch-derived proline is suggested to exert its function both as a compatible osmolyte and a ROS scavenger.

P182

OsNAC5 Overexpression Enlarges Root Diameter Leading to Enhanced Drought Tolerance and Increased Grain Yield in the Field

Jin Seo Jeong, Youn Shic Kim, Mark Redillas, Harin Jung, Seung Woon Bang, Yang Do Choi, Ju-Kon Kim

Seoul National University, Seoul, Republic of Korea

Drought conditions are among the most serious challenges to crop production worldwide. Here, we report the results of field evaluations of transgenic rice plants overexpressing OsNAC5, under the control of either the root-specific (RCc3) or constitutive (GOS2) promoters. Field evaluations over three growing seasons revealed that the grain yield of the RCc3:OsNAC5 and GOS2:OsNAC5 plants were increased by 9-23% and 9-26% under normal conditions, respectively. Under drought conditions however, RCc3:OsNAC5 plants showed a significantly higher grain yield of 22-63%, whilst the GOS2:OsNAC5 plants showed a reduced or similar yield to the non-transgenic (NT) controls. Both the RCc3:OsNAC5 and GOS2:OsNAC5 plants were found to have larger roots due to an enlarged stele and aerenchyma at flowering stage. Cell numbers per cortex layer and stele of developing roots were higher in both transgenic plants than NT controls, contributing to the increase in root diameter. The root diameter was enlarged to a greater extent in the RCc3:OsNAC5, suggesting the importance of this phenotype for enhanced drought tolerance. Microarray experiments identified 25 up-regulated genes by more than 3-fold ($P < 0.01$) in the roots of both transgenic lines. Also identified were 19 and 18 up-regulated genes that are specific to the RCc3:OsNAC5 and GOS2:OsNAC5 roots, respectively. Of the genes specifically up-regulated in the RCc3:OsNAC5 roots, GLP, PDX, MER15 and O-methyltransferase were implicated in root growth and development. Our present findings demonstrate that the root-specific overexpression of OsNAC5 enlarges roots significantly and thereby enhances drought tolerance and grain yield under field conditions.

P183

Foliar Antioxidant Status and Photosynthetic Performance of *Pisum sativum* Plants Exposed to Olive Mill Wastewater Irrigation

Georgia Ouzounidou¹, Maria Asfi^{1,2}, Kalliope Papadopoulou³, Fragiskos Gaitis⁴, Michael Moustakas²
¹Institute of Food Technology, Hellenic Agricultural Organization-Demeter,, Lycovrissi, Attica, Greece,
²Department of Botany, School of Biology, Aristotle University of Thessaloniki,, Thessaloniki, Greece,
³Department of Biochemistry and Biotechnology, University of Thessaly,, Larissa, Greece, ⁴Laboratory of Attica, Directorate of Laboratory Control, Hellenic Food Authority, Nea Philadelphia, Attica, Greece

The economic benefit of olive oil production is unfortunately associated with the generation of large quantities of olive-mill wastewater (OMW), a recalcitrant dark brown liquid waste with phytotoxic properties produced from the olive oil extraction process. OMW disposal presents a large-scale environmental problem due to its significantly high organic load, its relatively low biodegradability, a distinctive odor, an acidic pH (4.0-5.5) and the antibacterial phenolic compounds. To study the effect of using OMW for irrigation, we applied OMW dilutions 1:20 and 1:10 on pea plants. Root and shoot growth, chlorophylls, carotenoids, maximum PSII photochemical efficiency (F_v/F_m), operating PSII efficiency (Φ_{PSII}) and open reaction centers (q_p) decreased in a dose-dependent OMW treatment. Yet, CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and water use efficiency ($WUE = A/E$) also decreased. Non-photochemical quenching (q_N) increased only in the low OMW (1:10) dilution. In contrast to decrease in g_s , the increase in intercellular CO_2 concentration (C_i) with decreasing OMW dilutions, suggests metabolic causes, possibly domination of respiratory metabolism. However, the level of leaf lipid peroxidation decreased, suggesting a better homeostasis of reactive oxygen species (ROS) under OMW treatments. The latter could not be attributed to enzymatic antioxidant protection due to superoxide dismutase (SOD) activity, since all three SOD 1, SOD 2, SOD 3 isoforms were decreased in OMW-treated samples. Thus, despite the undesirable phytotoxic effects of OMW on photosynthetic performance of pea plants, OMW phenolic compounds resulted in an increased leaf antioxidant protection.

P184

Identification and Functional Study of an ABA-Activated MAPK Module Involved in Abiotic Stresses

Axel de Zelicourt^{1,2}, Agyemang Danquah¹, Marie Boudsocq¹, Nicolas Frei dit Frey¹, Nathalie Leonhardt³, Stephanie Pateyron¹, Frederik Gwinner⁴, Maria J. Marcote⁵, Heribert Hirt^{1,2}, Jean Colcombet¹

¹Unité de Recherche en Génomique Végétale, Institut National de Recherche Agronomique/Centre National de la Recherche Scientifique/Université d'Evry Val d'Essonne, Saclay Plant Sciences, Evry, France, ²Center for Desert Agriculture, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia, ³Institut de Biologie Environnementale et Biotechnologie, Centre National de la Recherche Scientifique/Commissariat à l'Energie Atomique/Université Aix-Marseille II, Saint Paul les Durance, France, ⁴Institut Pasteur, Paris, France, ⁵Biochemistry and Molecular Biology Department, Universidad de Valencia, Valencia, Spain

Abscisic acid (ABA) is the major phytohormone involved in long-distance drought signalling as well as other important stress-related and developmental processes. The identification of the ABA core signalling module highlighted the importance of phosphorylation events in ABA signalling and drought adaptation. Among them, the mitogen activated protein kinase (MAPK) cascades play important roles in regulating the signaling network. To date, however, only a handful of MAPKs have been identified and characterized in ABA signaling. Using transiently transformed mesophyll protoplasts generated from WT and mutant leaves, we report here the identification of a whole ABA-activated MAPK cascade of Arabidopsis, constituted of two MAP3Ks: MAP3KA and MAP3KB, one MAP2K and several MAPKs. In planta, MPK activation by ABA is blocked in map2k and map3kAmapk3kB double mutants. Mutants in the MAPK module also exhibit hypersensitivity to ABA and extended periods of drought stress. ABA-insensitive mutant analysis further shows that the MAPK pathway is activated by the ABA core module and that long time activation of the MAPK pathway depends on protein synthesis of MAP3KA/B, unveiling a novel mechanism for stress-induced long term MAPK activation. This work provides new insight how the ABA core module regulates a MAPK signalling pathway for adapting plants to drought stress.

P185

New Insights into Pot Size Effects on Oilseed Rape Vegetative Growth and Its Interaction with Response to Drought

Anaëlle Dambreville, Mélanie Griolet, Gaëlle Rolland, Myriam Dauzat, Alexis Bédiée, Anthony Rosello, Bertrand Muller, Denis Vile, Christine Granier
INRA, Montpellier, France

In the current context of reduction of the available freshwater, drought is one of the most studied environmental constraints restricting plant growth. Many plant phenotyping platforms have been developed to assess plant responses to drought. In these high-throughput platforms, plants are grown in pots and soil water content is monitored automatically. Several studies have reported a major effect of pot size and soil volume on plant growth. The interaction of the pot size with other growth conditions is often ignored even when plant growth response to drought is studied. We investigated whether pot size affects shoot growth responses to drought. Shoot, leaf and cell traits were studied for three genotypes of oilseed rape (*Brassica napus* L.) grown under two soil water regimes, in small and in large pots. Small pots led to a reduction of all growth-related traits. These traits were also decreased in response to drought, whatever the genotype or the pot size but the trait sensitivity to drought depended on the pot size. Depending on traits, allometric relationships were modified by pot size demonstrating complex interactions between pot size and plant responses to drought. Our results suggest that pot size has to be considered when defining the experimental set up as it may affect plant responses to other environmental conditions.

P186

Impact of a Humidity Shock on Immature Coffee Plants

Elhadji Thioune^{1,2}, James McCarthy², Thomas Gallagher¹, Bruce Osborne¹

¹University College Dublin-School of Biology and Environmental Science, Dublin, Ireland, ²Centre de Recherche Nestlé-Tours, Tours, France

Atmospheric water vapour (humidity) is an important environmental factor that influences plant growth and performance through its effect on water movement between the intercellular air spaces of the leaf and the atmosphere. Despite its global significance as a potentially important environmental constraint, the impact of variations in humidity has received little attention. We have observed that immature well watered *Coffea Canephora* plants, acclimatized to high humidity conditions (RH 99%, VPD 0.038 kPa), exhibited rapid leaf wilting and drooping, and reductions in leaf water content within 30 minutes after transfer to low humidity conditions (RH 33-36%, VPD 2.28 - 2.39kPa) and within two hours leaves had begun to desiccate. This was associated with a decrease in carbon assimilation and stomatal conductance, whilst photochemistry remained largely unchanged until severe leaf damage was observed. On return to RH 99% conditions most leaves recovered, however many leaves became necrotic even if leaf droop was no longer observed and the relative water content had recovered, indicating permanent damage after the humidity shock. Associated with these changes, several transcripts that display altered expression during long term soil water deficits, such as Dehydrin 1a (DH1a), Myb transcription factor 10 (Myb10) and Dehydration-Responsive Element-Binding protein1 (DREB1) also displayed altered expression during the short term humidity regime. This is an indication of some commonality in the response of coffee plants to atmospheric and soil water deficits

P187

Developing a Screening Protocol for Chilling Tolerance in Miscanthus

Simon Fonteyne¹, Peter Lootens¹, Tom De Swaef¹, Hilde Muylle¹, Isabel Roldan-Ruiz¹, Dirk Reheul²
¹The Institute for Agricultural and Fisheries Research (ILVO), Melle, Belgium, ²Ghent University, Ghent, Belgium

Miscanthus is a high yielding, low input biomass crop. At present, only one variety, the natural hybrid *M. x giganteus* is commercially grown. New varieties will be developed to increase yield, lower the risk of diseases and pests and adapt the crop to environmental stresses, such as drought, salt and low temperatures. The EU FP7 project OPTIMSC aims to accelerate miscanthus breeding by trialing new elite germplasm in multiple locations across Europe and by identifying key traits related to abiotic stress tolerance. As low temperatures limit the growing season and restrict the cultivation area, a set of 100 genotypes will be screened for chilling tolerance. Tolerance to low temperature will result in earlier emergence and better spring growth. Considerable variation in tolerance to low temperatures must exist in *M. sinensis* and *M. sacchariflorus*, given the large geographical distribution of these species. Three different screening methodologies were tested on six miscanthus genotypes (5 *M. sinensis* and 1 *M. sinensis* x *sacchariflorus* hybrid) grown at 20°C and 12°C : i) leaf elongation, ii) plant height ii) photosynthesis and iii) chlorophyll fluorescence. The genotypes showed large variation in chilling tolerance. Leaf elongation rate decreased less in chilling tolerant genotypes. While photosynthesis decreases significantly between the two tested temperatures, the difference was not significant between the tested genotypes. Chlorophyll fluorescence showed good correlation to growth at 12°C and could be used to screen for chilling tolerant genotypes.

P188

Effects of Cadmium on the Leaves of Maize Plants

Fabiana Hibary Kato Belini, Salete A Gaziola, Fernando A Piotto, Jade M Soares, Ricardo Antunes Azevedo

University of São Paulo, Piracicaba, São Paulo, Brazil

Soil contamination by heavy metals is a present problem. Cadmium (Cd) is extremely harmful heavy metal to cells which may eventually induce the excessive production of reactive oxygen species. The present study aimed to evaluate how the main antioxidant enzymes from leaves of maize plants respond when the plants were subjected to cadmium. Cadmium (30 mg/Kg) was applied to maize plants in the pre-fertilization period and 20 days after pollination the leaves were harvested and the antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX) activities, and malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) production were measured. The results of spectrophotometry and non-denaturing PAGE showed no significant difference in the activity and amount of the compounds analyzed in the leaves of plants subjected to cadmium and control plants. It is known that maize is generally more tolerant to cadmium than some other crops species and, in agreement with previous results not described here, most of the metal is retained in the plant roots, with little translocation of Cd to the above-ground parts. We are currently considering and testing different Cd concentrations, other stages in which Cd is supplied to the plants, among other variations, but it is clear that under the current conditions tested, Cd did not affect maize plants to a point that an antioxidant stress response was triggered.

Key-words: maize, cadmium, antioxidant enzymes

(Financial Support by FAPESP, Brazil).

P189

Endogenous Phytohormones and Cellular Redox Status in Ammonium-Induced Embryogenic Culture of *C. pepo*

Ales Pencik¹, Veronika Tureckova¹, Mihaela Glosova¹, Sandi Paulisic², Jakub Rolcák¹, Miroslav Strnad¹, Snjezana Mihaljevic²

¹Laboratory of Growth Regulators, Faculty of Sciences, Palacký University and Institute of Experimental Botany ASCR, Olomouc, Czech Republic, ²Institute Ruder Boskovic, Zagreb, Croatia

Ammonium, when supplied as the sole source of nitrogen, may trigger somatic embryogenesis induction in some plant species including pumpkin. To get an insight in the involvement of phytohormones in this adaptive developmental response, status of endogenous ABA, IAA, CKs and their conjugates was analyzed. Results were compared with phytohormone status in an embryogenic culture induced by exogenously supplied 2,4-D. Endogenous ABA peaked after 7 days of cultivation in 2,4-D-medium while in NH_4^+ -medium maximum of ABA is measured after 21 days of cultivation, what suggested the involvement of different stress response mechanisms. Furthermore, NH_4^+ -induced embryogenic cultures accumulated endogenous IAA to a level similar or higher than the level induced by exogenously supplied 2,4-D. Transfer from NH_4^+ -medium into the medium containing both ammonium and nitrate decreased free IAA content. A significant decrease in endogenous IAA was also observed when the acidification of NH_4^+ -medium during cultivation was prevented by 25 mM MES. On the other side, buffering of the culture medium increased endogenous ABA and CKs. Ammonium nutrition was also found to decrease total ascorbate and glutathione content as well as peroxidase activity what coincided with low mitotic index and growth retention of the NH_4^+ -induced culture, probably caused by unfavorable growth conditions. Results demonstrate that the high endogenous auxin content and the dominance of reduced forms of ascorbate and glutathione are key factors involved in the acquisition of embryogenic competence in ammonium-induced somatic embryogenesis in pumpkin. Interplay between ammonium nutrition, pH and hormone signaling during somatic embryogenesis induction in pumpkin is discussed.

P190

Nitric Oxide Induces the Alternative Oxidase Pathway under Phosphate Deficiency

Beatriz Royo^{1,2}, José F Morán², R George Ratcliffe¹, Kapuganti J Gupta¹

¹Department of Plant Sciences, University of Oxford, South Park Road, Oxford OX1 3RB, UK,

²Institute of Agro-Biotechnology, IdAB-CSIC-UPNa-GN, Public University of Navarre, Campus de Arrosadía s/n, E-31006 Pamplona, Navarra, Spain

Nitric oxide (NO) is a signal molecule that plays an important role in plant growth, development and stress physiology. In recent years the biosynthesis and action of NO in plant mitochondria has gained increasing attention. The plant mitochondrial electron transport has two pathways for the reduction of oxygen: the cytochrome pathway and the alternative oxidase (AOX) pathway. Both pathways are involved in mitochondrial NO metabolism, and the AOX pathway protects plants against the production of deleterious quantities of reactive oxygen species (ROS).

We studied the cytochrome and alternative pathways in *Arabidopsis thaliana* subjected to phosphate deficiency. WT (cv. Columbia) and *nia1,2* (nitrate reductase deficient mutant) seedlings were grown in axenic cultures that contained either 1 mM (+P) or 0 mM (-P) inorganic phosphate.

Phosphate starvation increased production of NO in WT, leading to increased capacity in the AOX pathway and decreased superoxide levels. In contrast, the *nia1,2* mutant failed to induce NO under phosphate starvation, leading to a lower AOX capacity and increased production of superoxide. As a result, the *nia* mutants had a lower biomass than WT plants under phosphate starvation. Furthermore the NO donor GSNO restored the *nia1,2* phenotype under phosphate starvation, confirming the requirement for NO for stress resistance during phosphate starvation.

P191

Ioxynil Xenobiotic Effect and Phosphorous Deficiency on Pea Plants during Ammonium Nutrition

Estibaliz Urarte, Raquel Esteban, Beatriz Royo, J.F. Moran
Institute of Agrobiotechnology, IdAB-CSIC-UPNA-GN, Public University of Navarre, Campus de Arrosadía s/n, E-31006 Pamplona, Navarra, Spain

Nitrogen oxidized species (RNS) are molecules involved in cell signalling, characterized for modulating plant responses to stressful conditions. An essential RNS in plants is nitric oxide (NO), known for mediating in numerous physiological processes. Despite the importance of NO for plant development, the existence of a mammalian-like nitric oxide synthase is doubtful and data on oxidative production of NO in plants are still vague (1). However, the detection of NO, nitrite and nitrate in plants grown under exclusive ammonium nutrition demonstrates the existence of oxidative reactions leading to the production of such molecules from the ammonium cation. It has been described that phosphorous deficiency enhances NO production (2) and that the enzymatic system cytochrome P450 (CytP450) is induced by phosphorous starvation (3). For that reason, the general aim of this work was to study the effect of the herbicide ioxynil, a CytP450 inhibitor, and phosphorous deficiency, which induces CytP450, monitoring NO and nitrate production in pea plants under ammonium nutrition. Phosphorous deficiency enhanced lateral root development and decreased growth, whilst ioxynil inhibited lateral root growth. The implication of CytP450 in nitrogen oxidation is discussed. The study of NO production under ammonium nutrition will have a clear impact in the knowledge of tolerance to ammonium in plants of agricultural interest, and it will surely contribute to ameliorate fertilization policies.

- 1) Gupta et al. (2011) Trends Plant Sci. 16: 160-168
- 2) Wang et al. (2010) New Phytol. 187: 1112-1123
- 3) Hammond et al. (2003) Plant Physiol. 132: 578-596

P192

The Polyadenylation Factor Subunit CPSF30: A Key Factor of Programmed Cell Death in Arabidopsis

Quentin Bruggeman¹, Marie Garmier¹, Linda de Bont¹, Ludivine Soubigout-Taconnat², Christelle Mazubert¹, Moussa Benhamed¹, Cécile Raynaud¹, Catherine Bergounioux¹, Marianne Delarue¹
¹*Biology Institute of Plants, CNRS, ORSAY, France*, ²*Unité de Recherche en Génomique Végétale, INRA, Evry, France*

Programmed cell death (PCD) is essential for several aspects of plant life, including development and stress responses. Indeed, incompatible plant-pathogen interactions are well known to induce the hypersensitive response, a localized cell death at or around the infection site and mutational analyses in plants have identified several key PCD components. In Arabidopsis (*Arabidopsis thaliana*), we recently identified the *mips1* mutant, which is deficient for the key enzyme catalysing the limiting step of myo-inositol (MI) synthesis. One of the most striking features of the *mips1* mutant is the light-dependent formation of lesions on leaves due to Salicylic Acid (SA)-dependent PCD, revealing roles for MI or inositol derivatives in the regulation of PCD. Here, we identified a regulator of plant PCD by screening for mutants that display transcriptomic profiles opposing that of the *mips1* mutant. Our screen identified the *oxt6* mutant, which has been described previously as being tolerant to oxidative stress. The *oxt6* mutation is due to a T-DNA insertion in the CPSF30 gene, which encodes a polyadenylation factor subunit homolog. We show that CPSF30 is required for lesion formation in *mips1* via SA-dependant signalling and that the pro-death function of CPSF30 is not mediated by changes in the cellular redox status. We also show that the *oxt6* mutation suppresses cell death in other lesion mimic mutants such as *lsd1*, *mpk4*, *cpr5* and *cat2*, suggesting that CPSF30 and thus the control of mRNA 3' end processing, through the regulation of SA production, is a key component of the plant immune response.

P193

Physiological Characteristics and Expression of Some Antioxidant and Cell Wall Related Genes during Desiccation of Sun and Shade *Haberlea rhodopensis* Plants

Katya Georgieva¹, Gergana Mihailova¹, Lars Dietzel², Claudia Büchel²

¹*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria,* ²*Institute for Molecular Biosciences, University of Frankfurt, Frankfurt, Germany*

The alterations in some physiological characteristics and gene expression during desiccation and following rehydration of shade and sun *Haberlea rhodopensis* plants were compared.

Upon desiccation the electrolyte leakage gradually increased in both sun and shade leaves and it was four fold higher in dry leaves compared to control, suggesting some membrane damage. However, the recovery after rehydration showed that this damage was reversible. The photosynthetic activity was higher in sun plants not only in control stage but also during desiccation. In addition, their recovery was faster compared to shade plants during rehydration. The quantum yield of PSII photochemistry in the light adapted state was also higher in control and moderately dehydrated sun plants as well as after their rehydration.

The pectate lyase gene was strongly upregulated during dehydration and also after rehydration. The ascorbate peroxidase and superoxide dismutase genes were upregulated but they were more expressed in sun plants. The changes in the expression of UDP xylose synthase gene were more pronounced in moderately desiccated and dried plants. The monodehydroascorbate reductase, NA2, thioredoxin-h and pectinase genes were more or less upregulated during dehydration in sun and shade plants, but the response in sun plants came earlier. The putative sucrose synthase/pectinesterase gene was upregulated during dehydration and after 1 day of rehydration and similarly to the others genes the pectinesterase was more expressed in sun plants. We found that the only not responsive tested gene was the chloroplast ERD1.

P194

UV-B Light Exposition Induces Phenylpropanoid Pathway Genes in Blueberry Leaves at the Short Term

Ana Luengo-Escobar^{1,3}, Laura Jaakola^{4,5}, Marjorie Reyes-Diaz^{2,3}, Miren Alberdi^{2,3}

¹Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de la Frontera, P.O. BOX 54-D, Temuco, Chile, ²Departamento de Ciencias Químicas y Recursos Naturales, Universidad de la Frontera, P.O. BOX 54-D, Temuco, Chile, ³Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile, ⁴Climate Laboratory, Department of Arctic and Marine Biology, University of Tromsø, NO-9037, Tromsø, Norway, ⁵Norwegian Institute for Agricultural and Environmental Research, Bioforsk Nord Holt, Box 2284, N-9269, Tromsø, Norway

Blueberry, *Vaccinium corymbosum* L., has been positioned as an important crop due to their high content on flavonoids. The flavonoids pathway is well understood in fruits and the key regulatory genes controlling this pathway have been identified in many species. However, there is less information regarding the influence of light quality on plant status, especially ultraviolet radiation (UV-B) and the mechanisms addressing this factor on gene expression level. In the present study, we show the expression of the phenylpropanoid pathway genes; phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and UDP-flavonoid glycosyl transferase (UFGT) in blueberry leaves under UV-B radiation. We found that the expression of PAL under UV-B was significantly increased after 6 hours of treatment, similar to CHS at the same time. At 18 h of treatment, PAL and CHS were no different to control, increasing around 3 fold at 42 h. Even, during the recovery time (42 h without UV-B radiation) the transcript levels remain higher compared to control plants. On the other hand, the expression of UFGT was highly upregulated at the first 6 hours of treatment (7 fold), decreasing to control values at 18 h and increasing at 42 h (2.5 fold). However, during the recovery time, the transcript levels were statistically no different from control values. We observed a particular trend in the expression of these general flavonoid biosynthetic genes under UV-B radiation, but most relevant, the role of glycosylation of flavonoids and therefore a photoprotection response under UV-B stress. Acknowledgments: CONICYT fellowships and FONDECYT N°1110726.

P195

Overexpression of AtBPM1 Gene in Arabidopsis Confers Salt and Osmotic Stress Tolerance

Kristina Majsec¹, Rosemary Vuković², Nenad Malenica¹, Bauer Nataša¹

¹*Department of Molecular Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia,*

²*Department of Biology, University of Osijek, Osijek, Croatia*

MATH-BTB proteins are identified in many eukaryotes as important regulators in various biological processes, including stress responses and development. A small family of six MATH-BTB genes is present in *Arabidopsis thaliana* genome. They encode proteins that directly interact with and target for proteasomal degradation transcription factor ATHB6 and assemble with members of the ERF/AP2 transcription factor family. To better understand the function of MATH-BTB proteins, we created plants overexpressing the AtBPM1-GFP fusion protein. Transgenic plants had shortened leaf petioles, rosette leaves curvature and showed premature flowering. AtBPM1-GFP recombinant protein localisation was monitored in different plant tissues during development. AtBPM1-GFP was expressed in chloroplasts, nucleus and cytoplasm during embryo development. It accumulated in the nuclei of the root stele and at lateral roots branching sites, as well as in nuclei of stomata cells. Comparative analysis in 14-day-old transgenic and wild type seedlings by qPCR revealed that overexpression of AtBPM1-GFP transgene decreased expression of other AtBPM genes. To investigate function of MATH-BTB proteins in more detail, we examined the effect of various stress stimuli on germination and growth of AtBPM1-GFP transgenic *A. thaliana*. When subjected to water deficit stress by mannitol, and salinity stress with NaCl and KCl, 14 day-old transgenic seedlings showed a considerable increase in tolerance compared with wild type seedlings. Furthermore, transgenic plants showed better germination and growth when subjected to elevated ABA concentrations.

Our results indicate AtBPM1 regulatory role towards other MATH-BTB family members and confirmed their pivotal role in stress response and ABA signalling.

P196

The Role of Auxin Transport in Plant Stress Adaptation

Agnieszka Bielach, Magda Zaoralová, Sharmila Madhavan, Vanesa Tognetti
Central European Institute of Technology, Masaryk Univeristy, Brno, Czech Republic

Plants are continuously exposed to a wide and diverse range of abiotic stress conditions which can lead to overproduction of reactive oxygen species (ROS). This results in the disruption of cellular and molecular processes. Modulation of plant development in response to the environment plays a major role in plant stress adaptation which is governed by changes in plant hormones homeostasis. Auxin is a main regulators of plant growth and development and recent studies show its emerging role in plant response to stress. However, molecular mechanism of ROS-auxin crosstalk remains scarce and in most studies has been identified as a side observation. Polar auxin transport mediated by PIN FORMED (PIN) transporters has been shown as a main modulator of plant development. Additionally, it has been proposed that some PIN transporters (PIN5, PIN6 and PIN8) participate in cellular auxin homeostasis.

Expression analysis of different promoter-GUS fusion lines grown under stress conditions reveals that PINs gene expression is differentially regulated by different stressors. Moreover, shoot phenotype of stressed -knock-out and -constitutively active overexpression Arabidopsis lines shows that pin6 and 35S::PIN5 are more tolerant to the applied stress. Photosynthetic pigments' (chlorophyll a, chlorophyll b and carotenoids) concentration is not altered in PIN mutants when comparing to the wild type. Interestingly, stress induced and auxin regulated anthocyanin's biosynthesis is decreased in pin6 mutant, which is more resistant to oxidative stress when comparing to the control plants. Our preliminary results indicate that PIN5 and PIN6 modulation of cellular auxin homeostasis could play important role during stress adaptation.

P197

Physiological Characteristics of *Lactuca sativa* Subjected to Drought Stress

Sandra Sakalauskiene^{1,2}, Ausra Brazaityte², Romualdas Juknys¹, Irena Januskaitiene¹, Ramunas Sirtautas², Alina Ceidaite², Viktorija Vaštakaite², Pavelas Duchovskis²

¹Vytautas Magnus University, Kaunas, Vileikos str. 8, Lithuania, ²Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kaunas district, Babtai, Kauno str. 30, Lithuania

The aim of the study was to investigate the effect of substrate moisture on the physiological indices of *Lactuca sativa* plants. The object of this study was two different genotypes of lettuce (green babyleaf and redbaby leaf). Experiment was performed in close growth chamber of controlled environment at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Plants were placed at 21/18 °C day/night temperature and 18 h photoperiod. High-pressure sodium lamps (SON-T Agro, Philips) were used for illumination ($150 \mu\text{mol m}^{-2} \text{s}^{-1}$). Experiment started after 16 days from germination. Plants were grown in peat substrate at two moisture regimes: normal (~35%) and drought (~10%) substrate. Different combinations of investigated factors had a significant influence on changes of *Lactuca sativa* plants physiological indices. The lack of substrate moisture reduced leaf area, fresh and dry biomass in the both lettuce genotypes, but under drought conditions increased accumulation of chlorophylls a, b and carotenoids. Lettuce exposed to drought conditions accumulated a larger amount of phenolic compounds, ascorbic acid, anthocyanins, however, their content in plants depended on genotyp. Redbaby leaf lettuce affected by drought conditions accumulated the significantly larger amount of total tocopherols.

Acknowledgements. *Postdoctoral fellowship is funded by Research Council of Lithuania according to the project "Postdoctoral fellowship Implementation in Lithuania".

P198

Drought-Stress Fundamentals: A Proteomic Approach to Compare the Arabidopsis Responses to Three Distinct Drought-Stress Induction Methods

Mafalda A. A. Rodrigues¹, M. Margarida Oliveira¹, Isabel A. Abreu^{1,2}
¹ITQB-UNL, Oeiras, Portugal, ²IBET, Oeiras, Portugal

Drought is a major environmental stress factor that severely affects plant growth and development. As a direct effect of impending climatic change, it will have tremendous impact on future crop productivity. Consequently, responses to drought stress have been widely studied on several plant models and crops. However, due to practical reasons, Water Withdraw, Dehydration, and PEG/Mannitol-induced stresses have been recurrently used to investigate drought stress responses in plants, regardless of the specific mechanisms that are being triggered by each of them. Consequently, many conclusions have been drawn, even regarding plant physiological responses, based on studies where non-physiological drought-stress induction was performed. This issue has been a subject of controversy in the past years, but no study was yet conducted to finally determine how similar or distinct are the plant responses to each of these distinct stresses. Drought stress can induce three main types of interactive modification in plants: (a) alteration in gene expression (transcriptome); (b) dynamics in protein production, modification and degradation (proteome) and (c) alterations in the metabolic pool (metabolome). Being proteins the true effectors of a plant's responses, by a proteomic analysis of water withdrawal; dehydration; and PEG-induced stress responses, we intend to identify the main players of these responses mechanisms, but also to conduct a fair comparison of these three distinct drought-stress induction methods.

P199

Red Light Effects on Nitrate Reduction in Lettuce

Sandra Sakalauskiene¹, Akvile Virsile¹, Giedre Samuoliene¹, Ausra Brazaityte¹, Ramunas Sirtautas¹, Jule Jankauskiene¹, Jurga Miliauskiene¹, Algirdas Novickovas², Aiste Bagdonaviciene¹, Pavelas Duchovskis¹

¹*Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kaunas district., Babtai, Kauno str. 30, Lithuania,* ²*Institute of Applied Research, Vilnius University, Vilnius, Sauletekio al. 9-III, Lithuania*

Red light is known to regulate nitrate reduction processes in plants, possibly through the action of photoreceptor phytochrome signaling cascade. In this study we revise the red LEDs light effect on nitrate reduction in red and green baby leaf lettuce in different growth and lighting environments. Red 640 nm LED light was investigated as the sole light source (I) and compared with the high pressure sodium lamp (HPS) illumination. The combination of red LED 627 nm light and HPS illumination (II) was evaluated. I and II experiments was carried out in the closed growth chamber for a whole lettuce growth cycle. The short-term pre-harvest 638 nm red LED light pre-treatment supplemental for HPS in the greenhouse (III). Red 640 nm light had no remarkable effect on nitrate contents in both lettuce varieties. Red 627 nm and HPS illumination resulted in 60% reduction in nitrate contents in red leaf lettuce, but had no effect in green leaf lettuce. At short-term red LED and HPS light in the greenhouse nitrate contents were 75% lower in red leaf lettuce as compared to sole HPS lighting. However, in green leaf lettuce slight increase in nitrate contents was observed. In summary, red light acts as photostressor for lettuce plants, thus its effects on nitrate reduction are apparent only when it is used in combination with broader spectrum lighting and it is efficient only for red leaf lettuce varieties, which accumulate higher contents of anthocyanins and other phenolic compounds and are more resistant to photostress conditions.

P200

Expression and Activity of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RUBISCO) in Perennial Ryegrass (*Lolium perenne* L.) Cultivars Exposed to Manganese Excess

Alejandra Ribera^{1,2}, Marjorie Reyes^{2,3}, Miren Alberdi^{2,3}, Daniela Alvarez⁴, María de la Luz Mora^{2,3}
¹Facultad de Ciencias Agropecuarias y Forestales; Universidad de La Frontera, Avenida Francisco Salazar 01145, Temuco, Chile, ²Center of Plant-Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Avenida Francisco Salazar 01145, Temuco, Chile, ³Departamento de Ciencias Químicas y Recursos Naturales; Facultad de Ingeniería, Ciencias y Administración; Universidad de La Frontera, Avenida Francisco Salazar 01145, Temuco, Chile, ⁴Laboratorio de Análisis de Suelo y Planta, Instituto de Agroindustria, Universidad de La Frontera, Avenida Francisco Salazar 01145, Temuco, Chile

Manganese play a key role in water-splitting system associated with photosystem II (PSII) and ATP synthesis. We found that photosynthesis decreases under Mn excess, being higher in Mn-sensitive cultivar of perennial ryegrass (Nui) compared to the Mn-tolerant (Kingston). These reductions can result in deleterious effects of Mn excess on RUBISCO kinetics. We evaluated the influence of Mn excess on gene and protein expression, and RUBISCO activity in ryegrass cultivars differing in their Mn-tolerance. Nui and Kingston cultivars were treated with normal doses (2.4 μM) or toxic (350 and 750 μM) of Mn for 14d under nutrient solution. Results indicated an increase of RUBISCO gene and protein expression in response to Mn toxicity in both cultivars, mainly in the Mn-tolerant.. It is showed that RUBISCO activity increased in Kingston in response to Mn excess, while in Nui it decreased. RUBISCO activation state was reduced in both cultivars in response to high Mn, but more markedly in Kingston. Results suggest that the increase of RUBISCO gene expression under Mn toxicity is due to higher levels of RUBISCO protein mainly in the Mn-tolerant cultivar. It can be one of the mechanisms that confer Mn-tolerance in Kingston, because can decrease the negative impacts of Mn excess on photosynthesis in this cultivar. Furthermore, the decrease of CO₂ assimilation detected in Mn sensitive cultivar under Mn excess could be attributed to a decrease in the RUBISCO activity. Further investigations are needed to determine the mechanism at which occurs the deactivation of RUBISCO. Acknowledgments: FONDECYT project 11100494.

P201

Genetic Response Variability of Tomatoes during Soil Drying and Repeated Cycles of Water Deficit and Recovery

Julie Ripoll^{1,2}, Laurent Urban², Béatrice Brunel¹, Alain Goujon³, Jean-Claude L'Hôtel¹, Mathilde Causse³, Nadia Bertin¹

¹INRA PACA, Plantes et Systèmes de Culture Horticoles (UR1115), 228 route de l'Aérodrome CS 40509, Domaine St Paul, Site Agroparc, Avignon, France, ²Université d'Avignon et des Pays du Vaucluse, Laboratoire de Physiologie des Fruits et Légumes (EA4279), 301 rue Baruch de Spinoza BP 21239, Site Agroparc, Avignon, France, ³INRA PACA, Génétique et Amélioration des Fruits et Légumes (GAFL), Domaine Saint Maurice, 67 Allée des chênes, CS60094, Montfavet, France

Under natural conditions, plants experience repeated cycles of water deficit and recovery, which could negatively impact on plant and fruit growth. Understanding the genetic variability in plant response and adaptation to such conditions is crucial for breeders. In this study, the 8 parents of the Multi-Parent Advanced Generation Inter-Cross population of tomato were subject to three periods of 15-days water deficit interrupted by two periods of 15-days recovery. In parallel tomato plant kinetic response during soil drying was characterized in order to identify response thresholds according to soil humidity. Independently of the genotype, a threshold of 20% of relative soil humidity was found in relation to the predawn water potential, and the plant performance index measured by fluorescence of chlorophyll a. Below this threshold, only individual variability was found and plant survival was endangered. The critical threshold of 20% was achieved during the alternating cycles of deficit irrigation and recovery. The response to water deficit during the alternating cycles was genotype dependent for intensity of response like to the decrease in estimated leaf surface area. However, recovery period smoothed the effects of deficit irrigation. So, plant predawn water potential and performance index were not significantly affected by the alternating treatment. Also, plant growth and fruit caliber were slightly, but not significantly reduced. On their whole, results indicated that the alternation of deficit irrigation and recovery periods may promote adaptation to water deficit without endangered for plant survival.

P202

Gradual Toxic Effect of Thallium Exposure on *Sinapis alba* L. Photosynthetic Activity in Relation to Distribution of the Two Chemical Forms: Tl(I) and Tl(III)

Radosław Mazur¹, Monika Sadowska², Lucja Rudowska³, Agnieszka Mostowska³, Beata Krasnodebska-Ostrega², Maciej Garstka¹

¹Department of Metabolic Regulation, Faculty of Biology, University of Warsaw, Warsaw, Poland,

²Laboratory of Applied Analytical Chemistry, Faculty of Chemistry, University of Warsaw, Warsaw, Poland, ³Department of Plant Anatomy and Cytology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Thallium is a toxic element introduced to the environment as a result of human activities. It occurs in two redox states: Tl(I) and Tl(III), the latter being unstable and existing mainly in complex compounds. Elevated concentrations of thallium were found in plants growing in areas polluted with thallium and some of these plants, especially species belonging to the Brassicaceae family, are able to cumulate extremely high amounts of thallium in their tissues. Previously we reported that *Sinapis alba* cultivated in the presence of high concentrations of monovalent thallium beside accumulation of Tl(I) additionally contained Tl(III). In the present study we analyzed the effect of different (100-1000 ppb) concentrations of monovalent thallium exposure on photosynthetic activity in relation to the distribution of two chemical forms: Tl(I) and Tl(III). Results show that increasing concentration of Tl has deleterious effect on plant growth, chloroplast structure and photosynthetic activity mainly due to the increase of non-photochemical quenching processes. However, at moderate (500 ppb) Tl concentration, the highest ratio of Tl(III) to Tl(I) was found in the least damaged leaves. It confirms the existence of detoxification mechanism based on oxidation of T(I) to Tl(III) and subsequent immobilization in a stable complex with yet unidentified compound.

Acknowledgements:

This work was supported by National Science Centre (NCN), Poland, Grant no. DEC-2011/01/B/NZ8/00052.

TEM images were performed in the Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology on JEM 1400 (JEOL Co. Japan) electron microscope.

P203

Interactive Effects of Salt and Boron Stresses on Lipid Peroxidation and Some Growth Parameters in Endemic Halophyte *Salvia halophila* Hedge from Turkey

Fazilet Ozlem Cekic, Alptekin Karagoz
Aksaray University, Aksaray, Turkey

Salinity is one of the most important problems in the agriculture areas. Boron (B) toxicity has been recognized as a serious problem in arid and semi arid regions of the world. Salinity and boron (B) stresses may have interactive effects on plant responses. To sustain future productivity, the characterization of salt tolerant halophyte plants is very important. The aim of this study was to determine the responses of Turkish endemic halophyte *Salvia halophila* Hedge to salt and boron stresses. Seeds of *S. halophila* Hedge were collected from the salty steppe habitat near Salt Lake in Turkey and were grown in a growth chamber. Effects of salinity (NaCl) and boric acid (H_3BO_3) on lipid peroxidation (MDA), leaf relative water content (RWC) and some growth parameters were investigated. Boron stress increased MDA content in *S. halophila*, however it was decreased in combined stress. Salinity and boron affected negatively shoot growth and RWC, however root growth increased during boron stress. From the results obtained in the present study, it can be suggested that *S. halophila* Hedge can be tolerant also to the combined stress by having less lipid peroxidation than single stress conditions.

P204

Effect of Exogenous Application of GABA on Prunus Rootstocks under Root Hypoxia Stress by Waterlogging

Ariel Salvatierra¹, Paula Pimentel¹, Rubén Almada¹, Manuel Pinto^{1,2}, Boris Sagredo^{1,3}, Patricio Hinrichsen^{1,2}

¹Centro de Estudios Avanzados en Fruticultura (CEAF), Rengo, Chile, ²INIA CRI La Platina, Santiago, Chile, ³INIA CRI Rayentué, Rengo, Chile

GABA shunt pathway occurs in a wide range of organisms, such as bacteria, yeasts, plants and animals. In plants, GABA level is normally low, but it quickly and extensively increases in response to stress (e.g., hypoxia). On two hypoxia contrasting rootstock genotypes, Mazzard F12/1 (sensitive) and Mariana M2624 (tolerant), a previous transcriptional study by RNAseq detected an up-regulation of gene expression of a glutamate decarboxylase (GAD), which encodes the enzyme for the first step of the GABA shunt pathway. An *in silico* search for orthologous genes of GABA shunt revealed four GAD isoforms in *Prunus persica* genome, which showed an organ-associated expression. In addition, these isoforms described different transcriptional profiles in roots of both rootstock genotypes under root hypoxia by waterlogging. In order to assess the effect of GABA levels on plant performance of these rootstocks, we watered plants of both genotypes with 500 mL of a 1mM GABA solution as pre-treatment before of the hypoxia stress. Exogenous GABA affected transient and positively gas interchange parameters of plants under stress and it reduced their H₂O₂ levels in roots. Transcript abundance of GAD was modified in an isoform dependent manner in GABA pre-treated plants. These results show physiological, biochemical and transcriptional effects of exogenous GABA application that would help to *Prunus* rootstocks to cope brief waterlogging events.

Acknowledgements. AS acknowledges to FONDECYT 31301384. PP acknowledges to FONDECYT 11110080. Plants were provided by Agromillora Sur S.A.

P205

OsbHLH148 Gene Regulates OsDREB1s in Response to Drought

Tae Young Um¹, Han Yong Lee¹, Hyung Il Kim¹, Ju Kon Kim², Yang Do Choi¹

¹*Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea,*

²*Green Bio Science & Technology, Seoul National University, Seoul, Republic of Korea*

OsbHLH148 gene encodes a transcription factor belonging to the basic helix-loop-helix (bHLH) family and is involved in jasmonate signaling. Expression of the OsbHLH148 gene is induced by various abiotic stress in rice, especially, in early phase. In transgenic rice over-expressing OsbHLH148 by the constitutive promoter OsCc1 showed significantly enhanced drought tolerance and OsDREB genes that are related to drought stress tolerance were up-regulated. Grain yield of over-expression lines of OsbHLH148 was increased with grain yield related parameters including panicle length, number of panicles per hill, number of spikelets per panicle, total number of spikelets, number of filled grain, filling rate, total grain weight, and 1000 grain weight compared with non-transgenic plants(NT) in normal and drought stress condition. To understand downstream genes regulated by OsbHLH148, cis-elements are identified by yeast one hybrid assay. bHLH transcription factors bind to E-box (CANNTG) element and mediate stress tolerance in rice. OsbHLH148 binds to GTGG and E-box sequence element as well. Further downstream target genes of OsDREB1s controlled by OsbHLH148 in rice is under study.

P206

Expression Pattern of OsCOIs in Developing Rice

Han Yong Lee¹, Taeyoung Um¹, Hyung il Kim¹, Jukon Kim², Yang Do Choi¹

¹Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea,

²Green Bio Science & Technology, Seoul National University, Seoul, Republic of Korea

There are three closely related COIs in the genome of *Oryza sativa*. They showed approximately 63~82% amino acid identities each other. To understand functions of these OsCOIs in defense and development, expression level was analyzed in various tissues. In 2-weeks-old rice seedlings, all of OsCOIs were expressed to similar level in various tissues such as root, shoot base, leaf sheath and leaf blade. In flowering stage (14-weeks-old) and milk stage plants (16-weeks-old), they showed different expression patterns. OsCOI1a and OsCOI1b were expressed in almost all tissues except stamens. OsCOI2 was, expressed in stamen as well as in other tissues of 14-weeks-old plants even though its expression level was lower than that of OsCOI1b in other tissues. In 16-weeks-old plants, OsCOI1b was expressed higher than OsCOI1a and OsCOI2 in almost all tissues. Three OsCOIs responded similarly to MeJA, SA, GA₃ and 6-BAP in 2-weeks-old wild type rice seedlings. However, OsCOI1b was more sensitive to ABA than OsCOI1a and OsCOI2. All three OsCOIs showed similar responses to drought, high salinity and cold. These results indicate that each OsCOI plays specific roles in development and various stresses responses.

P207

Cadmium and Oxidative Stress in Pepper

Milca Rolao, Fernando Piotto, Ricardo Azevedo
Universidade de São Paulo (USP/ESALQ), Piracicaba, SP, Brazil

Sweet pepper banana (*Capsicum annuum* L.), a important fruit crop grown in tropical and sub-tropical regions, is used worldwide in human nutrition and known for its biologically active compounds, such as antioxidants, vitamins and other phytochemicals (Rao et al., 2011). Heavy metals represent greatest industrial contaminants in the world and due to their wide release in the environment, affect the entire food chain. The presence of metals affects plant growth and causes lipid peroxidation, also influence nitrogen cycle and, therefore, all biomolecules depending on it (Gratão et al., 2012). This pepper presents a short life cycle (within 120 days) and also due to its short size is considered a good model for oxidative stress studies.

Our group is evaluating agronomic performance and characteristics of sweet pepper grown on the presence of cadmium (Cd). Initially, dry weight and cadmium quantification were assessed prior to further studies on oxidative responses, which involve lipid peroxidation, antioxidant compounds and enzymes activities, part from Cd translocation within the plant.

Following a dose-response experiment, plants were cultivated in hydroponic system and subjected to 0 and 25 μM CdCl_2 . Samples were collected at the 3th and 10th day of exposure. Data showed significant difference in dry weight both for leaves and roots at the 10th day of Cd exposure, consistent with results observed in H_2O_2 content and SOD activity. Leaves also exhibited signs of chlorosis. In conclusion, 25 μM CdCl_2 was mildly and highly toxic at the short (3th day) and longer (10th day) period analyzed, respectively.

P208

Modified Expression of the Alternative Respiratory Pathway in Arabidopsis Can Influence Abiotic Stress Tolerance

Kathleen Soole, Chevaun Smith, Colin Jenkins, David Day
Flinders University, Adelaide, Australia

Respiration is an important parameter in the growth and development of plants. Plants contain a non-phosphorylating alternative electron transport pathway in their mitochondria, which includes the alternative oxidase (AOX) and several alternative NAD(P)H dehydrogenases. There are seven genes which encode these dehydrogenases in Arabidopsis. Four of these dehydrogenases (ndb1,2,3 and 4) have been localised to the outside of the inner membrane, and three (nda1,2 and ndc1) have been localised to the inside of the inner membrane, bypassing complex I. Transgenic plants with reduced or over expression of particular NAD(P)H dehydrogenases provide the basis for linking these genes with their specific enzyme activity as well as for an analysis of their physiological role. We have been using over-expression and RNAi technology to examine the role of the mitochondrial cytosolic-facing NAD(P)H dehydrogenases in plant growth and abiotic stress tolerance. We have generated ndb4 and ndb2 suppression lines and over-expression lines of *A. thaliana* as well as double knockdown of the expression of these genes. Data will be presented to show that altered expression of these genes affects the corresponding mitochondrial activity and significantly affects plant growth and stress tolerance. In general, plants with altered expression of alternative NAD(P)H dehydrogenases and AOX display tolerance to stress such as salinity stress, probably by helping to reduce reactive oxygen species levels.

P209

KING1 Can Regulate the Function of SNF1-Related Protein Kinase 2 in Arabidopsis

Matleena Punkkinen¹, Jian-Kang Zhu², Hiroaki Fujii¹

¹University of Turku, Turku, Finland, ²Purdue University, West Lafayette, IN, USA

Since SNF1-related protein kinase (SnRK) 2.2, 2.3 and 2.6 are important components in the abscisic acid (ABA)-responsive pathways, their activity must be regulated sophisticatedly. Though protein phosphatase 2Cs and SnRK2-interacting Calcium Sensor have been reported to be important for the activity, the whole regulatory mechanisms of SnRK2s remain obscure. We try to analyze putative gamma subunits of the SnRK family as potential regulators of SnRK2s. Arabidopsis has several proteins similar to the gamma subunit of SNF1 complex in yeast, which is essential for the SNF1 function. One of them, KING1 binds to SnRK2.6. In vitro kinase assay shows that a recombinant KING1 inhibits the activity of SnRK2.6. Arabidopsis double mutant plants lacking KING1 and its closely related gene show hypersensitivity to ABA in the germination. These results suggest that KING1 negatively regulates the SnRK2 function.

P210

Proteome Analysis of Korean Maize Inbred Line under Drought Stress

Jin Seok Lee, Seong Hyu Sin, Sang Gon Kim, Jung Tae Kim, Hwan Hee Bae, Beom Young Son, Seong Bum Baek, Young Up Kwon

1National Institute of Crop Science, Rural Development Administration, Suwon, Republic of Korea

Drought stress greatly affects plant growth and crop yield. To understand the proteome dynamics in maize during 10 day drought stress at five or six leaf stage, protein expression profiling based on 2-DE combined with MALDI-TOF/TOF was compared between the well watered and the drought treated using an inbred line. Proteins were extracted from leaves with/without drought stress using phenol method, and separated by 2-DE. After staining of 2-DE gels with colloidal Coomassie brilliant blue (CBB), 29 spots were differentially expressed and were identified using MALDI-TOF/TOF mass spectrometry. They were major involved in 31% metabolism, 24% stress related proteins, 21% photosynthesis, 7% protein modification, and 14% unknown, respectively. Out of 29, 24 and 5 protein spots were found to be up and down-regulated, respectively. Interestingly, two pathogenesis-related proteins, an abscisic stress-ripening protein, and a heat shock 70 kDa protein were newly increased by drought stress. Our study provides a protein profiling of Korean maize inbred line during drought stress, which will be valuable for further study of the molecular mechanisms caused by drought in maize.

P211

Physiological Response to Drought Stress at Flowering Time in *Zea mays* cv. Ilmichal, a Korean Waxy Corn

Sang Gon Kim, Seong-Hyu Sin, Jin-Seok Lee, Jung-Tae Kim, Hwan Hee Bae, Beom-Young Son, Seong-Bum Baek, Young-Up Kwon

National Institute of Crop Science, Rural Development Administration, Suwon, Republic of Korea

Drought is a major environmental factor that severely limits plant productivity in Korea and worldwide. As global climate change progresses, agricultural production worldwide faces serious threats from frequent extreme weather events. Of maize (*Zea mays* L.), waxy corn is widely cultivated in Korea. Its production and yield stability are greatly affected by drought stress. Improving drought tolerance in maize has become one of the top priorities for maize breeding programs. In this report, we evaluated physiological response of "Ilmichal", a selection of maize waxy corn variety to 10 day drought stress at flowering time. After drought stress at flowering time, anthesis-silking interval was 7.3 times delayed compared to the well-watered, ear and tassel length was 12~13% and 10~12% decreased. And grain weight and number was 68% and 51% decreased, respectively. However, plant height, stem length, stem width, and leaf numbers were little different. Relative water content (RWC) was 41% decreased and photosynthetic rate was 28% decreased at flowering stage compared to control lines after drought stress. Chlorophyll contents (SPAD) and stomatal conductance of Ilmichal leaves were 31% and 55% decreased compared to the well-watered. These physiological responses to drought stress will be helpful to develop selective phenotypic markers for drought tolerant waxy corn varieties.

P212

The Study of Dehydrins Involvement in the Protective Action of 24-Epibrassinolide on Wheat Plants under Cadmium Stress

Chulpan Allagulova, Dilara Maslennikova, Azamat Avalbaev, Kristina Ivanova, Elvira Vyazova, Farida Shakirova

Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences, Ufa, Russia

Among heavy metals cadmium is the most toxic since it is non-essential element and negatively affects plant growth and development. Cadmium stress leads to water uptake imbalance causing plant dehydration and ABA accumulation. Dehydrins (group II of LEA-proteins) are the most commonly observed group of proteins in response to ABA and water-related stresses. Previously, we obtained data about activation of TADHN dehydrin gene transcription in wheat plants under cadmium stress and in response to 24-epibrassinolide (EB) which belongs to brassinosteroids phytohormones that are characterized by a wide range of physiological effects on plants including antistress activity. These results may indicate the dehydrins involvement in the EB protective action on wheat to cadmium. The aim of this study was the investigation of possible dehydrins involvement in EB protective action on wheat seedlings exposed to cadmium acetate. EB pretreatment decreased the cadmium damaging effect on wheat plants as indicated by the reduction in the extent of stress-induced electrolyte leakage and prevention of growth inhibition. We have found that dehydrin content with Mw 28 kDa markedly increased in response to 0.4 μM EB under normal conditions as well as to 1 mM cadmium acetate treatment. It is noteworthy that content of 28 kDa dehydrin was additionally increased in the EB-pretreated seedlings exposed to cadmium. Thus, it is reasonable to assume that 28 kD dehydrin might involve in the EB-induced protective reactions, contributing to the wheat tolerance to toxic effect of cadmium ions.

P213

Metabolic Response of Plant Cell to Osmotic Stress Involves the Mevalonate and Methylethylol Phosphate Pathways

Adam Jozwiak¹, Magdalena Kania², Witold Danikiewicz², Jaroslaw Poznanski¹, Ewa Swiezewska¹
¹*Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland,* ²*Institute of Organic Chemistry Polish Academy of Sciences, Warsaw, Poland*

Upon stress, complex metabolic changes occur involving the modulation of the primary metabolism and mobilization of secondary metabolites. Dolichols are the representatives of isoprenoids, the most numerous and diverse group of natural compounds. Earlier studies have demonstrated that plant polyisoprenoids are derived from two independent pathways responsible for the formation of their precursor, isopentenyl diphosphate (IPP): the cytosolic, mevalonate (MVA) pathway and the plastidial, methylethylol phosphate (MEP) pathway. The participation of polyisoprenoid alcohols in plant adaptive response to stress has been postulated earlier, however, it was not studied in detail.

Here we show that the osmotic stress affects the early steps of the dolichol biosynthetic pathway, i.e. the relative involvement of the MVA and MEP pathways in the formation of the dolichol molecule in *Arabidopsis thaliana* hairy roots. HPLC/ESI-MS analysis of metabolically labelled dolichols indicated the shift towards smaller m/z values of the dolichol isotopomers in the sorbitol treated hairy roots in comparison to control. These observations suggested increased contribution of the MEP pathway to biosynthesis of the dolichol molecule. Similar perturbations were also noted for phytosterols.

Our results demonstrate that osmotic stress regulates dolichol biosynthesis at the very initial steps of this process by affecting the exchange of intermediates between the MEP and MVA pathways and/or by modulating the activity of the pathway-specific enzymes. Such a profound effect of osmotic stress on regulation of the MEP–MVA cross-talk has never been reported before.

P214

The Influence of Oxidative Conditions on the Content and Profile of Polyisoprenoid Alcohols in *Arabidopsis thaliana* Roots

Joanna Komasyło¹, Ewa Swiezewska¹, Magdalena Kania², Witold Danikiewicz²

¹*Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland*, ²*Institute of Organic Chemistry Polish Academy of Sciences, Warsaw, Poland*

Cells have evolved a complex array of defense mechanism to control the level of reactive oxygen species (ROS). Protection of cellular membranes against peroxidation has recently been postulated as a new function of dolichol. Those isoprenoid alcohols are accumulated in plant roots, yeast and animal cells as mixtures of homologues. The structure of these lipids, multiple double bonds, makes them a perfect target for attack of ROS, which are produced continuously during the life span, especially in mitochondria, chloroplasts and peroxisomes.

Our studies were intended to test the susceptibility of polyisoprenoid alcohols to oxidation in order to analyze their putative function as a shield against ROS.

The biological model – in vitro roots culture of *Arabidopsis thaliana*, have been subjected to chemically generated singlet oxygen treatment. Generation of singlet oxygen was realized by UV irradiation in the presence of porphyrin in the atmosphere of oxygen.

Singlet oxygen generated during photocatalysis caused quantitative changes in the content of polyisoprenoid alcohols, sterols, esters of fatty acids and lipid peroxides (UV/HPLC, GC/FID, spectrophotometric method). The profile of oxidized derivatives of polyisoprenoid alcohols were analyzed with usage of ESI-MS method. Post-vivo experiments performed in this study confirmed the susceptibility of polyisoprenoids to oxidation with singlet oxygen and consequently their putative role in protecting cell components against ROS.

P215

Enhancing Spring Drought Tolerance of Oilseed Rape (*Brassica napus* L.)

Michele Faralli, Peter Kettlewell, Ivan Grove, Martin Hare
Harper Adams University, Newport, Shropshire, UK

Oilseed rape is regarded as an important break crop in the rotation of cereals, and has a primary role in the commercial production of biofuel, in the production of oil for food and in animal feed. A major limitation in the rapeseed yield appears to be drought stress during reproductive stages, leading to male sterility and pod abortion due to a reduction in water availability. Moreover in response to a decrease in soil water potential, plants accumulate abscisic acid, one of the factors affecting male sterility in *Brassica napus*.

Film-forming antitranspirants are natural compounds extracted from coniferous plants that create a water-proof film that mechanically close the stomata. Their use has been widely studied with satisfactory results, demonstrating their ability to decrease the accumulation of abscisic acid in plant tissues, decrease leaf transpiration and improving plant water status.

The preliminary experiments have focused on understanding the physiological response of the plants to antitranspirant treatment by measuring photosynthesis, transpiration, stomatal conductance and plant water status in relation to different spraying methods and nozzles. The data give us the opportunity to understand the plant's physiology in full watering regimes and to relate the data to leaf coverage and stomata physiology. Preliminary results show an increase in intrinsic water use efficiency in plants treated at low antitranspirant dose rate. The aim of further works is to test different film-forming antitranspirants at different growth stages and soil moisture deficits to understand the mechanism of action and the oilseed rape response to a stomata-closure treatment.

P216

Identification of Linkages between the End Products of Arabidopsis Cis-Prenyltransferase 7 Activity and Plant Resistance to Abiotic Stress

Liliana Surmacz, Hanna Siekierska, Daniel Buszewicz, Adam Jozwiak, Ewa Swiezewska
Institute of Biochemistry and Biophysics Polish Academy of Science, Warsaw, Poland

Polyisoprenoids are five-carbon unit linear polymers produced by all living organisms and classified into two groups: polyprenols and dolichols. They are accumulated in the cells as the mixtures (families) of homologues. The content of polyisoprenoids increases during life span and upon environmental stress. They modulate the properties of the biological membranes, participate in glycosyl-phosphoinositol membrane (GPI) anchor, protein C- and O-mannosylation, and protein N- and O-glycosylation. The key enzymes of polyisoprenoid synthesis are cis-prenyltransferases (CTPs), responsible for construction of the long hydrocarbon skeleton. Arabidopsis thaliana possesses a family of nine cis-prenyltransferase encoding genes – AtCPT1 to AtCPT9 which show the tissue-specific expression.

The main goal of our project is to characterize AtCPT7 and investigate the involvement of its products in stress tolerance.

In vivo AtCPT7 catalyzes the synthesis of a family of short-chain polyisoprenoid alcohols composed of 9 to 11 isoprenoid units in heterologous system. Furthermore, these specific products are absent in AtCPT7 T-DNA insertion mutant and over-accumulated in AtCPT7- overexpressing plants.

The effect of various stressors on polyisoprenoid accumulation and AtCPTs expression in Arabidopsis was studied. Under osmotic and heavy metal stresses the expression of AtCPT7 and dolichol accumulation were increased in Arabidopsis roots. Moreover, the expression of AtCPT7 was highly increased under heat stress conditions.

Currently, the studies on the influence of diverse stresses on AtCPTs expression and polyisoprenoids content in AtCPT7 insertion mutant and AtCPT7 overexpressing plants are in progress.

This research was supported by grant funded by the National Science Centre [DEC-2011/03/B/NZ1/00568].

P217

Functional Significance of Oxylin Induced Heat Shock Protein Accumulation

Miriam Münch, Elena Ferber, Stephanie Müller, Chih-hsuan Hsin, Martin J. Müller
University of Wuerzburg, Wuerzburg, Germany

Literature evidence suggests that oxylin confer basal thermotolerance in *Arabidopsis thaliana*. Elevated temperatures (37°C) have been shown to induce an accumulation of the jasmonates 12-oxo-phytodienoic acid (OPDA), jasmonic acid (JA) and jasmonic acid isoleucine (Ja-Ile). In addition, the Ja-Ile insensitive mutant *coi1-1* has been reported to be compromised in basal thermotolerance. Previously, we have shown that exogenously administered OPDA but not JA triggers heat shock protein (HSP) accumulation in the absence of heat suggesting that OPDA rather than JA might be a signal involved in thermoregulation.

About 40% of all OPDA regulated genes, in particular detoxification and general stress genes, are dependent on TGA transcription factors. In contrast, OPDA- and heat-responsive Hsp genes are TGA independent. We show that OPDA induced up-regulation of HSPs is mediated through the master heat shock factor genes HsfA1 and in part through HsfA2. However, short or long term elevated temperatures (37°C) did not trigger jasmonate accumulation. Moreover, a mutant, *dde2*, deficient in jasmonate biosynthesis displayed normal heat-induced HSP accumulation and a wild-type like phenotype in different thermotolerance assays. Hence, jasmonates appear not to be involved in thermoregulation.

Notably, a variety of abiotic and biotic stresses are associated with dramatically increased OPDA levels and HSP accumulation potentially increasing general stress resistance. Our results indicate that OPDA is as efficient as classical chemical HSP90 inhibitors in inducing HSPs. In the future we aim to further clarify the OPDA signal transduction mechanisms and the functional significance of the HSP-stress-response.

P218

Evaluation of Salt Tolerance in Miscanthus Using a Hydroponic Growth System

Chang-Lin Chen^{1,2}, Shiva Dehghan², Hanneke van der Schoot², Richard Visser², Gerard van der Linden²

¹Graduate School Experimental Plant Science, PO Box 386,6700AJ Wageningen, The Netherlands,

²Wageningen UR Plant Breeding, PO Box 386,6700AJ Wageningen, The Netherlands

Miscanthus is a woody rhizomatous C4 grass crop that has a high potential as a biofuel resource. It has potential to grow in marginal areas such as saline soils, avoiding competition for arable lands with food crops. This study explored genetic diversity for salt tolerance in *M. sinensis* to discover genotypes and traits that can be used to improve miscanthus salt tolerance. To assess the salt tolerance level of *M. sinensis*, two genotypes were grown and treated at two different salt levels (125mM & 250mM NaCl) in a hydroponic growth system. The results indicated that *M. sinensis* is more salt tolerant than rice and durum wheat, close to the tolerance level of barley. Seven genotypes were then screened in the same system at 150mM NaCl and evaluated for salt tolerance. Analyses of root and shoot growth traits and ion content revealed the existence of different tolerance mechanisms in the genotypes. Genotype Sh-104 had clearly lower Na⁺ and Cl⁻ contents in the shoots than other genotypes under salt stress, indicating that a Na⁺ exclusion mechanism was utilized to prevent Na⁺ accumulation in the leaves. Senescence symptoms were not increased and chlorophyll content was not changed under salinity in genotype Sh-110 but ion contents were average, indicating that this genotype may have osmotic tolerance. Tolerant genotype Sh-113 had relatively high Na⁺ content and may utilize a tissue tolerance mechanism. The results of this study revealed various salt tolerance mechanisms in *M. sinensis* germplasm, demonstrating genetic potential for improving salt tolerance.

P219

Acid Phosphatase Role in Oat (*Avena sativa* L.) Acclimation to Phosphate Deficiency

Ewa Zebrowska, Iwona Ciereszko

Plant Physiology Department, Institute of Biology, University of Białystok, Białystok, Poland

Plants have evolved various mechanisms that improve inorganic phosphate (Pi) acquisition, one of them is the induction and secretion of acid phosphatases. Oat cultivars (Arab, Krezus, Rajtar and Szakal) were grown for 1-3 weeks on nutrient media with contrasting phosphorus source: inorganic - KH_2PO_4 (control), organic – phytic acid (PA) and with no phosphate (-P). Growth parameters, Pi content and acid phosphatase activity (secreted and intracellular) were measured. Acid phosphatase activity localization was determined in root cross sections. Protein extracts from tissues were run on native PAGE to determine acid phosphatases isoforms. Pi deficiency in nutrient medium decreased shoot growth but increased root elongation, whereas plants grown on phytic acid were similar to control plants. Phosphate starvation decreased Pi content in shoots and roots of all the studied oat cultivars. -P plants showed increased activity of extracellular and intracellular acid phosphatases. The distribution of acid phosphatases activity differed between root tissues - the highest activity was found in the vascular tissues and epidermis of roots. Pi content and acid phosphatase activity in PA plants were similar to control plants. Three major acid phosphatase isoforms were detected in all oat plants; one was strongly induced in roots under Pi deficit. The results indicate that studied oat plants in general show similar acclimation mechanisms to phosphate deficiency; however they use different pools of acid phosphatases to acquire Pi from external or internal sources.

This work was supported by Grant DEC-2012/07/N/NZ9/00972 from the National Science Center (NCN), Poland.

P220

Aldehyde Dehydrogenases in the Mode of Action of Amino Acid Biosynthesis Inhibiting Herbicides

Miriam Gil-Monreal¹, Tagnon D. Missihoun², Dorothea Bartels², Ana Zabalza¹, Mercedes Royuela¹
¹*Public University of Navarre, Pamplona-Iruña, Navarra, Spain,* ²*Institute of Molecular Physiology and Biotechnology of Plants (IMBIO) - University of Bonn, Bonn, Germany*

Herbicide application is the main chemical treatment for weed control worldwide. Glyphosate (GLP) and Imazamox (IMX) inhibit amino acid biosynthesis and are among the most widely used herbicides. Application of GLP and IMX was found to induce aerobic fermentation and accumulation of acetate, suggesting a carbon flow from pyruvate to acetaldehyde (catalyzed by pyruvate decarboxylase) and from acetaldehyde to acetate (catalyzed by aldehyde dehydrogenases–ALDHs). This pathway is known as the pyruvate dehydrogenase bypass.

The objective of this study was to investigate the implication of selected ALDHs in the mode of action of GLP and IMX.

The expression pattern of ALDH2B4, ALDH2B7, ALDH2C4, ALDH3F1, ALDH3H1, ALDH3I1 and ALDH7B4 genes was measured by RT-PCR in *Arabidopsis thaliana* Col0 plants treated with 20mg/L GLP or 1.5mg/L IMX. The malondialdehyde (MDA) content was measured as a marker of lipid peroxidation, thus of sensitivity to herbicide-derived oxidative stress.

ALDH7B4 was the most affected ALDH by GLP and IMX treatment, as its relative expression was induced in both leaves and roots. This gene has been reported to be induced in plants exposed to different abiotic stresses and overexpression of ALDH7B4 in *A.thaliana* has conferred oxidative and abiotic stress tolerance. No MDA increase was detected in herbicide treated plants (except for the leaves of GLP treated plants). Our results indicate that the detected induction of ALDH7B4 is not related to lipid peroxidation, but likely to its involvement in the pyruvate dehydrogenase bypass.

Acknowledgments: Funded by Spanish Ministry Education and Science_AGL-2010-18621. M.Gil-Monreal was funded by Universidad Pública Navarra.

P221

Effects of Nanoparticles on Plant Metabolism

Radka Podlipná¹, Tereza Cyrusová^{1,2}, Premysl Landa¹, Tomáš Vanek¹

¹Institute of Experimental Botany, Czech Academy of Science, Prague, Czech Republic, ²Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

The recent rapid expansion of nanotechnologies has increased concern over the impact of engineered nanoparticles on the environment and on biota. The (eco)toxicity of nanoparticles has been investigated foremost under laboratory conditions for animals including humans and hence little is known about adverse environmental effects on plants. The effect of exposure to 100 mg/L zinc oxide (nZnO), fullerene soot (FS) or titanium dioxide (nTiO₂) nanoparticles on proteom in *Arabidopsis thaliana* leaves was studied using 2D- DIGE (2-D Fluorescence Difference Gel Electrophoresis). After 7 days of exposure to nTiO₂, nZnO, and FS we found at least 2.0-fold different expression between control and treated plants for 18, 18, and 16 resp. proteins. In the case of nZnO the bulc form of ZnO as the control was chosen.

In the following experiments we focused on the comparison of phytotoxicity of ZnO in bulc- and nano-form. The preliminary measurements on AAS (atomic absorption spectroscopy) show the higher (□ 2.0-fold) accumulation of zinc nanoparticles in comparison to bulc-form by *Arabidopsis* plant.

Acknowledgment: This study was supported by projects MYES of CR n.LD14100.

P222

Reactive Oxygen Species Metabolism in Sulphur-Deficient *A. thaliana* Leaves - The Role of Mitochondria

Monika Ostaszewska, Izabela M. Juszczuk

Institute of Experimental Plant Biology and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Reactive oxygen species (ROS) are produced mostly in the mitochondria as byproducts of normal cell respiration. It has been proved, that S-deficiency leads to severe changes in mitochondrial ultrastructure (Ostaszewska et al. 2014) and activities of the complexes in mitochondrial respiratory chain (mtETC; Juszczuk&Ostaszewska 2011). Defects in oxidative phosphorylation can increase ROS production, whereas ROS-mediated damage to biomolecules can have direct effects on the components of mitochondrion and other organelles. The aim of this study was to determine the role of mitochondria in ROS metabolism in S-deficient *Arabidopsis thaliana* leaves in terms of ROS production, scavenging and damage. Overreduction of mtETC in isolated S-deficient mitochondria resulted in higher generation of superoxide within the intermembrane space but similar to control in the matrix. Higher tissue levels of superoxide and hydrogen peroxide were detected. Total concentrations of glutathione and ascorbate decreased and their reduction increased. Superoxide scavenging enzymes activities, mitochondrial Mn-SOD and cytosolic Cu/Zn-SOD were significantly higher in S-deficient leaves. Cell lipid peroxidation remained unchanged while protein carbonylation level was higher due to higher oxidation of chloroplast proteins. Protease activity also was higher mainly to its increase in mitochondria. ROS signal from mitochondria in S-deficient *A. thaliana* leaf cells could allow to establish a new energetic and redox status that supports the growth of plants under shortage of one of the basic macronutrients.

Funding: Grant N/N303/800240 (I.M.J) from NCN, Poland and intramural grants DSM501/86-102342, DSM501/86-104952 (M.O.) from MSHE through FB, UW, Poland.

Juszczuk&Ostaszewska (2011) EEB,74:245-254

Ostaszewska et al. (2014) JPP,171:549-558

P223

SSR Markers Associated with Membrane Stability in *Triticum aestivum* L. Genotypes

Jalal Aliyev^{1,2}, Irada Huseynova¹

¹Department of Fundamental Problems of Biological Productivity, Institute of Botany, Azerbaijan National Academy of Sciences, 40 Badamdar Highway, Baku AZ1073, Azerbaijan, ²Department of Plant Physiology and Biotechnology, Research Institute of Crop Husbandry, Ministry of Agriculture of Azerbaijan Republic, Baku AZ1098, Azerbaijan

Drought is a serious problem significantly limiting yield of many crop plants including wheat in the world. Plant cell membrane stability (CMS) has been recognized as an important screening trait for drought tolerance in wheat. The objective of the present study was to determine the extent of genotypic differences for CMS in different wheat genotypes collected in Gene Pool at the Research Institute of Crop Husbandry by means of SSR markers which are linked to the membrane stability. The plant material used consisted of 64 wheat genotypes. The 156-bp PCR product from primer Barc108 which is diagnostic for the Xbarc108-7A locus associated with membrane stability and located on 7A chromosome, was found in all genotypes. This locus was identified in genotypes with high drought tolerance as well as in medium-tolerant and susceptible genotypes Giymatli-2/17, Qyrmyzy gul-1, 12nd FAWWON №97 (130/21). The specific locus Xwmc89-4A responsible for the membrane stability was identified using primer Wmc89. The amplification of 176-bp fragments responsible for the mentioned primer occurred in 77% of the studied genotypes. The microsatellite locus was localized on wheat 4A chromosome and was associated with all the important QTLs on 7.7 centiMorgan (cM) chromosome area under drought stress. This locus was not detected in drought-sensitive and medium-tolerant genotypes as well as in drought-tolerant Azamatli-95. Xwmc603-7A locus responsible for membrane stability and located on 7D chromosome was tested by the WMC603 marker that amplifies a band of the 173-bp in 60 out of 64 genotypes.

P224

Response of Perennial Ryegrass Populations to 5 Years of Simulated Grazing

Patrick Cashman^{1,2}, Trevor Gilliland^{2,3}, Mary McEvoy¹, Michael O' Donovan¹

¹Teagasc Animal & Grassland Research and Innovation Centre, Fermoy, Co Cork, Ireland, ²Queens Univeristy Belfast, Belfast, UK, ³Agri-Food Biosciences Institute, , Crossnacreevy, Belfast, UK

Perennial ryegrass (*Lolium perenne* L.) cultivars are a collection of phenotypically unique individuals, within which there is large variation. This variation creates the potential for population selection to occur, through loss of plants that don't survive. In commercial perennial ryegrass swards, selection could reduce the ability of a sward to express favourable agronomic characteristics, such as high yield, by loss of elite plant phenotypes. The objective of this study was to investigate if population selection does occur in perennial ryegrass cultivars under simulated grazing management. Twelve cultivars were sown in plots and managed under simulated grazing of 10 mechanical defoliations per year for 5 years. A population sample was then removed from the plots and established as spaced plants, along with a control population of the 12 cultivars established from seed. All space plants were subjected to a number of phenotypic measurements during the following year. Measurements included time of inflorescence emergence, plant height and plant width at time of inflorescence emergence. Ten of the cultivar populations taken from the simulated grazing management were found to be phenotypically distinct from the control population. Seven of the cultivar populations showed a change in plant height while 4 cultivar populations showed a change in plant width under simulated grazing when compared to the control cultivar populations. Results show that population selection can occur in commercially available cultivars, identifying a mechanism by which the agronomic characteristics of perennial ryegrass cultivars can change in swards over time.

P225

Protective Effect of 24-Epibrassinolide on Proteome and Phosphoproteome of Wheat Seedlings under Salinity

Azamat Avalbaev¹, Ruslan Yuldashev¹, Kristina Ivanova¹, Elvira Vyazova¹, Natalia Petrova², Evgenia Fedina², Regina Gilmanova², Fatima Karimova², Farida Shakirova¹

¹*Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences, Ufa, Russia,* ²*Institute of Biochemistry and Biophysics, Kazan Research Centre, Russian Academy of Sciences, Kazan, Russia*

Plants use signaling pathways to respond to a variety of extracellular signals, such as plant hormones and various stress factors, which then transformed to intracellular signaling that results in the induction of expression of specific genes. The transduction of the signal depends on the post-translational modifications (PTM) of proteins. The best-studied PTM is protein phosphorylation which can lead to changes in protein activity. Protein phosphorylation is considered to be a central mechanism for regulation and cellular signaling, and special attention is given to tyrosine phosphorylation. In our previous study we have found a pronounced growth-stimulating effect of 24-epibrassinolide (EBR), active representative of brassinosteroids, on wheat plants, and this EBR effect was accompanied by promotion of protein metabolism and changes in tyrosine phosphorylation of proteins. Meanwhile, we have observed protective effect of EBR on wheat plant growth under salinity conditions. It is not surprising that salinity has led to inhibition of protein metabolism. Moreover, we have revealed almost complete absence of tyrosine phosphorylation in salt-stressed wheat seedlings which indicates the dramatic disarrangement of cell signaling in them. Pretreatment with EBR resulted in adaptation of proteome and phosphoproteome of wheat seedlings to the adverse action of salinity which was evidenced by prevention by EBR of salinity-induced disturbance of protein metabolism and protein tyrosine phosphorylation. The obtained data suggests an important role of brassinosteroids in the protection of proteome and phosphoproteome of wheat seedlings under salinity.

This work is supported by Grant № 14-04-00731_a of the Russian Foundation for Basic Research.

P226

Mechanisms of NPR1 in Cellular Localization and Proteasome-Mediated Degradation at Nucleus and Chloroplast during Salt Stress Response in Tobacco Plants

So Yeon Seo, Soo Jin Wi, Ky Young Park
Sunchon National University, Suncheon, Republic of Korea

Reactive oxygen species (ROS) play a crucial role in response to biotic and abiotic stresses. Nuclear translocation of NPR1, which is a transcription coregulator, is a critical regulatory step against biotic stress with pathogen infection. Pathogen infection induces a change in cellular reduction potential, resulting in reduction from oligomeric NPR1 to a monomeric form. Many NPR1 researches focus on SA-related response pathway after pathogen infection. Therefore, we investigate regulatory mechanism of NPR1 in response to salt stress using 35S::NPR1-GFP construct. Salt stress induced strong GFP fluorescence in nucleus, indicating pathogen-induced regulatory mechanism of NPR1 was coincidentally functioned in response to abiotic stress. We also transiently detected very strong GFP fluorescence in chloroplast after 15 min of salt stress, indicating NPR1-GFP protein rapidly imported into chloroplast. NPR1-GFP in nucleus was transiently peaked at 12 h, after which NPR1 levels in chloroplast and nuclei were significantly reduced and then almost disappeared. However, only nuclear NPR1 level was increased again after 36 h of salt stress. We are very curious whether many researchers did not recognize this biphasic fluctuation of NPR1 accumulation or first increase of nuclear NPR1 was not occurred in response to biotic stress. NPR1 protein is more rapidly imported into nucleus after salt stress in TRX-overexpressed BY2 cell lines, compared to WT and TRX-antisense expressed plant lines. We will investigate the regulatory mechanism of stress-induced NPR1 translocation at chloroplast and nucleus, which is dependent on cellular redox potential, using western blot analysis with anti-GFP antibody.

P227

Effects of Polyamine Oxidase on Cellular ROS Accumulation in Response to Salt Stress

Yu Jung Kim, Soo Jin Wi, Ky Young Park
Sunchon National University, Suncheon, Republic of Korea

Reactive oxygen species (ROS) play a crucial role as signaling molecules in many cellular processes for development and defense response. Polyamines (PAs), such as putrescine, spermidine, and spermine, also implicate in a wide range of cellular physiological process. Stress-increased intracellular polyamines are important factors to inhibit or defense programmed cell death (PCD) by scavenging ROS. However, polyamine oxidase (PAO) localized at apoplast produces H_2O_2 by degradation of PAs. Also, we detected extracellular H_2O_2 imported into chloroplast, cytoplasm, and nucleus. When HR is induced, polyamine is accumulated in the apoplast, and then generates H_2O_2 as a result of PAs decomposition by PAO. Therefore, PAs can have dual roles as ROS-scavenging agents and ROS-generating sources. We are very curious whether or not ROS produced by PAO is a positive effector for stress resistance in response to abiotic and biotic stresses. In order to detect the signaling role of apoplastic PA-derived H_2O_2 under abiotic stress, we used a series of tobacco transgenic plants overexpressing or downregulating apoplastic polyamine oxidase (PAO-S and PAO-A plants) or overexpressing S-adenosyl-L-methionine decarboxylase (S16-S plants) for increasing PAs contents. In quantitative analysis with DCFH-DA, ROS levels was much reduced in S16-S, but was much increased in WT and PAO-S plants, In addition, stress-induced cell death was decreased in PAO-A and S16-S, but significantly increased in WT and PAO-S. Therefore, polyamines are more important functions as positive regulators for attenuation of stress-induced cell damage. We will provide the exact mechanisms of PAs-induced alleviation of stress-induced cell damage in plants.

P228

Effect of Continuous Light and Short-Term Low Temperature Treatments on Plant Cold Resistance

Elena Sherudilo, Tatjana Shibaeva, Alexander Titov

Institute of Biology, Karelian Research Centre, Russian Academy of Sciences, Petrozavodsk, Russia

Controlled-environment study showed that continuous light (CL) ($155 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at normal temperature of 26°C induced leaf cold resistance increase in tomato, pepper and eggplant. The combined plant treatment by CL and daily short-term temperature drop (from 26 to 10°C for 2 h) resulted in higher cold resistance than if only CL was used. In both cases cold resistance increase was accompanied by proline accumulation. The improvement of cold resistance by increased light quantity due to extended photoperiod at normal, nonhardening temperature indicates that light may induce nonspecific protection providing cross-resistance to cold. It is discussed whether photoperiod and low temperature act in an additive fashion or effects resulting from combined treatments are determined by the intensity of stress factors, when stronger effect of one of the factors (in this case, low temperature) overlaps the effect of the other one (CL). The reported study was partially supported by RFBR, research project 14-04-00840a.

P229

Effect of Prolonged Moderate Drought Stress on the Proton and Electron Transport in Wheat Leaves

Katarina Olsovska¹, Marek Zivcak¹, Hazem M. Kalaji², Hong-Bo Shao³, Marian Brestic¹

¹Dept. Plant Physiology, Slovak University of Agriculture in Nitra, Nitra, Slovakia, ²Dept. Plant Physiology, Warsaw Agricultural University SGGW, Warsaw, Poland, ³Yantai Institute of Coastal Zone Research, Chinese Academy of Science, Yantai, China

The photosynthetic electron and proton transport related responses were studied in conditions of long-lasting moderate drought stress in leaves of wheat (*Triticum aestivum* L.). The spectroscopic measurements of dark-interval relaxation kinetics of electrochromic bandshift (ECS) at 520 nm indicated a decrease of electric and an increase of osmotic component of the proton motive force (pmf) in drought stressed leaves, but neither the total pmf nor the thylakoid proton conductance (gH^+) were affected. We observed the enhanced protection against overreduction of PSI acceptor side in leaves of drought stressed plants. This was obviously associated with the rapid buildup of transthylakoid pH gradient, even at relatively low light intensities. It led to the steep increase of NPQ and the down-regulation of linear electron transport. It was further accompanied by the steep increase of redox poise at PSII acceptor side and PSI donor side. Based on analyses of P700 kinetics, we suggest that the early responses related to thylakoid lumen acidification in drought-stressed leaves could be associated with the activity of an enhanced fraction of PSI not involved in linear electron flow, which may have led to enhanced cyclic electron pathway even in relatively low light intensities. This may be also the reason of drought-induced decrease of IP-amplitude in fast chlorophyll fluorescence kinetics.

Acknowledgment: Supported by the research projects APVV-0197-10 and APVV-0661-10 and by the European Community under the Project No. 26220220180: "Construction of the "AgroBioTech" Research Centre".

P230

Plasticity in the Physiological Response to Salinity in the Invasive Species *Baccharis halimifolia*

Teresa Fuertes-Mendizábal, Lidia Caño, Carmen González-Murua, Mercedes Herrera, María Begoña González-Moro

University of the Basque Country UPV/EHU, Basque Country, Spain

Baccharis halimifolia is a woody shrub from the South of North America adapted to coastal environments, whose high tolerance to salt stress promotes its ability to invasion in Marsh environments. The soil salinity level could limit the ability of invasion of this species in most halophytic communities, even though it has been shown that *B. halimifolia* has a phenotypic plasticity that allows the tolerance to certain salinity degree allowing thus the invasion of salt marshes. The main objective of this study was to evaluate the effect of salinity on the growth and physiological response of this species, as well as the possible variability in the response among maternal lines coming from different environments, one more halophytic (high salinity genotype) and one less halophytic (low salinity genotype) from Urdaibai Reserve of the Biosphere (Basque country). The results obtained in this study showed that the physiological response of *B. halimifolia* to salinity is characterized by accumulate Na^+ ions in all the organs, behaving thus as salt-accumulator plant, wich also synthesize high levels of proline as osmolyte response to Na^+ accumulation. It also shows certain plasticity degree in leaf morpholgy increasing the leaf succulence and the stomatal density, and decreasing stomatal conductance probably as a mechanism to regulate transpiration in order to avoid a greater NaCl uptake. This species shows a different adpatation degree at intermediate salt levels, since plants of the low salinity genotype tended to accumulate higher Na^+ amounts, increase the proline synthesis and show a higher stomatal density.

P231

Genome-Wide Gene Expression Analysis of Genotype-Dependent Effects of CaCl₂ Treatment on Drought Adaptation in Barley (*Hordeum vulgare* L.)

Malgorzata Kaczmarek¹, Pawel Krajewski¹, Grzegorz Koczyk¹, Olga Fedorowicz-Stronska¹, Jan Sadowski^{1,2}

¹Institute of Plant Genetics Polish Academy of Sciences, Poznan, Poland, ²Institute of Molecular Biology and Biotechnology, Department of Biotechnology, Adam Mickiewicz University, Poznan, Poland

The objective of this study was the genome-wide analysis of the effect of CaCl₂ treatment on drought adaptation in barley. Caryopses of two barley genotypes (tolerant Sebastian and susceptible Georgie) were subjected to osmopriming with 50mM CaCl₂, previously chosen based on a pilot experiment. Drought stress was imposed on three-week-old plants by withholding water and their leaves were taken at three selected time points related to field water capacity (pF= 3.2, 3.6 and 4.2). GeneChip Barley Genome Array 22K (Affymetrix) were used to perform gene expression profiling. Analysis of variance set up groups of differentially expressed genes (DEGs) according to the studied factors and their interactions. Functional analysis of DEGs and their GO terms categorization was performed using Blast2Go (Conesa et al. 2005). Enrichment analysis was done using χ^2 test Bonferroni corrected and Wilcoxon Rank Sum test. MapMan and PageMan (Usadel et al. 2005) was used to visualize the cumulative results of our study. CaCl₂ treatment influence barley drought adaptation through modification of gene expression of genes encoding pectin esterases and enzymes related to cell wall degradation. On the other hand, changes in gene expression coding for PS light reaction and Calvin's cycle enzymes suggest independence of CaCl₂ treatment drought adaptation. The work was supported by the European Regional Development Fund through the Innovative Economy Program for Poland 2007-2013, POLAPGEN-BD no. WND-POIG.01.03.01-00-101/08 project.

P232

Sensitivity of Photosynthetic Apparatus to High Temperature Seem to be Independent on Biosynthesis of Cytokinins in Tobacco Leaves

Marek Zivcak¹, Marian Brestic¹, Katarina Olsovska¹, Marian Gabris¹, Radomira Vankova²

¹*Dpt. of Plant Physiology, Slovak University of Agriculture, Nitra, Slovakia,* ²*Institute of Experimental Botany, Czech Academy of Sciences, Prague, Czech Republic*

The role of cytokinins in plant high temperature tolerance has been documented by several studies. To verify these results we compared high temperature responses of wild type (WT) tobacco plants with a tobacco construct (line 303) overproducing cytokinins short time after treatment with dexamethasone. Plants grown in cultivation chamber at moderate temperature were divided into two groups: one group was supplied by solution of dexamethasone, the second one with pure water. Both groups were exposed to high air temperature levels 35°C, 38°C, 42°C, 45°C in growth chamber in light. After 10 hours of exposition, the measurements of chlorophyll fluorescence, PSI transmittance, gas exchange measurements as well as test of thermostability using treatment of leaf samples in dark at 35, 38, 42, 45 and 48°C for 30 minutes with measurements of fast fluorescence kinetics before and after heat treatment. High temperature led to the gradual decrease of the net photosynthetic rate and changes in chlorophyll fluorescence parameters, in line with expectations. A comparison of both types of tobacco showed very similar responses to high temperature. Dexamethasone treatment didn't lead to the increase of high temperature tolerance. However, in plants of line 303 treated by dexamethasone, the evident symptoms of cytokinin overexpression confirmed the effect of dexamethasone in treated plants. Our results suggest that the presence of different level of cytokinin biosynthesis had no direct effect on PSII thermostability neither on photosynthetic performance at high temperature. (Supported by the European Community under the Project No. 26220220180: "Construction of the "AgroBioTech" Research Centre").

P233

Root to Shoot Chemical Stress Signalling in *Humulus lupulus* under Drought

Halyna Korovetska¹, Ondřej Novák², Vít Gloser¹

¹Masaryk University, Department of Experimental Biology, Brno, Czech Republic, ²Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany Academy of Sciences of the Czech Republic, v.v.i. & Palacký, Olomouc, Czech Republic

Soil drying leads to the generation of long - distance chemical signals in plant roots that regulate water use of plant via control of the stomatal aperture in leaves. The aim of our work was to identify the presence and identity of potential chemical signals, their dynamics, and their relationship with transpiration rate during soil drying in hop (*Humulus lupulus* (L.)) plants. We used pressure chamber technique for measurement of shoot water potential and collection of shoot xylem sap. We analyzed concentrations of abscisic acid (ABA), nitrate, phosphate, sulphate and malate in sap and also the rate of whole plant transpiration. We also experimentally tested effects of some sap constituents and their interaction on transpiration of hop leaves.

Transpiration rate of whole plants decreased prior to changes in shoot water potential, which indicates presence of regulatory mechanism independent of shoot water status. The concentration of ABA in xylem sap continuously increased from early to later stages of water stress, whereas in leaves it increased only at later stages. Shoot sap pH increased simultaneously with the decrease of transpiration rate. Xylem sap alkalization was accompanied by a decrease in nitrate concentration and an increase in malate concentration. Concentration of sulphate increased in xylem sap during drying and sulphate in combination with a higher ABA concentration enhanced stomatal closure. We conclude that several compounds may act as early chemical signals in sap of hop plants during soil drying and their impact on transpiration may vary according to the stage of soil drying.

P234

Screening Genotypes of Candidate Energy Grasses for Flooding Tolerance

Poul Erik Laerke, Kirsten Kørup, Helle Baadsgaard, Yubaraj Karki, Uffe Jørgensen
Aarhus University, Tjele, Denmark

Low organic and often flooded soils are not suitable for food crop production. However, production of flooding tolerant perennial grasses aimed for bioenergy may be an alternative. Up to ten genotypes of each of reed canary grass (RCG), tall fescue, festulolium, miscanthus and cocksfoot were screened for tolerance to flooding at semi-field conditions. The grasses were grown in pots, which were placed in big water-filled containers. Ten days before the Flooding Treatment Initiation (FTI), all grasses were cut back and fertilised. At FTI the water table was gradually increased over a 13-days period until it reached the soil surface. Mean daily shoot growth rate (SGR) was calculated. In addition, leaf chlorophyll fluorescence (LCF) was measured four times and root porosity was quantified four weeks after complete flooding.

Before flooding, cocksfoot had significantly higher SGRs than the other four species, but shortly after FTI, SGR quickly decreased and cocksfoot ended up as the most flooding affected species. The SGRs of tall fescue and festulolium also decreased throughout the period, but at slower rates than cocksfoot. In contrast, SGRs of RCG and miscanthus increased after FTI. Some differences between genotypes within each species were also detected, but these were less significant.

Miscanthus seemed to be the most flooding tolerant of the five species, which was also supported by measurements of significantly higher root porosity. The LCF was not affected by flooding treatment except for miscanthus for which maximum quantum efficiencies of PSII significantly decreased after complete flooding was reached.

P235

Ozone Effects on Primary Metabolism in Three Poplar Genotypes Differing in Sensitivity

Jennifer Dumont^{3,1}, Sarita Keski-Saari¹, Markku Keinänen¹, Fabien Spicher³, Sari Kontunen-Soppela¹, Pierre Dizengremel², Yves Jolivet², Elina Oksanen¹, Didier Le Thiec³

¹University of Eastern Finland, Joensuu, Finland, ²Université de Lorraine, Nancy, France, ³INRA, Nancy, France

Tropospheric ozone acts as a phytotoxin producing an oxidative stress in plants. Two ways of defense are used, either by preventing ozone input through the regulation of stomatal conductance, or by detoxifying ozone and ROS in cells. It is known that stomatal conductance and photosynthesis are altered by ozone leading to energy deficiency especially when the needs for the detoxification mechanisms are increased. We performed a fumigation experiment on three euramerican poplar genotypes (*Populus deltoides* x *Populus Nigra* : 'Carpaccio', 'Cima' and 'Robusta') differing in their ability to cope with ozone stress, cultivated in pots in phytotronic chambers and submitted to 120 ppb ozone or filtered air. Leaf samples were collected 2, 4, 11, 15 and 17 days after the start of ozone treatment. Chemical analyses were made by GC-MS. We explored the effects of ozone on stomatal conductance, photosynthesis, chlorophyll content, biomass and primary metabolites and tried to relate them to ozone flux. The biggest changes in response to ozone were a strong increase in sugars and especially raffinose, and a decrease in most of the amino acids. The chemical distinction between the genotypes was mainly due to a delayed response in the most resistant genotype compared to the other two genotypes and to differences in the constitutive concentrations of some compounds such as sugars.

P236

High Temperature Sensitivity of Photosystem II and Environmentally Induced Increase of PSII Thermostability in Thirty Field-Grown Wheat (*Triticum aestivum* L.) Genotypes

Marian Brestic¹, Marek Zivcak¹, Katarina Olsovska¹, Zuzana Balatova¹, Petra Drevenakova¹, Pavol Hauptvogel²

¹Dept. Plant Physiology, Slovak University of Agriculture in Nitra, Nitra, Slovakia, ²National Production and Food Research Center - Research Center for Plant Production, Piešťany, Slovakia

High temperature resistance of photosystem II (PSII) as well as acclimation effects on PSII thermostability were analyzed in thirty field-grown winter wheat (*Triticum aestivum* L.) genotypes using a prompt chlorophyll a fluorescence kinetics. A gradual increase in temperature caused appearance of K-bands at 300 s on the chlorophyll fluorescence induction curve, indicating the impairment of the PSII donor side. The increase in basal fluorescence, commonly used as a criterion of PSII thermostability, was observed beyond the temperature threshold of 44 °C. Moreover, the acclimation shift (an increase of critical temperature) observed for K-band appearance was significantly higher than those for a steep increase of basal fluorescence F₀. The single temperature approach (40 °C) with regular weekly observations demonstrated that the acclimation effect was not gradual, but occurs immediately and it was associated with an increase of daily temperature maxima over 30 °C. In the non-treated plants the reduction of antennae size, increase of PSII connectivity and changes in the acceptor side occurred as a result of heat acclimation. The enhancement of PSII thermostability persisted over several weeks regardless of weather conditions. The genotype comparison identified three groups that differed either in initial PSII thermostability or acclimation capacity; these groupings were clearly associated with the origin of the genotypes.

Acknowledgment: Supported by the research projects APVV-0197-10 and APVV-0661-10 and by the European Community under the Project TRIVE , No.26110230085).

P237

Characterization of Selenium Binding Protein1 (SBP1) in *Trigonella foenum graecum* (Linn.)

Andri Georgiou, Chrysanthi Valasakis, Kosmas Charalampides, Andreas Roussis
University of Athens, Department of Biology, Section Botany, Athens, Greece

Trigonella foenum graecum (Linn.), commonly known as fenugreek, is an erect, annual aromatic herb of the Fabaceae family native to southern Europe and Asia which is extensively cultivated in China, Africa, Ukraine and Greece and represents one of oldest medicinal plants known to be historically recorded. Fenugreek or the “Greek hay” has been cultivated since ancient times, as it garners a range of properties characteristic of high quality crops and today is widely grown as a food, medicinal, dye, condiment and forage plant. We have shown that synthesis and excretion of the pterocarpan medicarpin, an isoflavonoid phytoalexin, is induced de novo in fenugreek by heavy metals, aluminum (Al) and more interestingly, by selenium (Se). While selenium naturally occurs as a trace element in soils, it has not been shown to be an essential nutrient for higher plants, although it can have a growth-promoting effect for many species, particularly Se hyperaccumulators. The mammalian selenium-binding protein 1 (SBP1) with no Se-amino acid in its sequence, is a protein that can directly bind selenium, with homologs found in many organisms and plant species. Furthermore, the Arabidopsis SBP1 has been shown to directly bind cadmium (Cd) and when overexpressed in transgenic plants to confer tolerance to otherwise toxic levels of selenium and drastically act in Cd detoxification processes. Taking into account the prospect of using selenium-containing plants both for biofortification and phytoremediation along with the economical, medicinal and dietary importance of *Trigonella foenum graecum*, we investigated the role of SBP1 in abiotic stress responses.

P238

Multilevel Analysis Revealed that Repeated Selection under Heat Stress Improves Heat Tolerance in *Arabidopsis thaliana*

Gaurav Zinta^{1,2}, Hamada AbdElgawad², Marios Nektarios Markakis², Ivan A. Janssens¹, Gerrit T.S. Beemster², Han Asard²

¹Research Group of Plant and Vegetation Ecology, Department of Biology, University of Antwerp, Antwerp, Belgium, ²Laboratory for Molecular Plant Physiology and Biotechnology, Department of Biology, University of Antwerp, Antwerp, Belgium

Plants are challenged to various biotic and abiotic stresses in their natural environment, and can adapt, to some extent, themselves to the stressful conditions. We tested whether selection for survival and fitness (seed biomass), under extreme heat, induces adaptive changes in *Arabidopsis thaliana* (Col-0). Selection was made repeatedly for three generations under heat and control conditions. We analysed fourth generation plants (G4), and found that heat-selected plants produced higher seed biomass, when exposed to heat stress in G4, and showed less oxidative damage as revealed by less electrolyte leakage, chlorophyll fluorescence, lipid peroxidation and protein oxidation. Moreover, they also showed enhancement in the antioxidant defense viz. total antioxidant capacity (FRAP), total phenolics and ascorbic acid. Genome-wide transcriptional analysis (via RNA-seq) revealed that under heat stress control- and heat-selected plants changed expression of thousands of genes, but the heat-selected plants had higher expression of 47 genes under control conditions. Together, these results suggest that stress can induce heritable changes (potentially adaptive) in plants, which can be artificially selected to allow increase in the yield potential and tolerance of plants under stress conditions.

P239

eQTL Analysis and Network Inference of a Drought Stressed Diploid Potato Mapping Population

Dennis van Muijen¹, Chris Maliepaard¹, AM Anithakumari^{1,2}, Ernest Aliche^{1,2}, Richard Visser¹, Gerard van der Linden¹

¹Wageningen UR Plant Breeding, Wageningen, The Netherlands, ²Graduate School of Experimental Plant Sciences, Wageningen, The Netherlands

Drought is one of the most devastating environmental stresses, affecting crop yield worldwide. Potato uses water efficiently for food production, but is sensitive to drought stress. Discovery of genetic factors that contribute to drought tolerance in potato will facilitate the breeding of tolerant cultivars. We previously evaluated the potato CxE diploid mapping population for drought tolerance, and found an important QTL locus on Chromosome 5 (1). We analysed the full transcriptome of 94 individuals of this population with micro arrays and used the dataset for eQTL analysis to find candidate genes for drought tolerance and traits contributing to drought tolerance. The data was analysed with bioinformatics tools like R, BLAST2Go, OmicsFusion, and an inference algorithm applied through the GenePattern95 DREAM network inference platform (<http://dream.broadinstitute.org/gp/pages/index.jsf>) (2).

A PCA with all expression data showed a distinct group of drought-stressed genotypes that also had relatively high yields, that specifically highly expressed a dehydrin (TAS14 homolog). In addition, eQTLs were mapped for the expression datasets of control, drought-stressed, and drought vs control plants. The most remarkable result is a peak of eQTLs clustering on a single eQTL hotspot on Chromosome 5, close to but not co-localizing with the maturity locus.

By network inference analysis a particular transcription factor was found to be a central node to the regulation of the genes with eQTLs in this hotspot. This transcription factor is an NF-YC homolog. Also, an ethylene response factor (ER24) appears to be directing the Heat Shock Protein response in the drought-stressed potatoes.

P240

Formation of Oxalate-Mn Internal Complex as a Potential Detoxification Mechanism of Mn Toxicity in Perennial Ryegrass

Karina Godoy^{1,2}, Daniela Alvarez³, María de La Luz Mora^{4,5}, Alejandra Ribera^{4,6}

¹Laboratorio de Citometría de Flujo-Unidad Biología Molecular, Hospital Regional Hernan Henríquez Aravena, Manuel Montt 115, Postal code 4781151, Temuco, Chile, ²Unidad de Equipamiento Mayor, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Av. Francisco Salazar 01145, Postcode 4811230, Temuco, Chile, ³Laboratory Soil-Plant Analysis, Instituto de Agroindustria, Universidad de La Frontera, Av. Francisco Salazar 01145, Postcode 4811230, Temuco, Chile, ⁴Center of Plant-Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Avenida Francisco Salazar 01145, Postc, Temuco, Chile, ⁵Departamento de Producción Agropecuaria; Facultad de Ciencias Agropecuarias y Forestales; Universidad de La Frontera, Av. Francisco Salazar 01145, Postcode 4811230, Temuco, Chile, ⁶Departamento de Ciencias Químicas y Recursos Naturales; Facultad de Ingeniería, Ciencias y Administración; Universidad de La Frontera, Avenida Francisco Salazar 01145, Postcode 4811230, Temuco, Chile

Manganese (Mn) toxicity is an important growth-limiting factor in acidic soils. The Mn-excess vacuolar chelation by oxalate constitutes a detoxification mechanism. Earlier results showed a link between an increased oxalate exudation and higher tolerance during Mn-excess conditions in ryegrass cultivar Kingston. Here we show the formation of Mn-oxalate complexes in ryegrass plants grown under Mn excess as an internal Mn-tolerance strategy. Two ryegrass cultivars (Kingston and Nui) were exposed to normal and toxic excess of Mn (2.4 and 750 μM) during 28 days (pH 4.8 in nutrient solution). Protoplast, vacuoles and chloroplasts were isolated, confirmed by Confocal Microscopy and Flow Cytometry, were only chloroplasts and protoplasts population had chlorophyll fluorescence. The yield of isolates vacuoles was around 50%. The foliar analysis confirmed that both cultivars had comparable levels of Mn uptake, but lipid peroxidation was higher in Mn-sensitive cultivar Nui. This suggests the existence of a Mn internal inactivation mechanism on Kingston cultivar. Finally, HPLC-DAD analysis indicated presence of oxalate in protoplasts and vacuoles, where the highest values were detected in the Mn-tolerant cultivar. The highest concentration of oxalate in vacuoles plus the higher Mn levels in leaves of Kingston shows that the formation of Mn-oxalate complexes could explain an internal detoxification mechanism of Mn excess in this cultivar. The effective standardization of protocol for protoplasts and vacuoles isolation, as well as the extraction of oxalate from these samples, is a breakthrough allowing more specific analyses to elucidate toxic effects in plants. Acknowledgments: FONDECYT Project 11100494.

P241

Metabolic Signatures of Oilseed Rape (*Brassica napus* L.) Leaves Development under Contrasted Nitrogen/Water Regimes

Benjamin Albert¹, Françoise Le Caherec¹, Laurent Leport¹, David Renault², Raphaël Lugañ³, Antoine Gravot¹, Alain Bouchereau¹

¹UMR 1349 IGEPP, INRA-Agrocampus Ouest-Université de Rennes 1, Le Rheu, F-35653, France,

²UMR 6553 EcoBio, CNRS-Université de Rennes 1, Rennes, F-35042, France, ³UPR 2357 IBMP, CNRS, Strasbourg, F-67084, France

Grain productivity of oilseed rape crop could be, in the near future, frequently impacted by periods of nitrate and/or water starvation. Insuring seed yield potential and plant tolerance with lower inputs should be possible by improving nitrogen (N) and water use efficiency through optimization of N and carbon (C) remobilization efficiency from senescing leaves to growing sink tissues and seed filling. In this study, leaf metabolic profiles were characterized by quantitative analysis of primary metabolites and compared between senescing source leaves, developing and mature sink leaves to assess metabolic networks of C and N recycling and remobilization processes. Plants were grown under controlled greenhouse conditions and subjected to nitrogen deficiency, water deficit and combined shortages at the vegetative stage. The project also aimed at determining whether water stress metabolic responses under different nitrogen fertilizer regimes may share possibly convergent or conflicting metabolic routes with those involved in the remobilization dynamics of N and C between sources and sinks. Metabolic profiling data were integrated into exploratory multivariate statistical analysis. Leaf status-specific and development-dependent metabolic signatures were pointed out and clusters of metabolic indicators could be assigned to sink/source status and nutrition conditions. Metabolic adjustments in response to drought were strongly affected by preliminary nitrogen availability and leaf development. In prospect, this work disclosed possible new components of the regulatory network for nitrogen use efficiency and plant stress response in oilseed rape.

P242

Effect of MeJA Applied Simultaneously to Application of Aluminum in *Vaccinium corymbosum* Leaves and Its Increase in Antioxidant Activity with Changes in Aluminum Content

Elizabeth Ulloa-Inostroza¹, Maria Graciela Muñoz-Pozo², Rayen Millaleo⁴, Cristian Meriño-Gergichevich⁴, Miren Alberdi^{3,4}, Marjorie Reyes-Díaz^{3,4}

¹Doctorado en Ciencias de Recursos Naturales Program, Universidad de La Frontera, Casilla 54-D, Temuco, Chile, ²Carrera de Biotecnología, Universidad de La Frontera, Casilla 54-D, Temuco, Chile, ³Ciencias Químicas y Recursos Naturales Department, Universidad de La Frontera, Casilla 54-D, Temuco, Chile, ⁴Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), Universidad de La Frontera, Casilla 54-D, Temuco, Chile

The exogenous application of methyl jasmonate (MeJA) can reduce toxic metals effects, increase antioxidant activity (AA), reducing the oxidative stress, and its Al content in plants. In this research an antioxidant response and changes in aluminum (Al) content of different MeJA doses applied simultaneously to the application of Al in leaves in *Vaccinium corymbosum* was to determine. Two year-old cultivars (Legacy Al-resistant and Bluegold Al-sensitive) were acclimated for seven days in greenhouse under controlled conditions. Plants were treated with and without toxic Al with the following treatments: a) control treatment (without Al and MeJA), b) Al treatment (100 μ M), and the combined treatments: c) 100 μ M Al+ 0.005 mM MeJA, d) 100 μ M Al + 0.01 mM MeJA and e) 100 μ M Al + 0.05 mM MeJA. The MeJA was applied by spraying in leaves simultaneously with Al application in the solution. The experiment was run for 48 h after to Al applied. The results showed decrease Al content, lipid peroxidation and hydrogen peroxide content, maintaining high AA and phenols content in leaves with low dose of MeJA in both cultivars. Therefore, we can conclude that the application of low doses of MeJA with Al in Legacy and Bluegold cultivars under Al stress could be a good alternative for reducing the alterations induced by Al toxic.

Acknowledgements: FONDECYT N°1120917, CONICYT fellowship, DI 13-2017 2013 Project, and Berries San Luis Farm.

P243

Methyl Jasmonate Application Differentially Affects Antioxidant Activity and Oxidative Damage of Highbush Blueberry (*Vaccinium corymbosum* L.) Exposed to UV-B Radiation

Marjorie Reyes-Díaz^{1,2}, Claudia Huanquilef³, Alejandra Ribera-Fonseca², Patricio Acevedo⁴, Elizabeth Ulloa³, María Graciela Muñoz-Pozo⁵, Miren Alberdi^{1,2}

¹Departamento de Ciencias Químicas Y Recursos Naturales, Universidad de La Frontera, Temuco, Chile, ²Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco, Chile, ³Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile, ⁴Departamento de Ciencias Físicas, Universidad de La Frontera, Temuco, Chile, ⁵Carrera de Biotecnología, Universidad de La Frontera, Temuco, Chile

Highbush blueberry (*Vaccinium corymbosum* L.) is widely cultivated in southern Chile, where higher ultraviolet B (UV-B) radiation doses are found. On the other hand, methyl jasmonate (MeJA) has proven to be an elicitor molecule able to participate in environmental abiotic stress responses (e.g. UV-B). In this context, the aim of this study was to study the effect of MeJA application on the antioxidant activity and oxidative damage in leaves of highbush blueberry exposed to UV-B radiation. Two-years-old plants of Legacy and Bluegold cultivars were grown under Hoagland solution during 7d under greenhouse conditions. UV-B irradiation and MeJA treatments were: a) without MeJA and without UV-B (CONTROL: -MeJA -UV-B); b) without MeJA plus UV-B (-MeJA +UV-B); c) 0.01 mM MeJA without UV-B (+0.01MeJA -UV-B); d) 0.05 mM MeJA without UV-B (+0.05MeJA -UV-B); e) 0.01mM MeJA plus UV-B (+0.01MeJA +UV-B) and f) 0.05mM MeJA plus UV-B (+0.05MeJA +UV-B). The results indicated that, contrarily to Bluegold, Legacy increased (3-fold) lipid peroxidation under UV-B compared to the control, decreasing with MeJA application in plants exposed to UV-B. Antioxidant activity was maintained in both cultivars. Total phenols increased 3-fold in Legacy plants treated with MeJA under UV-B, especially at the end of the experiment. In Bluegold, generally total phenols increased in all treatments at the end of the experiment. The results suggest that MeJA application decreased the oxidative damage in plants under UV-B, especially in Legacy. Acknowledgements: FONDECYT N°1120917, CONICYT fellowship, DI 13-2017 2013 project and Berries San Luis Farm.

P244

Transient Expression of Cyt-c in Tobacco Ameliorates Plant Responses to Salinity

Efthimios A. Andronis, Kalliopi A. Roubelakis-Angelakis
University of Crete, Herakleion, Crete, Greece

Cytochrome-c (cyt-c) is an electron carrier bound in the inner mitochondrial membrane. It is located between complexes III and IV of the mitochondrial electron transport chain (mETC) and facilitates transport of electrons between the two complexes. In earlier work we established that salinity stress led to the release of cyt-c in the cytoplasm, dysfunction of the mETC, mitochondrial membrane depolarization, and induction of PCD in tobacco. In order to further evaluate the role of cyt-c during salinity stress, a molecular approach was used involving agroinfiltration and transient expression of cyt-c in tobacco. Agroinfiltrated tobacco plants were subjected to salinity stress (250mM) for 3 and 24h and respiration efficiency, ADP/ATP ratio, ROS production, protease activity and PCD onset were monitored. Salinity stress led to mitochondria depolarization, release of cyt-c in the cytosol and impairment of respiration in both plants. Despite the release of cyt-c in the cytosol, the infiltrated plants maintained a high level of intra-mitochondrial cyt-c. These plants maintained a high ratio of ATP/ADP at both, control and stress conditions, whereas salinity stress led to a significant decrease of this ratio in WT plants. Infiltrated plants accumulated lower titers of ROS, compared to the WT plants, under salinity stress. Finally, PCD and increased caspase proteolytic activity were detected only 3h post-stress. In conclusion, transient expression of cyt-c in tobacco plants contributed towards maintaining a high level of intra-mitochondrial cyt-c, which in turn helped to maintain low ROS titers, high ATP/ADP ratio and prevented the onset of PCD 24h post-stress.

This work is implemented in the frame of ABISTOLE project KA3571 and the EU COSTFA06 action.

P245

UV-Sunscreens Effects on Physiological and Biochemical Responses in Highbush Blueberry Grown in Field Conditions

Claudio Inostroza-Blancheteau¹, Marjorie Reyes-Díaz^{2,3}, Francisco Musante¹, Ana Luengo-Escobar³, Patricio Acevedo⁴, Miren Alberdi^{2,3}

¹Núcleo de Investigación en Producción Alimentaria (NIPA-UCT), Escuela de Agronomía, Facultad de Recursos Naturales, Universidad Católica de Temuco, Temuco, Chile, ²Departamento de Ciencias Químicas y Recursos Naturales, Facultad de Ingeniería, Ciencias y Administración, Universidad de La Frontera, Temuco, Chile, ³Center of Plant, Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus (BIOREN), Universidad de La Frontera, Temuco, Chile, ⁴Departamento de Física, Facultad de Ingeniería, Ciencias y Administración, Universidad de La Frontera,, Temuco, Chile

Highbush blueberry (*Vaccinium corymbosum* L) productions are concentrated in south central Chile, where high UV-B radiation levels are reported in Spring and Summer. This radiation represents a serious risk for the biological processes, affecting negatively the yield production and quality of fruit. Therefore, aim of this work is to study the UV-Sunscreens effects on photosynthesis and phenolic compounds in leaves of twoighbush blueberry genotypes (Legacy and Bluegold) under field conditions. Three UV-Sunscreens (Control; -UV-B and -UV-A/B) was study. The photosynthesis (Pn), stomatal conductance (gs), transpiration (E), total phenols (TP) and antioxidant activity (AA) was evaluated. The results showed that both genotypes have differential responses to UV-Sunscreens. The Pn did not show significant changes between different sunscreens. However, gs and E was significantly lower ($P \leq 0.05$) in Legacy that in Bluegold. Otherwise, the TP and AA were higher in all UV-Sunscreens in Legacy compared to Bluegold genotype. According to our preliminary results, it is appears that Legacy has different strategies to cope UV radiation under field conditions, suggesting a better performance of this genotype.

P246

Water Stress Tolerance in Wheat Species Depends on Ploidy Level and Genome Content

Irina Kiselyova, Alexander Ermoshin, Olga Bondarenko
Ural Federal University, Ekaterinburg, Russia

Study of indicators of water stress and antioxidant elements in detached leaves of 14 wheat species under desiccation, showed that plants with different level of ploidy and genome content had specific ways of acclimation. It is shown that in diploid monogenome AA species and tetraallopoloids A^bA^bGG water deficit in leaves during desiccation developed faster, however, the level of lipid peroxidation increased much more slowly, then in tetraallopoloids A^uA^uBB and hexaploids A^uA^uBBDD . Common wheat (A^uA^uBBDD) and tetraallopoloid A^uA^uBB had smaller losses of water, but were less tolerant to desiccation. Species with genome A were characterized by a higher (18%-30%) level of proline in control conditions and under stress compared to other species. Species with genome A^bG revealed 1.3-1.8-fold higher total SOD-activity. In species with genomes A^uB and A^uBD GPO-activity was higher than in others in normal conditions and increased 1.2-1.5 times under stress. Leaf photosynthetic tissues (Khramtsova et.al, 2003; Khramtsova, Kiselyova, 2004) and stomata indicate more xeromorphic features in A^bG -genome wheat. Hexaploids A^uA^uBBDD compared to tetraploids A^uA^uBB had fewer stomata of the same size, therefore, had less potential capacity for transpiration, which probably explains the lowest values of water losses during desiccation. Obtained data show the ways of retaining water in wheat leaf tissues and tolerance to oxidative stress caused by drought.

P247

Physiological Efficiency in Water and Nutrient Utilization and Yielding Capacity of Fodder and Edible Peas under Water Shortage

Andrzej Gorny, Dominika Ratajczak, Magdalena Gawłowska, Wojciech Świecicki
Institute of Plant Genetics Polish Academy of Sciences, Poznan, Poland

In countries with light-textured podzolic soils, water and nutrient shortages appear to be essential constraints facing productivity of field pea (*Pisum sativum*), and better understanding of its morpho-physiological/genetic pieces under drought appears challenging. Hence, we examined a collection of fodder and edible pea cultivars grown in experimental pots (9dm³) under optimal and reduced water supply. Drought was applied during the whole generative growth phase. Negative relationship between drought resistance and yielding was observed. The less productive edible cultivars (mostly afilea types) transpired less water and were usually more resistant than the fodder (mostly conventionally leafed) ones. This variation was partly associated with leaf structure, stomatal functions and photosynthetic activity/efficiency of the tendrilled and/or conventional leaves. The season-integrated transpiration efficiency (TE) exhibited no close relationships with the instantaneous leaf TE and drought resistance. Most afilea cultivars exhibited high leaf TE, while the fodder cvs. tended to use water efficiently either at leaf level or in season-long scale. Efficiency of N and P utilization shown weak positive effects on the resistance, although yielding capacity under stress was related with seasonal TE and efficiency in P utilization, but not with N efficiency. The results suggest that genetic factors responsible for drought resistance and physiological efficiency in water and NP utilization are partially independent in pea. To some extent, observed variation in the efficiency and response of the examined peas to drought might be connected with breeding history and more or less conscious selection pressure during their development in specific environmental habitats.

P248

Using *In Vitro* Culture for Production of Regenerated Durum Wheat (*Triticum turgidum* subsp. durum Desf.) Plantlets under Salt Stress

Slama Olfa¹, Ayed Sourour², Slim-Amara Hajer¹

¹National Agronomic Institute of Tunisia, Tunis, Tunisia, ²Regional office of development agriculture semi arid, Kef, Tunisia

The genetic variability is considered as the major principle of plant breeding for durum wheat. This variability can be induced in vitro by selection pressure exerted by stress factors such as salinity and promoting regeneration of vitroplantlets tolerant.

This study aims in the first step in the regeneration of plantlets tolerant to salinity from mature embryos culture derived from two Tunisian durum wheat varieties : improved (Razzek) and local (Jenah Khotifa JK) varieties. The tolerance evaluation to salt stress was applied in vitro (100 mmol l⁻¹ NaCl) and is based on various parameters. Our results showed that JK variety is distinguished by a stable response for all parameters tested : average weight of callus (368.1 mg for control, 307mg under salt stress), callus regenerated percentage (36.6% for control and 35.7% under salt stress) and green shoots number /callus (17 for control and 17 under salt stress). This stability of response translates the adaptability of this variety to salinity.

In order to fix regenerated JK plantlets in single generation and obtain HDs homozygous stable lines, in vitro gynogenesis technical is tested for this genotype. The Evaluation of gynogenetic capacity focused on about 1200 unfertilized ovaries of JK and is based on its ability to induction, differentiation, development of green shoots, haploid plantlets regeneration and HDs production lines. JK showed a relatively good response to gynogenesis with a rate of regenerated lines HDs / total haploid plantlets of 60%.

P249

Investigation of Hydrophytes Antioxidant Status in Relation to Their Accumulative Capacity

Nadezhda Chukina, Galina Borisova, Maria Maleva

Ural Federal University named after the first President of Russia B.N. Yeltsin, Ekaterinburg, Russia

Aquatic macrophytes are the most important components of water bodies and take up metals from water due to their direct contact with the aquatic environment. They are able to accumulate heavy metals (HMs) several fold higher than their surroundings. We examined the uptake of Cu, Fe, Ni, Zn, and Mn, antioxidant systems parameters (enzymes activities, non-enzymatic antioxidants contents) of *Ceratophyllum demersum* L. and *Potamogeton alpinus* Balb. from Iset' river, Ural region, Russia. The studying territory is highly urbanized where the surface waters are contaminated by a wide spectrum of pollutants due to anthropogenic activity. Higher amounts of HMs accumulated in hornwort compared to pondweed was noticed. The maximum difference observed for Mn it was 7.9 times higher, for Cu - 3,3, for Fe- 3 times higher than in *P. alpinus*. In response to HMs stress, plants have evolved different detoxification mechanisms such as antioxidant inductions and complexation of HMs by phosphates and organic acids, metal-binding proteins.

It was shown that in leaves of *C. demersum*, ("accumulator") there were high amount of total phosphorus, nitrogen, organics acids and ash; high activity of guaiacol peroxidase; high content of non-enzymatic antioxidants viz., flavonoids, ascorbate, glutathione and proline; high amount of thiols compared to *P. alpinus* ("excluder"). Presumably, the most efficient functioning of the antioxidant system favors a better tolerance of plants to the impact of HMs. The study of plants physiological and metabolic functions could help to forecast the adaptive changes of biotic communities to increasing anthropogenic impact upon aquatic ecosystems

P250

Physiological and Biochemical Characters Involved in Drought Tolerance of Wild Tomatoes from Desert Habitat

Gerardo Tapia, Boris Muñoz, Oscar Arrey, Patricia Venegas
Instituto de Investigaciones Agropecuarias, Chillán, Chile

Wild tomatoes habitat include extended regions from humid zones in Ecuador until Atacama desert in North of Chile. The last zone is the habitat of *Solanum chilense* and *Solanum peruvianum*. Both species are adapted in different way to drought, salinity, cold and soil textures. In general they not share ecological niches, because *Solanum peruvianum* only reach until 1500 altitude meters, while *Solanum chilense* grow from sea level until 3500 meters. Differences with cultivated *Solanum lycopersicum* respect to tolerance to drought have been associated to deep roots and small leaves; however, detailed studies have not been realized.

Here we studied ten genotypes of *S. chilense*, *S. peruvianum* and *Solanum lycopersicum* var Money maker under different conditions of drought stress.

Under water restriction Money maker had the lower growth, while a *S. peruvianum* genotype was the highest. The wild tomatoes had reduced foliar area and a variable stomata density, although maintained a low stomata conductance during drought, which was associated with a high leaf temperature. The wild tomatoes showed a higher osmotic potential, which was associated with elevated soluble sugars and proline compared with cultivated tomato. Enzyme activity was dependent of genotype although we observed differences between both wild tomatoes. It was correlated in the majority of genotypes with lower levels of lipid peroxidation.

These results conclude the main differences between cultivated and wild tomatoes *S. chilense* and *S. peruvianum*, suggesting evolution mechanisms associated to drought tolerance.

P251

Physiological Changes and Initial Growth of Plants *Vatairea macrocarpa* (Benth) Ducke Submitted to Water Deficit

Evandro Alves Vieira¹, Maria das Graças da Silva², Daiane Salete Broch Mignoni¹

¹Institute of Botany, São Paulo, Brazil, ²State University of Mato Grosso do Sul, Coxim, Brazil

Drought is one of the major abiotic stresses that influence the distribution of plant species and limit the productivity of ecosystems. The aim of this study was to evaluate the physiological changes and growth in *Vatairea macrocarpa* plants subjected to water deficit. The experiment was conducted at the State University of Mato Grosso do Sul, Brazil. Plants were subjected to the treatments: control group (C) irrigated daily; treated with 75% field capacity-FC (T1), 50% FC (T2) and 25% FC (T3). The relative leaf water content (RWC), leaf water potential (Ψ_w), gas exchange, photosynthetic pigments content, relative content of amino acids and plant growth were recorded. The evaluations were performed within 0, 15, 30, 45, 60, 90 and 120 days. The RWC and leaf water potential decreased concomitant decrease in FC. The rate of transpiration in leaves decreased from 2.03 at time zero to $0.312 \mu\text{mol m}^{-2}\text{s}^{-1}$ at 25% FC. The same pattern was observed for net photosynthesis with a maximum value of $12.83 \text{ m}^{-2}\text{s}^{-1}$ for irrigated group and a significant decrease in T2 and T3 after 30 days. There was a decrease of 37, 86% for chlorophyll a T3 at 120 days compared to the control. Similar pattern was observed for the content of chlorophyll b and total chlorophyll with a considerable increase of carotenoids to the more restrictive treatments. Asparagine and glutamine increased after 45 days and significant increase of histidine and proline occurred after 60 days T3. Differences were observed in biomass allocation and growth, correlating higher values to higher FC soil.

P252

Physiological and Biochemical Changes in Leaves of *Pitcairnia lanuginosa* Ruiz & Pav. (Bromeliaceae): A Desiccation-Tolerance Species

Evandro Alves Vieira, Emerson Alves da Silva, Marcia Regina Braga
Institute of Botany of São Paulo, São Paulo, Brazil

Pitcairnia lanuginosa is a Bromeliaceae species able to survive leaf desiccation. In this study we evaluated physiological and biochemical changes in *P. lanuginosa* associated with the desiccation process and recovery after rehydration. Water stress was imposed to plants for 30 (T1) and 60 days (T2) by complete suspension of irrigation and subsequent rehydration. Control plants (C) were daily watered. We recorded leaf relative water content (RWC), leaf water potential (Ψ_w), gas exchange, quantum efficiency of PSII, content of photosynthetic pigments and carbohydrate reserves. The plants showed a delay in leaf dehydration with decrease in RWC and Ψ_w from the 13th day for T1 and T2, reaching 37.8% and 5.9 MPa respectively after 60 days of water suppression. Photosynthesis rate reached $8.56 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in the watered plants decreasing to $-0.292 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in T2. A significant decrease in chlorophyll content was observed only after 43 days in plants without irrigation followed by an increase in carotenoids. The intensification of the water deficit increased soluble carbohydrates, with predominance of sucrose in irrigated group and glucose and fructose after 38 days of water deficit (T2). The leaf starch content rapidly decreased after 22 days coinciding with increased in the sucrose levels in the same period. We concluded that increase in antioxidant compounds and osmotic adjustment allowed *P. lanuginosa* withstands severe desiccation, maintaining viable structures for metabolic restructuring after rehydration as described for resurrection plants (FAPESP, CNPq)

P253

RNA-Seq Transcriptome Profiling of Bread Wheat under Drought Stress

Birsen Cevher-Keskin¹, Yasemin Tuna¹, Oktay Kulen¹, Selma Onarici¹, Ismail Turkan², Askim Hediye Sekmen², Bayram Yuksel¹, Bugra Özer³

¹TUBITAK, Marmara Research Center, Genetic Engineering and Biotechnology Institute, Kocaeli, Turkey, ²Ege University, Faculty of Science, Department of Biology, Izmir, Turkey, ³TUBITAK, National Research Institute of Electronics and Cryptology, Kocaeli, Turkey

The production of wheat constrained by drought in many regions of the world. To make progress in breeding wheat for drought tolerance is laborious because of the complexity of measuring and quantifying drought traits and other parameters associated with the traits themselves. Identification of novel stress-responsive genes and their role in drought response is an important area for the improvement of the crops. Since the functions of some genes have not been completely identified yet, the knowledge of genes involved in drought response mechanism is still incomplete. Drought-stress related genes were identified for drought tolerant and non-tolerant *Triticum aestivum* (bread wheat) cultivars after different drought stress treatments by RNA Seq (Illumina) technology. Fifty-bp Illumina paired-end reads from a transcriptome library was constructed from hexaploid wheat poly (A) RNA. De novo assembly for 311 Gb was applied to short-read transcriptome data. For comparative bioinformatics analysis, de novo assembly was used as a reference genome. Root proteome profiling was performed by LC-MS/MS to get a lot of details of the molecular mechanisms of bread wheat in response to drought and show complementarities between two levels of cellular organization. Responses of antioxidative defence system -the activities of antioxidant enzymes and peroxidation levels of lipids in cell membranes (TBARS content) of drought tolerant and non-tolerant cultivars were also investigated for different drought stress. All these analyses will allow us to get a better idea about the possible role of these genes in the drought-response mechanism.

P254

Differences in Acclimatization to High Light of the Photosynthetic Apparatus in C4 Plants of NADP-ME Type

Alicja Buczynska, Elzbieta Romanowska

Department of Molecular Plant Physiology, Faculty of Biology, Warsaw University, Warsaw, Poland

In C4 plants the photosynthetic apparatus is partitioned over two cell types called mesophyll (M) and bundle sheath (BS) which have different structure and specialization of the photosynthetic thylakoid membranes. Excess light leads to photoinhibition and decline of photosynthetic efficiency. We examined the chloroplasts of *Echinochloa crus-galli*, *Digitaria sanguinalis* and *Zea mays* in response to treatment high light leaves from growth conditions, (from 200 to 1600 $\text{mmol photons m}^{-2} \text{ s}^{-1}$). Results show that short-term irradiation of high light caused changes of: fluorescence in room and low (77K) temperature, photochemical activities of PSII and PSI, and electron transport. Phosphorylation of PSII proteins show changes. Highest phosphorylation of the D1 protein was observed in M and BS chloroplasts of *E. crus-galli*, whereas phosphorylation of LHCII was more pronounced in thylakoids of *D. sanguinalis*. It suggest that the phosphorylation level, as well as turnover of photosystem II depend on the structure and metabolic status of thylakoids. TEM microscope show quantitative differences in the amount of grana in BS chloroplasts. Protein complexes revealed no significant changes in organization of the supercomplexes in M and BS thylakoids. Highest PSI activity was in BS chloroplasts of maize and it was two times higher than in other species. PSII activity was 3 - 6 times higher in M than in BS chloroplasts, and was lowest in maize BS chloroplasts. We show the differences in the cell-type specific organization and relative functioning of PSI and PSII. Data suggest existence of different protective mechanisms against photoinhibition in investigated species.

P255

Over-Expression of CaMSRB2 Gene Confers Tolerance to Drought Stress by Maintaining Chloroplast Function in Transgenic Rice

JS Lee¹, HM Park¹, YH Kim¹, SB Lee¹, JS Kim², YK Kim³

¹National Institute of Crop Science, Rural Development Administration, Suwon, Republic of Korea,

²Division of Bioscience and Bioinformatics, Myong Ji University, Yongin, Kyonggido, Republic of

Korea, ³Genomics Genetics Institute, GreenGene BioTech Inc., Yongin, Kyonggido, Republic of Korea

The oxidative modification of amino acids residues, including the oxidation of methionine residues by the reactive oxygen species (ROS) can lead to protein conformational changes and the loss of function. Methionine sulfoxide reductases (MSRs) catalyze the reduction of methionine sulfoxide back to the methionine residue. To assess the role of this enzyme, we generated transgenic rice using a pepper CaMSRB2 gene under the control of the rice Rab21 (responsive to ABA protein 21) promoter. Rice transgenic plants over-expressing the pepper CaMSRB2 gene perform better against drought stress compared to their WT counterparts. Several tests indicate that transgenic lines show less oxidative stress symptoms and a strengthened PSII quantum yield under stress conditions, and increased survival rate and chlorophyll index after the re-watering. The results from immunoblotting using a methionine sulfoxide antibody and nano-LC-MS/MS spectrometry suggest that porphobilinogen deaminase (PBGD), which is involved in chlorophyll synthesis, is a putative target of CaMSRB2. The oxidized methionine content of PBGD expressed in *E. coli* increased in the presence of H₂O₂, and the Met-95 and Met-227 residues of PBGD were reduced by CaMSRB2 in the presence of dithiothreitol (DTT). An expression profiling analysis of the overexpression lines also suggested that photosystems are less severely affected by drought stress. These results suggest that CaMSRB2 might play an important functional role in chloroplasts for conferring drought tolerance in rice.

P256

Pro-Survival Protein Tudor Staphylococcal Nuclease (TSN) is Essential for Structure and Function of Stress Granules in Arabidopsis

Emilio Gutierrez Beltran¹, Panagiotis N. Moschou¹, Andrei P. Smertenko², Peter V. Bozhkov¹
¹*Department of Plant Biology, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, SE-75007, Sweden, Sweden,* ²*Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA, USA*

Tudor staphylococcal nuclease (TSN) is an evolutionarily conserved protein found in all eukaryotic lineages. Animal TSN (also known as SND1 and p100) is a well-characterized component of several post-transcriptional processes, such as mRNA splicing and silencing. Molecular functions of plant TSN remain elusive, although it is essential for normal plant development and stress tolerance. Stress response induces formation of specialized messenger ribonucleoprotein (RNP) complexes known as stress granules (SG) and processing bodies (PB), sites of post-transcriptional gene regulation. We have established a paradigm for studying dynamics of SG during heat stress response in *Arabidopsis thaliana*. Assembly of SG is initiated immediately upon heat stress, whereas disassembly takes several hours. We show that TSN is a component of both SG and PB, essential for the maintenance of structural integrity of these complexes. TSN associates with SG post assembly where it is likely to perform a scaffolding role. This stage of SG formation requires dynamic microtubules, since it is inhibited by reduction or stimulation of microtubule stability. N-terminal region of TSN encompassing tandem repeat of four SN domains and possessing nucleolytic activity is sufficient for SG-specific localization and pro-survival function of TSN, whereas C-terminal Tudor domain is functionally redundant. Our findings establish TSN as an integral component of plant SG, providing mechanistic explanation for the role of TSN in stress tolerance.

P257

Different Inductions of the Detoxification System in Poplars Due to Copper and Zinc Treatments

Ágnes Gallé, Dániel Benyó, Tünde Leviczky, Kinga Takács, Irma Tari, Laszlo Erdei, Jolán Csiszár
Department of Plant Biology, University of Szeged, Szeged, Hungary

The physiological stress responses and effective acclimatisation are usually characterized by the activation of certain groups of genes, which are important targets of researches to help even the breeding process. Heavy metal (HM) stresses can result in the accumulation of toxic metabolites in plants, which are mainly eliminated by different types of glutathione transferases and ABC (ATP Binding Cassette) transporters. The heavy metal-binding ligands, as phytochelatins and metallothioneins, play important roles in the protection against deleterious effects of HMs. Other major mechanisms behind HM toxicity have been attributed to oxidative stress. Our aim was to characterise some parts of the detoxification system, which might play prominent roles in the successful zinc and copper stress acclimatisation processes. Redox properties and ability to generate reactive radicals are different of the two metals.

In our experiments, cuttings of two *Populus deltoides* and one *Populus x canadensis* lines were treated with 3 and 30 μM of the redox active or inactive metal. The high copper concentration caused more drastic effects than zinc treatment. According to our results, the two *Populus deltoides* lines have more effective detoxification mechanism (quick inductions in the metallothionein and ABC transporter transcript amounts) than the *P. x canadensis* line, in which the investigated transcripts exhibited lower changes and higher malondialdehyde levels. Based on our research, *P. deltoides* clones are recommended to be utilized for phytoremediation purposes on heavy metal contaminated sites.

This work was supported by the Hungarian National Scientific Research Foundation (OTKA K 105956).

P258

The Impact of Salinity Stress on Photosynthetic Apparatus and Hormone Levels in Genus Triticum

Ivana Safrankova¹, Radomira Vankova², Jana Kujalova³

¹University of Hradec Králové, Hradec Králové, Czech Republic, ²Institute of Experimental Botany AS CR, Prague, Czech Republic, ³University of Hradec Králové, Hradec Králové, Czech Republic

Salinity represents worldwide spread abiotic stress, significantly limiting crop yields and agricultural production. One of the most widely grown crops is wheat. This crop represents a major resource for food and feed, especially in Europe and North America.

The aim of this study has been to evaluate the effect of salinity (150 mM NaCl) on photosynthetic performance and hormonal pools in wheat (*Triticum aestivum* L.) cv. 'Sirael'. The effect of exogenous application of abscisic acid (ABA, 10^{-5} M) and 24-epibrassinolide (10^{-9} M) on plant stress tolerance was compared. Both hormones were applied by spraying at the very beginning of the experiment, prior to the evocation of salinity. During the eight-day salt stress, responses of photosynthetic apparatus were followed, together with phytohormone profiling.

Salinity caused strong, transient elevation of ABA content, which was accompanied with mild increase in active cytokinin and auxin levels. ABA supplementation substantially enhanced its endogenous level, which under salinity strengthened the stress response. Simultaneously, active cytokinins transiently decreased. The initial ABA elevation in stress conditions was accompanied by transient decrease of jasmonic acid. Application of 24-epibrassinolide had at the presence of NaCl negative effect on ABA level, but positive effect on active cytokinins. The results indicate that the application of exogenous phytohormones seems to attenuate the negative effects of the salt stress, as indicated by the improvement of the photosynthetic parameters and total chlorophyll. The mechanism of action is, however, different.

P259

Role of Symplast Transfer in Regulation of Plant Root Water Permeability under Low Temperature and Osmotic Stress

Irina Ionenko, Timur Sibgatullin, Nailiya Dautova

Institute of Biochemistry and Biophysics, Kazan Scientific Center of the Russian Academy of Sciences, Kazan, Russia

Information about cell and tissue permeability mechanisms which enable water balance maintenance of plants under unfavorable environmental factors, such as low temperature and water deficiency can be derived from the analysis of temperature dependences of plant water transport. Temperature dependences of water permeability of intercellular transport pathways within the temperature range from 5 to 35 °C in plant roots differing with respect to sensitivity to low temperature and water shortage were measured using the spin-echo NMR method with pulsed magnetic field gradient. To estimate the symplast transfer, non-penetrating into cells paramagnetic complex Gd-DTPA was used for the suppression of apoplast water magnetization signal.

Obtained results point to the presence of thermo induced changes in activation energy (E_a) of water transport along different pathways in plant tissues with different sensitivity to low temperature and water deficiency. For the root samples exposed preliminarily to cold (5 °C, 3 days) and water deficiency (PEG-6000, -0.4 MPa), the discovered differences in the temperature dependences give evidence of the higher stability of the composite water permeability in roots of resistant plants under the temperature changes. Low E_a (17 ± 2 kJ/mol) of transmembrane water transfer in resistant plants points to the channel mechanism (aquaporin mediated) of water transport. Permeability regulation under the temperature changes in roots of the resistant plants results from the symplast permeability regulation (plasmodesmata transition from close to open state).

Acknowledgements: This research was supported by grants № 13-04-01203 from the Russian Foundation for Basic Research

P260

Investigating the Interaction of Metacaspase 5 and DAD1 during ER Stress in *A.thaliana*

Mohamad Zulfazli Bin Mohd Sobri, Patrick Gallois
University of Manchester, Manchester, UK

Many abiotic stresses such as drought cause endoplasmic reticulum (ER) stress, which in turn can induce PCD. Metacaspases have been shown to be involved in PCD in response to various biotic and abiotic stresses. Identification of protein substrates is important to understand Metacaspase mechanism of action. Here we report an anti-PCD protein, Defender against Apoptotic Death-1 (DAD1) as a putative substrate for Arabidopsis Metacaspase 5. DAD1 is part of the glycosylation complex (OST), which resides in the ER membrane. Treatment of DAD1::GFP-overexpressing seedlings with an ER stress inducer, tunicamycin showed that DAD1::GFP was gradually cleaved during PCD, in vivo. Furthermore, we demonstrated that there was a cleavage and degradation of DAD1 protein during incubation with metacaspase 5 and which did not occur when we mutated a cysteine residue in the catalytic site of metacaspase 5. In addition, we found that metacaspase5 is localised in the cytoplasm of the cells.

P261

Strigolactone Analogues as Molecular Probes in Chasing the Receptor/s: Design and Synthesis of Fluorescent Labeled Molecules

Beatrice Lace^{1,2}, Cristina Prandi¹, Ernesto Occhiato³, Helena Rosso¹

¹University of Turin, Department of Chemistry, Turin, Italy, Italy, ²University of Turin, Department of Life Sciences and Systems Biology, Turin, Italy, Italy, ³University of Florence, Department of Chemistry, Sesto Fiorentino, Florence, Italy, Italy

Originally identified as allelochemicals involved in plant-parasite interactions, more recently, Strigolactones (SLs) have been shown to play multiple key roles in the rhizosphere communication between plants and mycorrhizal fungi. Even more recent is the hormonal role ascribed to SLs which broadens the biological impact of these relatively simple molecules. In spite of the crucial and multifaceted biological role of SLs, there are no data on the receptor(s) which bind(s) such active molecules, neither in the producing plants nor in parasitic weeds or AM fungi. Information about the putative receptor of SLs can be gathered by means of structural, molecular, and genetic approaches. Our contribution on this topic is the design and synthesis of fluorescent labeled SL analogs to be used as probes for the detection *in vivo* of the receptor(s). Knowledge of the putative receptor structure will boost the research on analogs of the natural substrates as required for agricultural applications.

P262

P23 Co-Chaperone Modulates Auxin Controlled Root Growth in Arabidopsis

Stefano D'Alessandro, Fiorella Lo Schiavo, Michela Zottini
Department of Biology, University of Padova, Padova, Italy

Homologues of the p23 co-chaperone of HSP90 are present in all eukaryotes, suggesting conserved functions for this protein throughout evolution. Although p23 has been deeply studied in animals, little is known about its function in plants. *Arabidopsis* owns two isoforms of p23 and single knockout mutant lines of the two paralogues show short root phenotype. We demonstrated that the two proteins are both required for auxin-controlled root growth showing no redundancy in this function. The impaired root growth of the knockout mutants is linked with an altered auxin homeostasis. In fact, in the root of mutants a varied auxin distribution, depending on a not working polar auxin transport, was observed. HSP90 plays a dual role in auxin control both in auxin distribution and perception. Our analyses suggest p23 could modulate HSP90-regulated auxin distribution via TWD1-ABCB efflux carrier. We further found that while knockout lines show a short root phenotype the overexpression of the two p23 induces longer root.

P263

Disentangling Who Is Who during Rhizosphere Acidification in Root Interactions: Combining Fluorescence with Optode Techniques

Stephan Blossfeld, Marc Faget, Philipp von Gillhaussen, Ulrich Schurr, Vicky Temperton
Forschungszentrum Jülich, IBG-2: Plant Sciences, Jülich, Germany

Plant-soil interactions can strongly influence root growth in plants. There is now increasing evidence that root-root interactions can also influence root growth, affecting architecture and root traits such as lateral root formation. Both, when species grow alone or in interaction with others, root systems are in turn affected by as well as affect rhizosphere pH. Changes in soil pH have knock-on effects on nutrient availability. A limitation until recently has been the inability to assign species identity to different roots in soil. Combining the planar optode technique with fluorescent plants enables us to distinguish between plant species grown in natural soil and in parallel study pH dynamics in a non-invasive way at the same region of interest (ROI). We measured pH in the rhizosphere of maize and bean in rhizotrons in a climate chamber, with ROIs on roots in proximity to the roots of the other species as well as not-close to the other species. We found clear dynamic changes of pH over time and differences between the two species in rhizosphere acidification. Interestingly, when roots of the two species were interacting, the degree of acidification or alkalization compared to bulk soil was less strong than when roots were not growing in the vicinity of the other species. This cutting-edge approach can help provide a better understanding of plant-plant and plant-soil interactions.

P264

Effects of Wild Boar Grazing on *Olea europaea* L. Cultivar Growth in Mediterranean Ecosystem

Costantina Barbarisi, Carmen Arena, Domenico Fulgione, Anna De Marco
Department of Biology, University of Naples Federico II, Naples, Italy

The wild boar (*Sus scrofa*) grazing represents one of the main disturb for soil and plants in Mediterranean ecosystems. The boar grazing may alter soil structure and function, causing changes in compaction, nutrient cycling and microbial biomass as well as changes in plant community. These effects depend on grazing pressure. Moderate boar rooting can stimulate the nutrient turnover increasing their availability, whereas intensive grazing may be responsible for both decrease of microorganism amount and soil organic matter loss. The goal of this research was to study the seasonal effects of wild boar rooting on an *Olea europaea* L. cultivar widely diffused in the Southern Italy and assess the relationships among rooting impact and soil properties (chemical and biological), olive functional leaf traits and olive fruit characteristics. In general the wild boar activity determined a decrease in organic matter content and cationic exchange capacity (CEC) in soils. In particular, during the summer we observed, an increase of fungal mycelium, microbial biomass and microbial activity in grazing soils compared to ungrazing ones. In addition the olive trees showed leaves with the highest relative water content (RWC), the lowest specific leaf area (SLA) and C/N ratio as well as fruits with reduced pulp/stone ratio, dry matter content, total polyphenols amount and antioxidant activity.

P265

Regulatory Roles of the *Arabidopsis* CRK5 Protein Kinase in Root Gravitropism and Abiotic Stress Responses

Gábor Rigó¹, Ferhan Ayaydin¹, Olaf Tietz², Laura Zsigmond^{1,6}, Hajnalka Kovács¹, Anikó Páy¹, Zsuzsanna Darula⁵, Katalin Medzihradzky F.^{5,3}, László Szabados¹, Klaus Palme², Csaba Koncz^{4,1}, Ágnes Cséplő¹

¹Institute of Plant Biology, Biological Research Center, Szeged, Hungary, ²Institute of Biology II/Molecular Plant Physiology, Faculty of Biology, Albert-Ludwigs-University, Freiburg, Germany, ³Department of Pharmaceutical Chemistry, University of California, California, USA, ⁴Max-Planck Institute für Züchtungsforshung, Cologne, Germany, ⁵Laboratory of Proteomics Research, Biological Research Center, Szeged, Hungary, ⁶Department of Plant Biology, University of Szeged, Szeged, Hungary

Roots are vital organ system of plants due to their involvement in e.g. water and nutrient acquisition, anchorage, propagation and storage functions. Signals such as gravity, light, water, and touch stimulate changes in the direction or rate of growth. Gravitropic responses, directing downward and upward bending of horizontally placed roots and shoots, respectively, are controlled by asymmetric distribution of plant hormone auxin. We recently published a paper about the *Arabidopsis thaliana* CRK5 protein kinase (Rigo et al. 2013, TPC, 25:1592-1608). The CRK5-GFP indicates a unique plasmamembrane localization in root cells. Inactivation of CRK5 protein kinase inhibits root gravitropic response. The auxin efflux transporter PIN2 protein had abnormal localization pattern in *crk5* root cells resulting in delayed gravitropic response. Endocytosis of PIN2 was decelerated by BFA treatment in *crk5* mutant, and CRK5 phosphorylates PIN2 protein *in vivo*. Thus, delayed gravitropic response and deceleration of PIN2 recycling may be explained by inappropriate phosphorylation of PIN2 in *crk5* mutant. Our preliminary results suggest that CRK5 might regulate signaling through reactive oxygen species (ROS). Hydrogen peroxide content levels in *crk5* roots were found to be altered. It is very intriguing that CRK5 might be a novel player of ROS signal transduction.

Acknowledgements. This work was supported by the OTKA Grant K81765 to G.R., TET_12_RO-1-2013-0010 to G.R., H.K. L.Zs. and Á.Cs., and OTKA K68226 and NKFP4-038-04 Grants to L.Sz., and SFB635 and AFGN (KO 1438/12-1) Grants from the Deutsche Forschungsgemeinschaft to Cs.K.

P266

Ethylene is Differentially Regulated during Sugar Beet Germination and Affects Early Root Growth in a Dose Dependent Manner

Willem Abts¹, Bram Van de Poel², Bert Vandenbussche³, Maurice De Proft¹

¹KULeuven, Leuven, Belgium, ²University of Maryland, Maryland, USA, ³SESVanderHave N.V., Tienen, Belgium

A fast and effective growth and development of the primary root after germination is crucial for initial plant growth, especially for crops grown in the field. An important field crop is sugar beet (*Beta vulgaris* L.) which yields 30% of world sugar production. A close relation was found between ethylene production and seedling growth of sugar beet, yet the exact function of ethylene during this developmental stage is still unclear. While ethylene is mostly considered to be a root growth inhibitor, we found that external ACC stimulates root growth in sugar beet in a concentration depend manner, where low concentrations stimulate root growth while high concentrations inhibit root growth. These results reveals that ethylene action during root elongation is strongly concentration dependent. Furthermore our detailed study of ethylene biosynthesis kinetics revealed a very strict gene regulation pattern of ACS and ACO. We found that ACO is rate limiting during early germination and determines the start of ethylene production, while ACS is the rate limiting step during further seedling growth. In conclusion, our results suggests ethylene is an important factor in the process of root elongation. We also showed that ethylene biosynthesis is differentially regulated during germination in which both ACS and ACO have a rate limiting function.

P267

PIP Aquaporins Facilitate Lateral Root Emergence

Jin Zhao¹, Leah Band², Malcolm Bennett², Anton Schäffner¹

¹*Helmholtz Zentrum München, Neuherberg, Germany,* ²*University of Nottingham, Nottingham, UK*

Lateral root (LR) formation to achieve repetitive branching is initiated in pericycle cells located deep within the primary root. LR emergence (LRE) requires the LR primordia (LRP) breaking through the overlaying tissues, in which the cell separation is triggered by the phytohormone auxin. Aquaporins are membrane channels that facilitate water movement across cell membranes. We recently established a link between aquaporin-dependent tissue hydraulics and LRE using a major root isoform, PIP2;1, in *Arabidopsis thaliana* (Péret et al. 2012, *Nat Cell Biol.* 14, 991). To comprehensively understand the role of aquaporins in LRE, the spatio-temporal expression patterns of individual plasma membrane-located aquaporins (PIPs) during LRE were examined using PIPpro:GUS fusions. PIP isoforms expressed in LRP and overlaying tissues have been identified. Modelling predicts that loss-of-function of LRP-expressed PIPs should slow down LRE, whereas mutations of overlaying tissue-expressed PIPs should favor it. Consistent with the prediction, mutation(s) in single, double or multiple LRP-expressed PIP(s) resulted in delayed LRE with PIP2;1 having the strongest impact. In contrast to the model prediction, LRE was also retarded in mutants of PIPs expressed in overlaying tissues. Modifications of the current model will be discussed. Since the numbers of emerged LRs and total LRs were not altered in pip mutants showing delayed LRE, PIP aquaporins are executors, but not regulators of the LR formation.

P268

TRH1 Forms Homodimers Regulating Root Hair Morphogenesis Autonomously of the Intrinsic Developmental Pathway

Gerasimos Daras, Stamatis Rigas, Polydefkis Hatzopoulos
Agricultural University of Athens, Athens, Greece

TRH1 is a member of the AtKT/AtKUP/AtHAK gene family encoding for potassium transporters in Arabidopsis and acts as regulator of auxin efflux facilitator PIN1 localization, controlling root-hair growth and gravitropism. Trichoblasts of *trh1* mutant plants form root hair initiation sites that fail to undergo tip growth resulting in a “tiny root hair” phenotype. Genetic analysis was performed to elucidate the role of TRH1 in root hair development. Crosses were performed between *trh1* and root-hair mutants affecting cell fate or root hair initiation. All double mutants exhibited additive phenotypes suggesting that TRH1 acts independently and developmentally downstream of root hair initiation, participating in auxin homeostasis and signaling processes that control root hair morphogenesis. In addition, to elucidate the function of TRH1, the dimerization assembly was assessed through Bimolecular Fluorescence Complementation (BiFC) and Yeast Two-hybrid system. Upon TRH1-cYFP and TRH1-nYFP co-transformation into tobacco epidermal cells, fluorescence was observed, indicating TRH1 protein homodimerization. Yeast Two-hybrid analysis revealed that the polypeptide region located at the carboxy end of the last predicted transmembrane domain, extending from amino acids R565 to A729, strongly self-interacts. This domain could modulate the co-assembly of TRH1 forming an active K⁺ transport system within cellular endomembrane structures. These results support the central role of TRH1 acting developmentally downstream of root hair initiation as a convergence point between the intrinsic developmental pathway and the environmental/hormonal signaling pathway. The co-assembly could modulate TRH1 sensing properties of external K⁺ to preserve auxin homeostasis ensuring plant adaptation and survival in changing environments.

P269

Molecular Insight into Strigolactone Signaling Network in *Arabidopsis thaliana*

Sylwia Struk^{1,2}, Carolien De Cuyper^{1,2}, Alan Walton^{1,2}, Annick De Keyser^{1,2}, Stephen Depuydt^{1,2}, Fromentin Justine^{1,2}, Belen Marquez Garcia^{1,2}, Lingxiang Jiang^{1,2}, Matthys Cedrick^{1,2}, Goormachtig Sofie^{1,2}

¹Department of Plant Systems Biology, Flanders Institute for Biotechnology, Ghent, Belgium,

²Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium

Strigolactones (SLs) are a class of phytohormones that are negative regulators of shoot branching. They also determine general plant architecture since SLs influence root morphology as well: they have a profound impact on root length by affecting the meristem composition, on root hairs and on lateral root number and length. Hence, they may function as mediators for environmental stimuli to adapt root growth.

Responses to SLs require the F-box protein MAX2 and the α/β -hydrolase D14. Perception of SLs by D14 triggers MAX2-mediated degradation of the target proteins activating physiological responses.

Here, the tandem affinity purification (TAP) technique is used as a unique approach to identify possible target proteins of AtMAX2 and AtD14 as well as downstream SLs signaling components. Preliminary results of MAX2 tandem affinity purification experiments have revealed both general SCF complex components as well as novel MAX2 interactor proteins. Confirmation analyses are ongoing and interesting interactor candidates are further analyzed in more detail at the molecular and physiological level.

Overall, these newly identified interactor proteins will help to better understand how strigolactones are perceived and will improve our knowledge of the strigolactone signaling cascade in *Arabidopsis thaliana*.

P270

Molecular and Physiological Aspects of the Role of Root Hairs during Drought Stress in Barley

Mirosław Kwasniewski¹, Agata Daszkowska-Golec¹, Agnieszka Janiak¹, Karolina Chwiałkowska¹, Michał Słota¹, Steven Timmermans², Iwona Szarejko¹

¹*Department of Genetics, University of Silesia, Katowice, Poland,* ²*Flanders Institute for Biotechnology and Ghent University, Ghent, Belgium*

The importance of root hairs in water uptake is well embedded in general knowledge, but no experimental data linking the presence of root hairs and a general response of a plant to water deficits, measured on molecular and physiological levels, are available. Here, using series of physiological tests and the global transcriptome differentiation methods in the root hairless mutant and its wild-type parent variety we analyzed the role of root hairs under drought stress conditions. The comparison of transcriptional changes specifically induced by drought in the wild-type 'Karat' variety and in the root hairless mutant *rhl1.a* resulted in the identification of genes that were specifically affected by water deficit in both genotypes. The lack of root hairs during drought results in reduction of water uptake in the mutant, which in turn leads to much higher level of transcriptome changes in the mutant leaves and roots. The processes of cellular defense against ROS, DNA and other macromolecule damage, or the secretion of cytotoxic compounds, are specifically induced in the root hairless mutant, but not in the wild type with normal root hairs. On the other hand, more processes of drought tolerance are actively induced in both, leaves and roots of the wild type. These results are in agreement with the much stronger damage of photosystem II under drought in the *rhl1.a* mutant than in its parent variety. Consequently, our analysis confirms the importance of root hairs in plant survival during drought stress.

P271

Genome Wide Analysis of *Arabidopsis thaliana* Reveals High Frequency of AAAGN7CTTT Motif

Rajesh Mehrotra, Vishesh Jain, Sandhya Mehrotra
Birla Institute of Technology and Science, Pilani, Rajasthan, India

The promoter region of many genes contain multiple binding sites for the same transcription factor. One possible explanation for this is that the individuals with multiple, redundant binding sites have higher fitness. Cis regulatory element multiplicity has been correlated with several gene functionality like promoters containing multiple sites evolve more slowly. Sequence specific elements in DNA regulate transcription by recruiting transcription factors. The Dof proteins are a large family of transcription factors that share a single highly conserved zinc finger. The core to which Dof proteins bind has a consensus AAAG or ACTTTA sequence. These motifs have been over represented in many promoters. We performed a genome wide analysis of AAAG repeat elements increasing the spacer length from 0 to 25. Similar analyses was done with AAAG-CTTT motifs. We report unusual high frequency of AAAGN7CTTT in *Arabidopsis thaliana* genome. We also conclude that there is a preference for A/G nucleotides in spacer sequence between two AAAG repeats.

P272

Genome Assembly of Perennial Ryegrass, *Lolium perenne*

Ewan Mollison^{1,3}, Susanne Barth³, Dan Milbourne³, Linda Milne¹, Claire Halpin², Matthew McCabe⁴, Chris Creevey⁴, David Marshall¹

¹James Hutton Institution, Dundee, UK, ²University of Dundee, Dundee, UK, ³Teagasc, CELUP Oak Park Research Centre, Carlow, Ireland, ⁴Teagasc Animal and Bioscience Research Department, Dunsany, Ireland

Although native to Europe, Asia, and North Africa *Lolium perenne* is widely grown worldwide. Its rapid growth, adaptability, and digestibility make it especially suited to usage as forage for livestock, turf for recreational and soil conservation purposes, and also as a source of biofuel. An assembled genome for *Lolium*, in particular its gene-containing regions, will be of value as a resource for future research into the genetics and breeding of this important forage species. The objective is to assemble a “working draft” of the *Lolium* gene-space that can be used for future genome annotation, study of target gene families, and as a reference sequence for genotyping by sequencing. *De novo* assembly of the *Lolium* genome gave a 1.1 Gbp assembly, with contig N50 of 3,790 bp and scaffolded N50 of 25,212 bp, and with GC content of 44.16%. *Ab initio* gene prediction using Augustus with a maize-based training set identified 144,022 potential gene models within the assembly, and mapping of RNA-seq reads, from 22 experiments, to the genome assembly indicated 67,287 expressed regions. Comparison with barley full-length cDNAs matched 24,027 of 28,620 (83.95%) sequences within the genome assembly, suggesting a good overall coverage of the *Lolium* gene-space.

P273

A High Density Genetic Linkage Map of Perennial Ryegrass Using Genotyping by Sequencing

Janaki Velmurugan^{1,2}, Dan Milbourne¹, Susanne Barth¹, Bridget Lynch², David Marshall³, Linda Milne³, Ewan Mollison^{1,3}, Christine Hackett⁴, Chris Creevey⁵

¹Teagasc, Carlow, Ireland, ²chool of Agriculture and Food Science, University College Dublin, Dublin, Ireland, ³James Hutton Institute, Invergowrie, UK, ⁴BIOS, James Hutton Institute, Dundee, UK, ⁵The Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK

Perennial ryegrass underpins pasture-based production of milk and meat in Ireland and genetic improvement of the species is pivotal in increasing productivity of this system. In animal systems, biotechnology-based approaches such as genomic selection (GS) are revolutionising breeding, and developing and deploying GS-based approaches in grass breeding is a national priority. As a necessary precursor for this strategy, a next generation sequencing (NGS)-based approach for genome-wide surveying of single nucleotide polymorphisms (SNPs) originally developed in cereal species (Elshire et al. 2011) is being adapted for perennial ryegrass. This approach, called genotyping by sequencing (GBS), can identify thousands of SNPs in large numbers of genotypes in a cost and time-efficient manner—a key prerequisite for applying GS in breeding. To validate and illustrate the utility of the GBS approach we have developed a high density genetic linkage map of *Lolium perenne* in an F2 genetic mapping population of nearly 200 genotypes at a fraction of the cost and time than possible using previous methods. In total, 192 individuals from an F2 mapping population of *L. perenne* previously used at Oak Park research centre were used to construct GBS libraries. The methylation sensitive restriction enzyme PstI-6bp frequent cutter was used. Samples were multiplexed and sequenced on two channels of an Illumina HiSeq 2000 platform. Sequencing resulted in 345 million reads with ~1.35 million reads per individual. In total, 2019 SNPs and 7429 presence/ absence variants were identified using a bioinformatics pipeline and a high density genetic linkage map is created using these markers.

P274

PasturebaseIreland - a National Grassland Database

Vincent Griffith, Michael O'Donovan, Laurence Shalloo, Anne Geoghegan
Teagasc, Animal and Grassland Innovation Centre, Teagasc Moorepark, Fermoy, Co Cork, Ireland

The future of an efficient low cost milk production system will depend on low cost feed in the form of grazed grass been converted to milk. On many farms some level of grassland measurement is being completed. To date in Ireland there has been no central database to retain and archive this data for research purposes. The creation of PastureBase Ireland (PBI) in January 2013 addresses this issue. PastureBase Ireland is a web based grassland database which has an inbuilt grassland decision support tool. The aim of the system is to build a large national grassland database which will increase the level of grass utilisation and production at farm level. A key aspect of PBI is that the grassland farmer inputs the data at farm level, through measuring farm grass covers regularly on the farm. A subset of farms using PBI in 2013 was selected for the purposes of this paper, all farms had a high level of grassland measurement to ensure the data used was accurate. The average DM production on the farms was 12.5 t ha⁻¹. There was significant variation in the amount of grass produced between farms ranging from 16 t ha⁻¹ to 8 t ha⁻¹. There was also significant variation between the average number of grazings per paddock achieved between farms ranging from 9.1- 4.1. There is a requirement to understand why such variations in grass growth data, within and between farms are taking place, this will be discussed in this paper.

P275

Monitoring Spring Growth of Perennial Ryegrass *Lolium perenne* Using Thermal Imaging

Tiina M.H. Lynch^{1,2}, Olga M. Grant³, Philip J. Dix², Susanne Barth¹

¹Teagasc, Teagasc, CELUP Oak Park Research Centre, Carlow, Ireland, ²National University of Ireland Maynooth, Biology Department, Maynooth, Co. Kildare, Ireland, ³University College Dublin, Agriculture & Food Science, Belfield, Dublin 4, Ireland

For ryegrass, the most economically important grass species grown in Ireland, growth season begins in February. Monitoring spring growth can provide information on productivity and reveal differences between ryegrass genotypes. Thermal imaging, which is quick and non-invasive, can be used to measure stomatal aperture and therefore provide information on IG values (an index proportional to the leaf conductance to water vapour transfer) and Crop Water Stress Index (CWSI). The objective of this study was to use thermal imaging to compare the stomatal conductance of 30 ryegrass varieties in a cool temperate climate and use IG values as indication of growth. The experiment was carried out at two Department of Agriculture, Food and the Marine (DAFM) field sites in Moorepark farm, Co. Cork and Backweston farm, Co. Dublin, where the late perennial ryegrass trials were sowed in 2011. Thermal imaging was carried out from February to April in 2013 and 2014. Results from previous experiments indicate that since there are differences in IG and CWSI values between ryegrass genotypes, thermal imaging does offer a practical method of observing spring growth. However, the results must be carefully correlated with weather conditions since wind speed, direct sunlight and temperature can all have an effect on the measurements.

P276

Synthesis of CdS Quantum Dots Using Biological Systems: From Bacteria to Plants

A. Yemets², M. Borovaya², A. Naumenko¹, T. Krupodyorova², Ya. Pirko², Ya. Blume²

¹Taras Shevchenko National University, Department of Physics, Kiev, Ukraine, ²Institute of Food Biotechnology and Genomics, Natl. Acad. Sci. Ukraine, Kiev, Ukraine

The development of eco-friendly technologies in material synthesis is of considerable importance to expand their biological applications. Respectively, the synthesis of designer solid-state materials like fluorescent semiconductor nanocrystals by living organisms is an emerging field in nanobiotechnology. The fluorescence color of these nanoparticles depends on their chemical composition, surface chemistry and size. Therefore semiconductor quantum dots (QDs) can be effectively used in biology and biomedicine as an alternative to traditional dyes since they have high levels of photostability and resistance to photobleaching.

Here, we show how bacterial, fungal and plant systems can be exploited to produce luminescent water-soluble CdS QDs. At first CdS QDs were synthesized using *Escherichia coli* and fungus *Pleurotus ostreatus* as biological capacitances. These results represent the first report about obtaining QDs using *Basidiomycetes* fungus. QDs were synthesized by cultivation of the mycelium in presence of 0.25M CdSO₄ and 0.5 M Na₂S. Using optical spectrophotometry, the absorption maximum (453 nm) and luminescent peaks (431, 462 and 486 nm) of QDs were established. By transmission electron microscopy it was found that the size of individual QDs ranges from 5 to 8 nm. The similar method was applied for CdS QDs synthesis using *E. coli*. The absorption maximum of obtained QDs of same size (5-8 nm) corresponded to the wavelength 425 nm and the luminescent peak - 431 nm. We found that obtained QDs were stable after 1 and 3 months. Some of these approaches were transferred for producing of CdS QDs by plant cell and hair root cultures.

P277

***Rosa damascena* Mill. f. *Trigintipetala* - The Role of Tissue Cultures As an Alternative for Production of Plant Metabolites**

Antoaneta Ginova, Violeta Kondakova
Agrobiointitute, Sofia, Bulgaria

Rosa damascena Mill. f. *Trigintipetala*, named Kazanlak oil - bearing rose is a symbolic plant species for Bulgaria with very important economical sense. The production of rose oil is enshrined deeply in Bulgarian traditions. The Bulgarian rose oil is highly appreciated from world perfume companies due to its quality. It is used in food and pharmaceutical industry. It has proven therapeutic properties - anti-HIV, antibacterial, antioxidant activities etc.

The methods for propagation have a key role in maintenance and improving the quality of oil rose. The propagation by tissue culture is such method. Plant tissue cultures are widely used to produce various active compounds. The oil - bearing rose is related to the group of recalcitrant plants. It is very rich of phenolic compounds which cause problems during *in vitro* cultivation - top necrosis, problematic rooting et c. We managed to overcome those problems by the successful introduction of a temporary immersion system of micropropagation - bioreactor "RITA" and optimizing the culture media. We present the optimized media for proliferation and for rooting based on QL medium. In addition we performed metabolic analysis of *in vitro* propagated plants by GC-MS with respect to carbohydrates, amino acids, organic acids, alcohols, phenolic compounds, etc.

Our results will allow effective use of tissue cultures from *Rosa damascena* Mill. for micropropagation and for production of various metabolites.

P278

Application of LED and Fluorescent Light Sources on Common Dandelion (*Taraxacum officinale*) and Purple Coneflower (*Echinacea purpurea*)

Donato Castronuovo, Adriano Sofo, Stella Lovelli, Vincenzo Candido, Antonio Scopa
University of Basilicata, Potenza, Basilicata, Italy

Artificial light supplied by conventional lamps, such as fluorescent lamps, has been positively utilized in the last years, especially for integrating natural sunlight during the greenhouse cultivation of flowers and crops. In the last years, the interest of researchers and business have been moved on the use of light emitting diodes (LEDs) such as an innovative artificial light source. LEDs technology is appreciate to be high efficient and to have a long lifetime. Moreover, LEDs have the possibility to emit light at a specific wavelength and are able to provide precise light quality and quantity to crops. To assess the effect of different artificial light supply on photosynthesis and chlorophyll content of common dandelion (*Taraxacum officinale*) and purple coneflower (*Echinacea purpurea*), an experiment was conducted in controlled conditions using two black chambers equipped respectively with LEDs and fluorescent lamps (fluora). Plants were divided into two groups on the basis of lighting type plus a control with plants kept under natural lighting. Leaf gas exchange and chlorophyll content were recorded before, during and after the light treatments. Results pointed out both the species had a different photosynthetic response to LEDs and fluora. Negative gas exchange performances of common dandelion were recorded on both LEDs and fluora light supply. On the other hand, net assimilation, transpiration and stomatal resistance of purple coneflower were positively affected by fluora lighting. For both the species, SPAD values (correlated to chlorophyll content), after a first increase, has progressively reduced till the end of the experiment.

P279

A Plant-Transferable Transient Expression System in the Deep Sequencing Era

Patrícia Duarte¹, Diana Ribeiro^{1,3}, Inês Carqueijeiro^{1,2}, Francisco Lima^{1,2}, Ana Luísa Silva^{1,2}, Sara Bettencourt^{1,2}, Mariana Sottomayor^{1,2}

¹*IBMC - Instituto de Biologia Molecular e Celular, Porto, Portugal*, ²*Faculdade de Ciências da Universidade do Porto, Porto, Portugal*, ³*Departamento de Biologia, Universidade do Minho, Braga, Portugal*

The post-genome era is changing fast from a situation in which genome/transcriptome information was available only for a limited number of model species, to a scenario where the increasing accessibility to high throughput technologies, e.g. deep sequencing and RNA-seq, is leading to an incredible wealth of information in gene sequences for virtually any species of interest. This means that the study of gene function can now be widened from the previously limited number of plant models to any non-canonical species. However, the study of gene function in any target plant species is still highly hindered by the availability of customized molecular tools. Here, a highly efficient methodology for the isolation and transformation of protoplasts easily adaptable for different plant species is reported. The transient expression system described is straightforward and inexpensive and, being plant-transferable, constitutes an invaluable test-system for the functional characterization of any novel candidate gene in the precise plant species under scrutiny, even if this is a poorly studied species. Transient transformation is a powerful tool to characterize gene and protein functions, protein subcellular localization, *in vivo* protein-protein interactions, etc, and may also be used for silencing experiments. Moreover, it offers the major advantage of generating results within hours or days, compared to weeks or months needed with stable transformation, only accessible for widely studied plants. Therefore, the methodology described here may become strategic for the study of nonmodel plants for which gene information is now easily accessible.

P280

Monitoring Programmed Cell Death of Living Plant Tissues in Microfluidics Using Electrochemical and Optical Techniques

Christina Mark¹, Kinga Zór², Arto Heiskanen², Jenny Emnéus², Martin Dufva³, Christine Finnie¹
¹*Agricultural and Environmental Proteomics, Department of Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark,* ²*Bioanalytics, Department of Micro- and Nanotechnology, Technical University of Denmark, Kongens Lyngby, Denmark,* ³*Fluidic Array Systems and Technology, Department of Micro- and Nanotechnology, Technical University of Denmark, Kongens Lyngby, Denmark*

This project focuses on developing and applying a tissue culture system with electrochemical and optical detection techniques for tissue culture of barley aleurone layer to increase understanding of the underlying mechanisms of programmed cell death (PCD) in plants.

The major advantage of electrochemical detection systems is that they can be miniaturized, multiplexed and automated without losing their performance[1,2]. Combining tissue culture with electrochemical and optical detection allows implementation of a wide range of assays for online, real-time, parallel analysis of important parameters such as redox activity, O₂ and H₂O₂ concentration, pH, cell viability and release of target enzymes such as α -amylase.

We have optimised an intracellular, whole-cell redox activity assay[3] that detects changes in redox activity in barley aleurone layer during PCD. The assay uses a double mediator-system to electrochemically measure redox activity via changes in the NADP:NADPH ratio. Experiments show that redox activity changes depend on phytohormone activation or inactivation of aleurone layer metabolism and subsequent PCD.

We have also successfully detected PCD induced by phytohormones in barley aleurone layer using an optical double-fluorescent probe-system[4].

Currently, we are working on integrating both detection methods into a tissue culture system for immobilised plant tissues.

References

- [1] Xu et al, *Talanta*, 2009, 80, 8-18, 2009
- [2] Sassa et al, *Electrophoresis*, 2008, 29, 1787-1800, 2008
- [3] Heiskanen et al, *Anal Biochem*, 2009, 384, 11-19, 2009
- [4] Fath et al, *Plant Physiol*, 126, 156-166, 2001

P281

EU-OPENSREEN – A European Infrastructure for Enabling Innovative Plant Chemical Biology Research

Torsten Meiners¹, Dominique Audenaert², Ronald Frank¹

¹*EU-OPENSREEN, FMP Leibniz-Institute of Molecular Pharmacology, Berlin, Germany,* ²*Compound Screening Facility (CSF), VIB - Ghent University, Ghent, Belgium*

Plant Chemical Biology applies bioactive small molecules as tools to study cellular networks and developmental processes by selective interference with target activities. While many biological processes and pathways cannot be addressed via genetic means due to gene redundancy and/or embryonic lethality, small molecules can overcome these limitations because their application can be readily dosed and controlled in a temporal and spatial manner.

EU-OPENSREEN is the largest emerging academic Chemical Biology research infrastructure initiative in Europe. As a joint effort of national networks in 16 European countries, EU-OPENSREEN will collaboratively develop novel molecular tool compounds with external users from all life sciences to address challenges in e.g., systems and network biology (directed and selective perturbation of signalling pathways), structural biology (compound-target interactions at atomic resolution), and plant biology (response of wild or crop plants to environmental and agrichemical substances).

EU-OPENSREEN supports all stages of a tool development project, including assay adaptation, high-throughput screening, and chemical optimisation of the 'hit' compounds. All tool compounds and data will be made available to the scientific community.

EU-OPENSREEN integrates high-capacity screening platforms throughout Europe, which jointly use a rationally selected compound collection, comprising up to 300.000 commercial and proprietary compounds collected from European chemists.

EU-OPENSREEN was included in the European ESFRI (European Strategy Forum for Research Infrastructures) Roadmap in 2008 and many national roadmaps. It plans to start operating as a permanent ERIC (European Research Infrastructure Consortium) in 2016. First calls for projects will be released in 2015.

P282

Enzymatic Hydrolysis of Biotin Labeled Polysaccharides Detected via Bio-Layer Interferometry

Lucia Marri, Marie Bøjstrup, Morten M. Nielsen, Finn Lok, Ole Hindsgaul
Carlsberg Laboratory, Copenhagen, Denmark

Starch is the most abundant storage carbohydrate in plants. It consists of 20-30% amylose, a linear polysaccharide of $\alpha(1\rightarrow4)$ -linked glucose units, and 70-80% amylopectin, where approximately 5% of the of $\alpha(1\rightarrow4)$ linked glucose units contains $\alpha(1\rightarrow6)$ -branches.

A pool of hydrolytic enzymes, α - and β -amylases, pullulanase/limit dextrinase and α -glucosidases, acts together in starch breakdown, for the degradation of starch into smaller fermentable sugars.

These starch hydrolytic enzymes find broad applications in industrial processes, as for the production of syrups for food sweeteners or during mashing in brewing. In these processes, a key role for the complete conversion of the branched polysaccharides into small sugars is played by pullulanase or limit dextrinase, $\alpha(1\rightarrow6)$ -debranching enzymes.

Here we describe an assay for limit dextrinase and pullulanase using biotinylated polysaccharides as a substrate. The activity of the hydrolytic enzymes can be monitored by the use of Bio-Layer Interferometry on a FortéBio OctetRED96 system, due to the decrease in size of the immobilized substrate.

Biotin labelled pullulan is selected as the substrate and it is immobilized on streptavidin biosensors. The rate of hydrolysis of the immobilized substrate allows the detection of enzymes activity, both commercial pullulanase (*K. planticola*, Megazyme) and recombinantly expressed limit dextrinase from barley.

The limit dextrinase activity present in barley malt extract can be quantitated by detecting the degree of substrate hydrolysis. The activities in malt samples are compared to existing commercial limit-dextrinase assay proving the reliability of the assay.

P283

Development of Colorectal Carcinoma Vaccines using Plant Expression System

Se Hee Park, Ah Young Kim, Hyun Min Kim, Sang Hoon Ma, Seo Young Park, Ji Sun Park, Mi Jin Jeon, Young Hee Joung*
Chonnam National University, Gwangju, Republic of Korea

Production of vaccine in plants has become an important issue in plant biotechnology field. The GA733-2 is one of the Epithelial Cell Adhesion Molecule and colorectal carcinoma-associated antigen, which is highly expressed in human colorectal carcinoma cell surface. So, the GA733-2 is one of the candidate proteins to development colorectal cancer vaccine. To development of colorectal cancer vaccine in plant, the rGA733-2/rGA733-Fc genes were cloned into plant expression vectors, and introduced into tobacco using stable transformation and transient transformation. To improve the recombinant protein expression, signal peptide and ER retention signal were fused to the rGA733-2/rGA733-Fc gene. We observed that expression level of rGA733-2/rGA733-Fc protein were higher in the transient expressed leaves than the stable transgenic leaves. The recombinant protein expression levels were different between expression vector types and also expression level affected T-DNA length of expression vectors.

Key words: colorectal carcinoma antigen GA733-2, transgenic tobacco, polyclonal antibody against GA733-2, Agro-infiltration

*Corresponding author: Tel 82-62-530-5202, e-mail: yhjyoung@jnu.ac.kr

P284

The Use of Spectroscopic Techniques to Elucidate Metal Homeostasis in *Arabidopsis thaliana* Mutants

Camille Larue¹, Mike Haydon^{1,4}, Hiram Castillo-Michel³, Detlef Rogalla², Hans-Werner Becker², Ute Kraemer¹

¹Ruhr Universität Bochum, Department of Plant Physiology, Bochum, Germany, ²Ruhr Universität Bochum, RUBION, Central unit for ion beams and radionuclides, Bochum, Germany, ³European Synchrotron Radiation Facility, beamline ID21, Grenoble, France, ⁴University of York, Department of Biology, York, UK

To date, the limited accessibility of spatially resolved techniques for the correct determination of metal localization, speciation and concentration has been a major bottleneck in our understanding of plant metal homeostasis. Recent advances in spectroscopic techniques, such as the use of cryo-stages, higher spatial resolution or enhanced sensitivity, increasingly permit to apply them more broadly for addressing biological questions.

In order to identify novel genes of the molecular network achieving zinc and iron homeostasis in plants, we conducted a mutant screen using an EMS-mutagenized ZIF1promoter-GUS line of *Arabidopsis thaliana*. One mutant was selected for the detailed characterization by a multidisciplinary approach combining spectroscopy with molecular biology.

Data will be presented from synchrotron-based micro X-ray fluorescence (μ XRF) experiment on ID21 beamline (ESRF) for the analysis of Fe distribution in both root and shoot of the *Arabidopsis thaliana* mutant in comparison with the wild-type. Fe distribution and also Mn and P distributions were altered in this mutant. In parallel, Fe speciation in situ was recorded by micro X-ray absorption near edge structure spectroscopy (μ XANES). The local elemental quantification will be performed on a nuclear microprobe (RUBION, Ruhr University Bochum) by micro particle induced X-ray emission (μ PIXE) coupled to Rutherford backscattering spectroscopy (RBS). Furthermore, mutant and wild-type seedlings were phenotypically characterized after being grown on different media (basal, depleted of Fe and supplemented with excess Zn).

P285

Fast Estimation of Heat Stability of Plants Using PlanTherm Device

Petr Ilik¹, Martina Spundova¹, Helena Melkovicova¹, Michal Sicner², Martin Trtilek²

¹Palacky University, Olomouc, Czech Republic, ²Photon Systems Instruments, Drasov, Czech Republic

PlanTherm device allows fast and easy estimation of heat stability of plants. A plant segment is immersed in a water bath with deionised water and gradually (linearly) heated (25-70°C, duration approx. 25 minutes). During the heating, changes in the conductivity of the bath are continuously monitored. The measured conductivity-temperature curve enables the determination of the threshold temperature of the leakage of ions from cells, which is taken as a measure of the heat stability of plants. Simultaneous detection of chlorophyll fluorescence intensity (so called minimal fluorescence level - F_0) during the heating provides information on heat stability of primary photosynthetic processes in the plant material. Here, we present a comparison of the heat stability data obtained for various plants using PlantTherm and the traditional method, which is based on preheating and soaking of the samples in water bath with the subsequent conductivity measurement of the bath at room temperature. We also demonstrate an application of PlantTherm for the estimation of both constitutive and inducible heat stability of plants. The PlantTherm device can be viewed in the exhibition stall of Photon Systems Instruments. This work was supported by Grant Agency of Czech Republic (project No. 13-28093S) and by Technological Agency of Czech Republic (project TA01010254).

P286

Identification of Carbon Allocation Pattern in Higher Plants Based upon IR-Spectroscopy

Jennifer Albrand^{1,2}, Heiko Wagner¹, Christian Wilhelm¹

¹University of Leipzig - Department of Plant Physiology, Leipzig, Germany, ²German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Carbon allocation pattern in plants changes in terms of altered cellular protein, lipid and carbohydrate contents and is strongly depending on ecological conditions. Thus growth, development, reproduction as well as defense and acclimation of higher plants are influenced by environmental factors. Though these impact cellular carbon pools can change between structural carbohydrates for growing and biomass production or proteins for housekeeping processes and at least a shift towards carbon storage pools. Besides the different partitioning of carbon pools within cells, the distribution of carbon compounds can be altered also in different plant tissues (e.g. shoot to root distribution). The partitioning of carbon into these different pools and plant tissues can then be used as physiological traits in the context of ecological questions. This may lead to a better understanding of community structure and species interaction with regard to biodiversity analysis especially for field samples. To capture these changes in carbon allocation we used Infrared-(IR)-spectroscopy as a high-throughput method to quantify different carbon compounds in higher plants. With IR-spectroscopy a wide range of compounds can be analyzed like structural and soluble polysaccharides, proteins, lipids, organic acids as well as secondary metabolites (e.g. phenolics and pigments). The applicability of IR-spectroscopy has already been proved for qualitative analysis. However, if using IR-spectroscopy for quantitative analysis of the different carbon pools, the method has to be calibrated, which has been performed by traditionally biochemical methods. Based on these biochemical analysis robust mathematical models have been developed for quantification of these compounds in unknown samples.

P287

Chlorophyll Fluorescence Imaging: A Powerful Tool or a Tightrope Walking

Alicia Perera-Castro, Patricia Brito, J. Roberto Lorenzo, Águeda M^a González-Rodríguez
Departamento Biología Vegetal, Universidad de La Laguna, Spain

Chlorophyll a fluorescence has been extensively used during last decades as an indirect, sensitive and non-invasive measurement of physiological state of plants. In the last years, the development of imaging tools has allowed an entire view of leaf heterogeneity, instead of choosing blindly a small dot which could be not representative of the rest of the leaf, especially under stress conditions. However, the use of this technic requires a suitable use of the instruments which will be reflected in each measured parameter. Despite the availability of a lot of reviews and information about this technic, its tuning is not as simple as it is supposed and can became into a tightrope walking, in which any wrong assumption can involve an spectacular fall.

In this work, we analyze different fluorescence parameters in a high light adapted species using an imaging PAM fluorometer (MINI-Version, blue light). Blue light is used not only for generation of the pulse modulated measuring light, but also for actinic illumination and for saturation pulses. This energetic light must be used carefully in order to avoid potential untoward effects. Determination of standard F_v/F_m values showed lower values than those reached with others conventional fluorometers and R_{fd} values estimated under continuous saturation actinic light resulted in an additional harmful in the leaf. Furthermore, calculation of F and F_m factors in order to correct the effect of both measuring light and LEDs heating, became more relevant when a long uninterrupted use of the instrument is required.

Project CGL2010-21366-C04-04 (MCI)

P288

DNA Methylation within Regulatory Region of the VRN Gene in Contrasting Wheat Genotypes

Anna Dimitrova¹, Ákos Boldizsár², Gábor Kocsy², Kiril Mishev¹, Tatjana Petrova³, Ganka Ganeva¹

¹*Institute of Plant Physiology and Genetics, BAS, 1113 Sofia, Bulgaria,* ²*Agricultural Institute, ATK.*

MTA, 2462 Martonvásár, Hungary, ³*Dobrudzha Agricultural Institute, 9200 General Toshevo, Bulgaria*

Frost is one of the major abiotic stresses that reduce wheat growth and production. Wheat varieties exhibit different sensitivity to low temperature. Some wheat varieties (referred to as “winter type”) require long period of cold treatment (vernalization) to their reproductive development. The vernalization response of wheat is controlled by the allelic variation of VRN-1 gene (designated VRN-1A, VRN-1B, and VRN-1D). The dominant VRN alleles determine spring phenotypes and recessive *vrn* alleles define winter phenotypes. Vernalization is induced by environmental signals and could be controlled by epigenetic mechanisms. Investigations show that low temperature induces variation in DNA methylation pattern as well as in histone methylation of promoter region of VRN-1 chromatin. We hypothesized that short-term cold treatment would lead to reduced levels of DNA methylation in the promoter region of the VRN-1 gene. This study examined the differences in the earliest stages of the DNA methylation pattern of the regulatory region of VRN-1 gene between winter and spring wheat genotypes in response to low temperature. DNA methylation profile of this gene was analyzed by PCR-amplification on bisulfite-treated DNA of wheat varieties carrying diverse VRN-1 alleles, which were submitted or not to a 72 hours cold treatment.

This work was supported by the grant №BG051PO001-3.3.06-0025, financed by the European Social Fund and Operational Programme Human Resources Development (2007–2013) and co-financed by Bulgarian Ministry of Education and Science and by the Bulgarian-Hungarian Joint Academic Reserch Project (25/2010).

P289

Genome-Wide Analysis of Chromatin Changes during Early Flower Morphogenesis

Julia Engelhorn¹, Robert Blanvillain¹, David Pose², Felix Ott², Hugues Parrinello³, Emeric Dubois³, Sabine Nidelet³, Marine Rohmer³, Markus Schmid², Cristel Carles¹

¹Laboratoire Physiologie Cellulaire Végétale, CNRS-UMR5168, Université Grenoble Alpes, CEA-iRTSV, INRA, 38054 Grenoble Cedex 9, France, ²Max-Planck-Institute for Developmental Biology, Department VI - Molecular Biology, 72076 Tübingen, Germany, ³MGX-Montpellier GenomiX; c/o Institut de Génomique Fonctionnelle, 34094 Montpellier Cedex 5, France

Activation of organ identity genes at the shoot apical meristem is largely regulated by changes in the chromatin state of the respective loci. We study the dynamics of such activation events in an inducible system for flower development (Wellmer et al., 2006), in which idle flower development can be resumed by dexametasone treatment. We generated a developmental time series starting from leaf tissue and covering the complete process of flower morphogenesis.

In this series, we performed quantitative analysis of repressive (H3K27me3) and activating (H3K4me3) histone marks by ChIP-seq and transcriptome analysis. The generated dataset allows direct, genome-wide correlation of chromatin states and expression levels, with temporal resolution during gene activation processes.

Interestingly, our (so far analysed) ChIP-seq data show that unanticipated chromatin changes accompany gene activation: while changes in H3K27me3 and H3K4me3 levels during flower morphogenesis affect around 4000 and 1200 genes, respectively, we observed only very subtle changes in H3K27me3 after two days of induction, although expression of floral organ identity genes is already strongly elevated. Only 14 genes were significantly changed at this time point. However, more than 100 genes seem to change in H3K4me3 levels and in a more pronounced manner than for H3K27me3. These results suggest that activation of a locus could be predominantly accompanied by deposition of activating marks, while removal of repressive marks occurs later in time. I will discuss how this first dataset, together with identification of transcriptional phases, will contribute to resolve the temporal succession of gene activation events at the chromatin.

P290

Changes in DNA Methylation and *MET1* Expression Accompany Developmental Programmed Cell Death of Tapetum Cells

María-Teresa Solís, María C. Risueño, Pilar S. Testillano
Biological Research Center, CIB-CSIC, Madrid, Spain

The tapetum, nursing tissue inside anthers undergoes cellular degradation by programmed cell death (PCD) during late stages of microspore development. Despite the key function of tapetum, little is known about the molecular mechanisms regulating this process in which profound nuclear and chromatin changes occur; no evidences on the epigenetic regulation of PCD have been reported.

DNA methylation establishes the functional status of chromatin domains. It is accomplished by DNA methyltransferases. *MET1* is a maintenance methyltransferase in plants, also showing de novo methyltransferase activity.

In this work, epigenetic changes during PCD of tapetal cells have been investigated. Dynamics of DNA methylation and expression pattern of *MET1* in relation to cellular changes during PCD have been characterized in *Brassica napus* and *Nicotiana tabacum*.

Results showed that tapetum PCD progresses with increase in global DNA methylation and *MET1* expression, epigenetic changes that accompanied reorganization of the nuclear architecture, high chromatin condensation, activity of caspase 3-like proteases, and cytochrome C release. This data indicates a relation between the PCD process and DNA methylation dynamics and *MET1* expression in tapetal cells, suggesting a possible new role of the epigenetic marks in the nuclear events occurring during this cell death process and giving new insights in the knowledge of the epigenetic control of plant PCD.

SOLÍS MT, CHAKRABARTI N, CORREDOR E, CORTÉS-ESLAVA J, RODRÍGUEZ-SERRANO M, BIGGIOGERA M, RISUEÑO MC, TESTILLANO PS. (2014) Epigenetic changes accompany developmental programmed cell death in tapetum cells. *Plant and Cell Physiology*. 55, 16-29.

Funding by Spanish MINECO (BFU2011-23752) and CSIC (PIE 201020E038).

P291

Characterization of MUT9, a Novel Kinase Family in Arabidopsis

Izabel Moraes¹, Zhen Wang², Heriberto Cerutti², J. Armando Casas-Mollano¹

¹University of Sao Paulo, Sao Paulo, SP, Brazil, ²University of Nebraska-Lincoln, Lincoln, NE, USA

The MUT9 kinase, recently identified in *Chlamydomonas*, is responsible for phosphorylation of histone H3 threonine 3 (H3T3), a modification associated to the chromatin of transcriptionally silent transgenes and transposons. In *Arabidopsis*, four putative MUT9 orthologs have been identified: At2g25760, At3g13670, At5g18190 and At3g03940. We aim to functionally characterize MUT9 genes and understand their roles in the plant life cycle. To achieve these goals, we are: 1) generating promoter fusion lines with GUS and GFP to characterize the temporal and spatial expression of MUT9 genes; 2) analyzing mutants, overexpressed and complemented transgenic lines and 3) characterizing MUT9 biochemical activity. Preliminary results revealed that At3g13670 localizes within the nucleus distributed either as speckles or diffuse signals. Histochemical assays of transgenic plants expressing promoter fusions indicate that At3g13670, as well as At2g25760, are expressed in the vascular tissue of leaves, flowers and roots. Kinase assay revealed that recombinant At3g13670 is able to phosphorylate H3T3 in vitro. Moreover, a T-DNA mutant for At3g13670 displayed a late flowering phenotype suggesting this gene is involved in the regulation of flowering in *Arabidopsis*. Flowering time of the T-DNA mutant and complemented lines is being studied under different photoperiods, vernalization and GA treatments to understand in which pathway At3g13670 participates.

P293

Role and Molecular Mode of Action of the Plant Topoisomerase VI in Response to Oxidative Stress

Cécile Vriet¹, Dorine Achard¹, Christian Breuer², Keiko Sugimoto², Christophe Laloi¹

¹Laboratoire de Génétique et Biophysique des Plantes (UMR 7265), CEA-CNRS-Aix Marseille Université, Marseille, France, ²Cell Function Research Team, RIKEN Center for Sustainable Resource Science, Yokohama City, Japan

Most biotic and abiotic stresses lead to an increased production of reactive oxygen species (ROS) in plants that are involved in plastid-to-nucleus retrograde signaling. In order to identify new signaling components that regulate ROS-responsive gene expression in the model plant *Arabidopsis thaliana*, a genetic approach has been undertaken. One of the mutant identified from this genetic screen is a weak mutant allele of the A subunit gene of the plant topoisomerase VI (TopoVI) (Šimková et al., 2012), a DNA topology modifying enzyme previously shown to be involved in the control of endoreduplication (Breuer et al, 2007). Results of the transcriptome analyses we carried out demonstrate that Topo VI also acts as an integrator of multiple ROS signals generated in response to adverse environmental conditions and eventually regulates the expression of ROS-responsive genes. Interactome studies suggest that this role could be achieved via the direct interaction of TopoVI with the transcription machinery and components of DNA and histone methylation pathways. The functional characterization of these TopoVI interactors is underway. Furthermore, genome-wide chromatin immunoprecipitation and nucleosome positioning assays are being performed to elucidate the molecular mechanisms by which TopoVI, the associated factors, and the resulting chromatin modifications, regulate gene expression changes in response to oxidative stress. Altogether, our results are providing novel insights into the role and molecular mode of action of the plant Topo VI as a transcriptional activator and/or repressor connecting chromatin remodeling with cellular metabolism, stress response, hormonal pathways, and the control of endoreduplication and cell size.

P294

Comprehensive Profiling of MicroRNA Transcriptome in Hot Pepper (*Capsicum annuum*) Suggests the Implication of Epigenetic Regulation and Plant Defense

June Hyun Park, Jae Yun Lim, Chanseok Shin

Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea

MicroRNAs (miRNAs) are a class of non-coding RNAs approximately 21 nt in length which play important roles in regulating gene expression in plants. Although many miRNA studies have focused on a few model plants, miRNAs and their target genes remain largely unknown in hot pepper (*Capsicum annuum*), one of the most important crops cultivated worldwide. Here, we employed high-throughput sequencing technology to identify miRNAs in pepper extensively from 10 different libraries, including leaf, stem, root, flower, and six developmental stage fruits. Based on a bioinformatics pipeline, we identified 29 and 35 families of conserved and novel miRNAs, respectively. Northern blot analysis was used to validate further the expression of representative miRNAs and to analyze their tissue-specific or developmental stage-specific expression patterns. Moreover, we computationally predicted miRNA targets, many of which were experimentally confirmed using 5' rapid amplification of cDNA ends analysis. One of the validated novel targets of miR-396 was a domain rearranged methyltransferase, the major de novo methylation enzyme, involved in RNA-directed DNA methylation in plants. In addition, our results suggested that interaction between miR-482 family and disease resistance proteins is likely to serve as a conserved trigger for plant defense mechanism. This work provides the first reliable draft of the pepper miRNA transcriptome. It offers an expanded picture of pepper miRNAs in relation to other plants, providing a basis for understanding the functional roles of miRNAs in pepper.

P295

Transcriptome-Wide Analysis of Rice Non-Coding RNAs Involved in Nitrogen-Starvation Stress Response and Nitrogen Use Efficiency

Sang-yoon Shin¹, Jae Yun Lim¹, Jin Seo Jeong², Ju-Kon Kim², Chanseok Shin¹

¹Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea, ²Seed Biotechnology Institute, Green Bio Science and Technology, Seoul National University, Pyeongchang-gun, Kangwon-do, Republic of Korea

Nitrogen is a key component of crop plants growth. For increasing the crop yield, the enormous amount of nitrogen source-containing fertilizer is used, which increases the total production cost and leads to environmental pollution caused by residual nitrogen source which crop doesn't take up. For these reasons, many researchers are considering the increase of crop's nitrogen use efficiency as a solution for reducing the amount of used nitrogen fertilizer. Both microRNA and long non-coding RNA are a class of regulatory non-coding RNAs regulating the expression of target genes. Recent studies showed that the expression pool of both microRNA and long non-coding RNA was changed in response to nutrient-deficient conditions. In this study, we aim to identify and characterize rice non-coding RNAs, of which expression changes in response to condition of nitrogen starvation and supplement. We performed RNA-Seq and small RNA-Seq for nitrogen-deprived and re-supplied rice samples to investigate plant responses in a whole transcriptome level. We observed the expression of some genes involved in nitrogen assimilation was changed in a deprivation and re-supplied time-dependent manner. We also found that the expression of considerable numbers of microRNAs and long non-coding RNAs was dynamically changed in a nitrogen supply state-dependent manner, and especially, the expression level of some genes targeted by up- and down-regulated microRNAs was changed in a microRNA-dependent manner. Our study suggests that non-coding RNAs may have roles in regulating the response of rice to nitrogen supply state and modulating nitrogen use efficiency.

P296

DMR-RDA: Identifying Differentially Methylated Regions in Non-Model Systems using Representational Difference Analysis

Pavlina Sasheva Naydenova^{1,2}, Iliana Ionkova², Ueli Grossniklaus¹

¹*Institute of Plant Biology, University of Zurich, Zurich, Switzerland*, ²*Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria*

Thracian flax (*Linum thoricum*, Linaceae) endemic to the Balkan peninsula can synthesise the aryltetralin lignan podophyllotoxin (PT). PT is a plant secondary metabolite with a broad spectrum of medical applications, including that as an anticancer and antiviral agent.

Introducing the Thracian flax into tissue cultures caused a time-dependent increase in the PT level, which was additionally accompanied by dramatic changes in the ratio of PT to the closely related 6-methoxy podophyllotoxin (6-MPT), which is derived from the same precursor. The observed shift in the PT/6-MPT ratio mirrored the lignan profiles of the root-leaf axis of the mother plant, which may be linked to epigenetic changes of cells grown in vitro.

To investigate possible epigenetic changes occurring in tissue culture, we established a Differentially Methylated Regions - Representational Difference Analysis (DMR-RDA) method for plant genomes. RDA is a powerful method allowing the identification and isolation of genomic differences. In combination with the use of methylation-sensitive enzymes, DMR-RDA is a PCR-driven subtractive hybridisation technique that is designed to kinetically enrich the differentially methylated CG-rich regions. DMR-RDA is a robust and cost-effective technique regardless of the genomic information availability and genome complexity.

We are currently testing a hybrid protocol, bringing together the DMR-RDA and the Suppression Subtractive Hybridisation (SSH) techniques, promising to be an effective and time-saving approach with increased performance.

P297

MSAP-Seq: Direct Sequencing of MSAP Markers for Fast and Reliable Analysis of Methylation Polymorphism in Species with Large Genomes

Mirosław Kwasniewski, Karolina Chwiałkowska, Urszula Nowakowska, Iwona Szarejko
Department of Genetics, University of Silesia, Katowice, Poland

DNA cytosine methylation is a heritable epigenetic modification that links DNA sequence and transcription regulation. DNA methylation plays an important role in plant development, contributes to genome stability. It may be influenced and changed by environmental conditions, thus it can play a role in adaptation and evolution. The distribution of cytosine methylation is highly variable within plant species. Moreover, tissue-dependent variation in methylation profiles was observed, although its specific role in plant development is largely unknown. Therefore, analyses of global DNA methylation profiles and their developmental or environmentally induced variations, coupled with other genome-scale functional genomics studies have been conducted recently to assess the role of DNA methylation in a plant genome. Several methods of DNA methylome analysis are currently used in plant epigenomics, including genome-scale bisulfite sequencing. However, this most advanced, direct approach is feasible only for model species with small genomes - for other species, including cereal crops, indirect techniques of DNA methylome analysis are widely used. One of these is a simple, but reliable Methylation Sensitive Amplified Polymorphism (MSAP) method, that is particularly useful in studies of methylation variation. Here we propose a great improvement of MSAP method, where the conventional separation of MSAP amplicons on polyacrylamide gels is replaced by their direct sequencing using Next Generation Sequencing methods and automated data analysis (MSAP-Seq). Detailed analysis of variation in DNA methylation profiles caused by drought stress in barley carried out with our MSAP-Seq method reveals its usefulness in studies of methylome polymorphism in crops with large genomes.

P298

Climate, Soil and Management Effects on Ecosystem Carbon Balance in Grazed Temperate Grasslands

Orlaith Ni Choncubhair¹, James Humphreys², [Gary Lanigan](#)¹

¹Teagasc Johnstown Castle, Wexford, Ireland, ²Teagasc Moorepark, Cork, Ireland

Temperate grasslands constitute over 30% of the Earth's biomes and make an important contribution towards the mitigation of anthropogenic greenhouse gas emissions. In managed pasture systems, carbon balance is strongly influenced by climate, soil type and management activities, such as harvests, grazing events and the deposition or application of organic matter. In this study, the controls and drivers of carbon dynamics in grazed and cut grasslands in Ireland were examined. The sites experience similar temperate climatic regimes but differ in soil texture, water drainage and management intensity (N fertilisation and biomass removal from grazing and cutting). Eddy covariance measurements of net ecosystem exchange of carbon were complemented by regular assessment of standing biomass, leaf cover, harvest exports and organic amendment inputs. Management practices during the active growing season exerted a strong influence on both the direction and the rate of C exchange in the grassland systems, with a strong dependence on the timing and intensity of the management effect. Reductions in productivity and net C uptake were generally greater for harvested as opposed to grazing-induced removals. Seasonal and inter-annual shifts in weather patterns, especially during dry summer periods were observed to particularly impact on the balance between gross primary productivity (GPP) and total ecosystem respiration (TER) at the poorly drained sites. The potential of vegetation indices as a means of tracking sward development will also be investigated, with the aim of improving our understanding of the impact of vegetation dynamics on measured ecosystem carbon fluxes.

P299

Estimated Carbon Sequestration Benefit under Climate Change: A Case Study of the Mediterranean Shrublands

Loretta Gratani, Rosangela Catoni, Carlo Ricotta, Laura Varone
Department of Environmental Biology, Sapienza University of Rome, Rome, Italy

Climate projections in the Mediterranean region point to a potential acceleration of climatic trend, with rising air temperatures (1-6 °C) in the 21st century and a precipitation amount decrease by 22% from April to September. Climate change could significantly affect carbon (C) acquisition in Mediterranean areas considering that spring rainfall is more important than total annual rainfall. Climate change could make these ecosystems more vulnerable because in contrast to forests growing on comparable soils, shrubs are less capable of exploring deeper soil layers for water. The contribution of Mediterranean ecosystems to the global carbon cycle has received low attention in the past and few estimates have been carried out to date. Species, composition and structure of the Mediterranean shrublands developing along the *Latium* coast (Italy) were analyzed in order to evaluate their contribution to C sequestration, also taking into consideration the economic benefits at a national level. The total yearly CO₂ sequestration was calculated multiplying the total photosynthetic leaf surface area of each species by the mean yearly photosynthetic rate and the total yearly photosynthetic activity time (in hours). Taking into account the frequency of species, the total CO₂ sequestration of the Mediterranean maquis was quantified by 80 Mg CO₂ ha⁻¹year⁻¹, corresponding to 22 Mg C ha⁻¹year⁻¹. From a monetary point of view, this quantity could be valued to more than 500 US\$ ha⁻¹ year⁻¹. Extending this benefit to the Mediterranean shrublands throughout the whole country, we obtained a nationwide estimated annual benefit in the order of \$500 million

P300

Soil N₂O Emission and Microbial Activity in a Potato Cropping Fertilized with DMPP-Added Nitrogen Fertilizers

Luca Vitale¹, Anna De Marco², Franca Polimeno³, Giuseppe Maglione³, Paul Di Tommasi¹, Vincenzo Magliulo¹

¹CNR-ISAFOM, Naples, Italy, ²University of Naples Federico II, Naples, Italy, ³CNR-ISAAM, Naples, Italy

In this work we evaluated the effects of 3,4-dimethylpyrazole phosphate (DMPP), a nitrification inhibitor, on N₂O emission and microbial activity of a soil cropped to potato in Southern Italy. The experiment consisted in two treatments applied: control (C) and DMPP plots. The nitrogen fertilizer was supplied in three events: at 0 Day After Sowing (DAS; 100 kg N ha⁻¹), at 57 DAS (30 kg N ha⁻¹), and at 71 DAS (30 kg N ha⁻¹). Soil N₂O emission was monitored by both dynamic and static chambers. Microbial biomass and basal respiration were determined as CO₂ evolution. No statistical difference in N₂O fluxes measured with dynamic chambers between C and DMPP plots was found. However, after the first fertilization event, the average N₂O fluxes measured with static chambers were higher in DMPP plots than in C plots. Microbial biomass significantly decreased in DMPP plots as compared to C plots, whereas an opposite trend for basal respiration was observed. After 57 and 71 DAS the microbial biomass was similar between C and DMPP plots, whereas basal respiration resulted statistically lower in DMPP plots than C plots. During these periods, average DMPP N₂O fluxes were also comparable or lower. Overall, our data evidence a stressful condition for soil microbes and in particular for nitrifiers when a higher DMPP quantity is supplied. On the contrary, when lower quantities of DMPP-added fertilizers are supplied the effectiveness of DMPP in reducing soil N₂O emission is guaranteed by reducing the nitrifiers activity without negatively affecting their growth.

P301

Salinity is an Agent of Divergent Selection Driving Local Adaptation of *Arabidopsis thaliana* to Coastal Habitats

Sílvia Busoms¹, Xinyuan Huang², Kirsten Bomblies³, Charlotte Poschenrieder¹, David E. Salt²
¹Universitat Autònoma de Barcelona, Barcelona, Spain, ²University of Aberdeen, Aberdeen, UK,
³Harvard University, Cambridge, USA

Local adaptation is a well-established driver of population differentiation. Here, we took advantage of the natural variation between inland and coastal populations of *Arabidopsis thaliana* to determine traits for adaptation to these local environments. A Species Distribution Model (SMD) was created and used to locate 45 stands of wild *A. thaliana* in north eastern Spain (Catalonia). To determine if stand genotypes are differentiated by geography we scored 254 genome-wide SNPs in 65 individuals from different 20 stands. Clustering of genotypic data indicated no differentiation by geography. Reciprocal transplant experiments into coastal and inland experimental plots revealed that progeny from coastal stands perform better in a coastal habitat than those from inland stands (local vs. foreign). Further, progeny from coastal and inland stands performed better in their local environment (home vs. away). Similar results were also obtained in greenhouse common garden experiments using soil excavated from coastal and inland experimental plots, confirming that the local adaptation observed in the field is driven by soil factors. We observed a gradient of sodium accumulation in both plants and soils collected from the field, increasing from inland towards the coast. Progeny from coastal stands were also observed to outperform those from inland stands when grown together in hydroponic culture with an elevated concentration of sodium chloride. Taken together our data supports the conclusion that *A. thaliana* is locally adapted to coastal environments, and this adaptation is driven, at least in part, by the elevated salinity of these coastal soils acting as a selective agent.

P302

Root Morphology Adaptation to the Nitrate Supply in *Arabidopsis* Natural Populations

Jérôme De Pessemier¹, Pascal Tillard², Philippe Nacry², Jim Haseloff³, Malcolm Bennett⁴, Christian Hermans¹

¹Université Libre de Bruxelles, Lab Plant Physiology and Molecular Genetics, Brussels, Belgium,

²Université de Montpellier II, Biochimie & Physiologie Moléculaire des Plantes, UMR CNRS-INRA-SupAgro, Montpellier, France, ³University of Cambridge, Biology Dpt, Cambridge, UK, ⁴University of Nottingham, Plant Sciences Division, School of Biosciences, Nottingham, UK

Arabidopsis thaliana has a broad geographical distribution and consequently is subject to varying environments which makes it a useful model for studying adaptation and selection. The natural populations which grow on a wide range of soil conditions could provide a rich source of genetic diversity to explore potentially adaptive differences in root architecture in response to nitrate availability. We described the root phenotypes of a core collection of 24 accessions grown *in vitro* upon contrasted nitrate supplies (10 μ M and 10 mM). That study illustrated that natural variation existed within *Arabidopsis* for root traits (primary root length, number and length of lateral roots), which were primarily genetically determined (*Mech. Dev.* 130: 40-53). Differences between accessions were somewhat more pronounced at low than at moderate N supplies. In addition, nitrate uptake was measured using ¹⁵N tracer in order to corroborate if accessions with a highly branched root system have higher N uptake efficiency. Interestingly, no robust correlation was found between root traits and N uptake. The identification of root morphology ideotypes in the N response was the foundation for further analysis of quantitative traits for root morphology. Currently, we are fine-mapping several QTL intervals, which were defined by screening recombinant inbred lines generated from the cross between contrasted (Bur-0, Cvi-0, Jea, Nok-1, Oy-0 and Tsu-0) and reference (Col-0) accessions. In parallel, a larger data set was generated with ~350 accessions for genome-wide association studies. The initial output supports the nomination of a number of *loci* identified in the cell cycle and hormonal pathways.

P303

Differences in the Flavonoid Profile of Hungarian Sour Cherry Genotypes

Nora Papp¹, Attila Hegedus², Eva Stefanovits-Banyai¹, Laszlo Abranko^{1,3}

¹Corvinus University of Budapest, Department of Applied Chemistry, Budapest, Hungary, ²Corvinus University of Budapest, Department of Genetics and Plant Breeding, Budapest, Hungary, ³Research Centre for Natural Sciences, Institute of Organic Chemistry, Budapest, Hungary

Sour cherry is a widespread and beloved fruit in Hungary and a huge number of Hungarian cultivars are available for the fruit growers and customers. Sour cherries are rich in natural sources of flavonoids and phenolic acids. In order to be able to study the real health beneficial properties of these polyphenols it is essential to get detailed information on the actual chemical forms they present in the sample. Nowadays, liquid chromatography-mass spectrometry (LC-MS) based analytical techniques are the most preferred methods for polyphenol profiling.

In this work, we studied 13 Hungarian sour cherry genotypes to identify the differences in their flavonoid profiles using high performance liquid chromatography-time-of-flight mass spectrometry (HPLC-TOFMS). The analytical approach was based on a previously developed protocol suitable for a comprehensive profiling and tentative identification of intact forms of flavonoid glycoconjugates of various fruits. As an example, we tentatively identified five different quercetin (Que) derivatives. Based on the most abundant quercetin derivative the studied sour cherry genotypes can be classified into two groups. In the first group, the major quercetin derivative is a Que-deoxyhexoside (dH)-hexoside (H), while in another group it is a Que-dH-H-H. Another interesting example is the case of cyanidin (Cy) derivatives. Cy-dH-H is characteristic for one group of sour cherries, while Cy-dH-H-H is the major cyanidin compound in another group.

This study was financed by the OTKA PD 100506 and OTKA K84290 and János Bolyai Research Scholarship grants.

P304

'Stabilstroh': A New Hope for Lodging Resistance

Aleksandra Muszynska¹, Andreas Börner¹, Gilbert Melz², Marion S. Röder¹, Twan Rutten¹, Kirsten Hoffie¹, Marion Benecke¹, Michael Melzer¹

¹Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany,

²Monsanto Saaten GmbH, Nienstädt, Germany

Lodging resistance is one of the main breeding objectives in crop production, especially in rye (*Secale cereale* L.), where yield losses due to lodging can be as high as 75%. 'Stabilstroh', a recently identified genotype of rye, has the best lodging resistance and simultaneously it is characterized by the longest tillers among the German cultivars of rye.

The transverse sections of the basal internodes of segregating F₂ population and its parental lines: 'ms135' ('Stabilstroh') and 'R1124' (wild type) were analysed using Light Microscopy, Scanning Electron Microscopy, and Transmission Electron Microscopy in order to identify traits responsible for lodging resistance. Analyses of tissue distribution, cell size, and cell wall thickness revealed that the 'Stabilstroh' genotype has a significantly higher sclerenchyma/parenchyma ratio while both sclerenchyma cell walls and inner periclinal cell walls of the epidermis are significantly thickened in comparison to the wild type. Phloroglucinol staining confirmed a higher content of lignin in 'Stabilstroh' tillers. Invaginations of the stem, an important factor enhancing mechanical stability, were also much more abundant and pronounced in the genotype 'Stabilstroh'. It is expected that these traits are significantly improving lodging resistance and biomass production in rye.

Currently the F₂ population and its parental lines are being genotyped using microsatellite (SSR) markers. The QTL-mapping will be based on the inheritance of traits affecting mechanical stability of tillers (stalk invaginations, thickness of cell walls, lignin content, and sclerenchyma/parenchyma ratio).

P305

The Site of Oil Synthesis Changes along the Leaf Development in the Diesel-Tree (*Copaifera langsdorffii*, Leguminosae)

Tatiane Rodrigues, Plácido Buarque, Silvia Machado
Universidade Estadual Paulista- UNESP, IBB, Botucatu, São Paulo, Brazil

Copaifera langsdorffii, popularly named diesel-tree, is an economically important legume in Brazil. The oils produced in leaves and stem are exploited by drugs, cosmetic and biodiesel industries, and protects the plant against herbivores and pathogens. Studies showed that the chemical composition of the diesel-tree's oil changes during the leaf development. We aimed to study the structural features of oil production during the development of leaves in *C. langsdorffii* along different seasons. For this, we applied conventional techniques in plant anatomy and transmission electron microscopy. In the Brazilian cerrado, *C. langsdorffii* leaves arise in the beginning of spring and drop in the winter. Actively secreting oil cavities, constituted by uniseriate epithelium and lumen, were present in the mesophyll since the leaf primordium stage; such glands exhibited typical cell machinery of oil synthesis such as abundant smooth endoplasmic reticulum and elaioplasts. The cavities remained active until the fully expansion of the leaves during the summer. Mature leaves in spring and summer showed mesophyll parenchyma cells with dense cytoplasm and typical chloroplasts. In a later developmental stage of the leaves, during the autumn, the epithelial cells ceased their secretory activity and showed an empty aspect. Simultaneously, the chloroplast of the parenchyma cells in the mesophyll changed to elaioplasts producing large amount of oils. Lipid bodies were abundant and filled the vacuoles and periplasmic and intercellular spaces of the mesophyll. These findings are novelty for this species and open new avenues to study the natural variations in the oil synthesis along the leaf development.

P306

Variation of Morpho-Physiological Characters in *Sambucus nigra* L. Populations Growing in Portugal

Eunice Bacelar¹, Berta Gonçalves¹, Ermelinda Silva¹, Helena Ferreira¹, José Moutinho-Pereira¹, Carlos Correia¹, Fernando Nunes²

¹CITAB, Universidade de Trás-os-Montes e Alto Douro, UTAD, Vila Real, Portugal, ²CQVR, Universidade de Trás-os-Montes e Alto Douro, UTAD, Vila Real, Portugal

Recently, there has been a growing interest in alternative plants. Elderberry (*Sambucus nigra* L.), has high potential as an alternative culture since almost all its parts can be used as a food source, as flavouring or a food dye, or in traditional medicine. In Portugal, the elderberry cultivation is mainly found in the Varosa Valley (Douro-Sul region). This region is surrounded by several mountains and has excellent edaphoclimatic conditions for the development of this species. A three-year field experiment is being conducted in a mature commercial elderberry orchard to compare and characterize the leaves and fruits of 18 elders belonging to 3 different populations of the Varosa Valley known as 'Sabugueira', 'Sabugueiro' and 'Bastardeira'. Morpho-physiological traits including thickness of the mesophyll tissues, relative water content, specific leaf area, succulence and the contents of epicuticular waxes, total proteins, total soluble sugars, starch, total phenols, photosynthetic pigments and thiobarbituric-acid-reactive substances (TBARS) were evaluated. In the first two years of the experiment significant differences were found between the leaves of the 3 populations, namely in epicuticular waxes, photosynthetic pigments, total phenols, total proteins and TBARS contents. In general, trees of 'Bastardeira' seem to be the more adapted to the edaphoclimatic conditions of the region since their leaves are more protected against dehydration and their berries had high nutritional value.

Aknowledgments: funding by Project QREN 23109 I&D co-promotion, financed by Fundo Europeu de Desenvolvimento Regional (FEDER) through ON2 - Programa Operacional Regional do Norte (PO Norte), managed by Agência de Inovação (ADI)

P307

Photosynthesis Peculiarities of Some Halophytes of the White Sea Coast

Evgenia Markovskaya¹, Anatoly Kosobrykhov²

¹Petrozavodsk State University, Petrozavodsk, Russia, ²Institute of Fundamental Problems of Biology, Russian Academy of Sciences, Puschino, Russia

The work was conducted on the obligate halophytes *Acter tripolium*, *Triglochin maritimum*, and *Salicornia europaea* in the intertidal zone of the White Sea (64°14'N 35°52'E) in July during low tide. The photoperiod was 22 h, light intensity 1200-1400 $\mu\text{mol m}^{-2}\text{s}^{-1}$, air temperature (day/night) 25-28/18-20°C. Analysis of the photosynthetic apparatus activity (measured with the LCpro+Portable Photosynthesis System) at saturating light intensity with the use of Farquhar model have shown that *A.tripolium* had the highest net photosynthetic rate (Pn), electron transport rate (ETR), and the rate of triose phosphate utilization, but lowest CO₂ compensation point compared to other species. There was no difference between species in maximum RuBPCO activity, but its efficiency was higher in *T.maritimum*, which had high values of CO₂ compensation point and dark respiration. *S.europaea* had low values of Pn and ETR, similar to *T.maritimum*, and low values of carboxylation efficacy and dark respiration similar to *A.tripolium*. Comparative study of this three species has revealed that *A.tripolium* leaves at high latitudes have more active photosynthetic apparatus compared to *T.maritimum* and *S.europaea*. Supported by RFBR project 14-04-01008-a.

P308

Functional Limits of Plants in the Arctic

Evgenia Markovskaya¹, Natalja Shmakova²

¹*Petrozavodsk State University, Petrozavodsk, Russia,* ²*Polar Alpine Botanical Garden – Institute, Kola Research Centre, Russian Academy of Sciences, Apatity, Russia*

When moving towards higher latitudes, plant size, biomass, pigment content become smaller. Of particular interest are plant functional limits. Research objects were the annual plants *Koenigia islandica* and *Euphrasia frigida* on Spitsbergen Archipelago. Plants of *K.islandica* were 2-3 cm of height with fresh weight (FW) 26 ± 2 mg/plant. Total chlorophyll (chl) content was 0.83 ± 0.03 mg/gFW, carotenoids (car) content - 0.29 ± 0.02 mg/gFW; chl/a/b - 2.2, chl/car - 2.9. *Eu.frigida* is a rare species that can be found sporadically. Plant height was 2.0 ± 0.5 cm, FW 7.0 ± 0.8 mg/plant. Total chl content was 0.39 ± 0.03 mg/gFW, car content 0.18 ± 0.03 mg/gFW, chl/a/b - 1.1, chl/car - 2.1. The obtained data were compared with the data for other common species of Spitsbergen flora. Obtained data were similar to the low values of chl content of *Saxifraga cespitosa* and *Silene acaulis* (0.39 and 0.44 mg/gFW). This suggests that the minimum limit for total chl content of vascular plants in West Spitsbergen is 0.4 mg/gFW, which can provide the functional integrity of the photosynthetic apparatus and ensure ontogenetic development in the Arctic. Supported by RFBR project 14-04-01008-a.

P309

Analysis of the Genetic Diversity and Structure in a Collection of Pepper (*Capsicum annuum*) Landraces from Spain

Susana Gonzalez-Perez¹, Ana Garcés-Claver², Cristina Mallor², Oreto Fayos², Fuencisla Merino¹, Federico Pomar¹, Cristina Silvar¹

¹Universidad da Coruña (Facultade de Ciencias), A Coruña, Spain, ²Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain

Pepper (*Capsicum* spp.) is one of the most important crops in the family Solanaceae. *Capsicum annuum* is the most widely cultivated species and it represents the main subject of breeding programs. In the majority of growing regions, high-yielding commercial cultivars have replaced traditional landraces. However, these cultivars possess a reduced genetic basis, which make them more susceptible to biotic and abiotic stresses. Therefore, increasing the genetic variability within commercial cultivars appears compulsory. Genetic resources stored at germplasm banks constitute a valuable source of natural variation that could be incorporated into elite varieties. The aim of the present work was to analyze the genetic diversity and structure of a collection of Spanish peppers as well as to investigate their relationship to a group of worldwide accessions. Fifty-two *C. annuum* accessions were selected from the BGHZ (Banco de Germoplasma de Horticolas de Zaragoza). The collection was genotyped with a set of fifty-five microsatellites markers previously described in the literature. The thirty-three informative loci amplified 151 alleles in the whole collection, with an average of 4.57 alleles per locus. Fifty-two alleles were recorded as rare alleles (those with frequencies <5%) and sixty-seven alleles were considered private alleles (specifically present in only one group). Cluster and STRUCTURE analysis showed a clear differentiation between worldwide and Spanish annuums. Although clear cut-offs could not be established, the pattern of clustering within Spanish accessions seems to be related to fruit shape morphology and organoleptic traits, such as pungency.

Acknowledgments: this work was funded by MICINN-INIA-FEDER (project RTA2011-00118)

P310

Chemical and Geographical Diversity of Wild Lavender (*Lavandula angustifolia* Miller)

Yolande Despinasse¹, Sandrine Moja¹, Catherine Soler¹, Cyrille Conord¹, Frédéric Jullien¹, Bernard Pasquier², Jean-Marie Bessière³, Sylvie Baudino¹, Florence Nicolé¹

¹Université de Lyon-Laboratoire BVpam EA2061, Saint Etienne, France, ²CNPMAI, Milly-la-Forêt, France, ³ENSCM, Montpellier, France

Lavandula angustifolia Miller is a Mediterranean aromatic shrub belonging to the Lamiaceae family. It produces large amounts of Volatile Organic Compounds (VOCs), particularly terpenoids. Here, we studied the variability of the bouquets of VOCs of fourteen wild populations, originated from all along the geographical range of the species. VOC's were analyzed and quantified using Gas Chromatography coupled to Mass spectrometry. We identified 65 compounds, which represents 60%-92% of the total extract. Unsupervised clustering method (k-mean) was processed and the 62 accessions were divided into four groups. These groups fitted perfectly with the original localisation of the wild populations. On the basis of these clusters, PLS-DA (Partial-Least Squares-Discriminant analysis) was performed to identify discriminant compounds. The results show that discrimination involved many compounds (36), and is especially explained by strong qualitative differences between clusters. Finally, we found a 1,8- cineol gradient from North to South, where regions exhibit quite different climatic conditions. Since all our individuals were sampled in a common garden to prevent us from any impact of abiotic factors, our results indicate that the highlighted variations are constitutive, i.e. explained by local adaptation rather than by plasticity in VOC emission of the species.

P311

Genetic Analysis of Populations and the Relationships of Species in Genus Tulipa, Subgenus Eriostemones

Natalia Kutlunina

Ural Federal University, Ekaterinburg, Russia

Genus *Tulipa* L. includes more than 100 species, some of them - the well-known ornamental plants. Many species of tulips belong to rare and endangered plants and are listed in the Red Books of different regions. Despite this, the genetic diversity in populations of wild tulips, interspecific hybridization, phylogenetic relationships of the species are not studied enough. We studied 26 populations of species closed to *Tulipa biebersteiniana*, subgenus *Eriostemones* from different regions. Karyological method and flow cytometry identified diploid and triploid level of *T. biebersteiniana*. *T. scythica* and *T. patens* are stably diploids and *T. riparia* is triploid. Allozyme analysis shows high level of polymorphism in populations of tulips. The lowest level of genetic polymorphism is revealed in *T. patens* growing in the Southern Urals in the form of small isolated populations. Populations of tulips have significant differentiation and the most part of diversity belong to interpopular level. *T. patens* shows the highest genetic subdivision of populations and the lowest gene flow. Allozyme and AFLP analysis revealed the genetic separateness of *T. patens* from the other species of the complex. *T. scythica* showed the important geographic differences. Populations from the Volgograd region form a single cluster, the Urals populations are closed to *T. biebersteiniana*. The northernmost populations of *T. biebersteiniana* genetically differed from other populations and according to the AFLP analysis are closed to *T. riparia*.

The reproductive biology of four species was also studied.

The work was supported by The Russian Foundation for Basic Research (grant 13-04-01458).

P312

Perspectives of Using Botanical Species of Pear for Fruit Cultivar Breeding

Ales Matejcek, Jitka Matejickova, Jiri Kaplan, Frantisek Paprstein

Research and Breeding Institute of Pomology Holovousy Ltd., Holovousy, Horice, Czech Republic

Pears are the second most grown pome fruit species from Rosaceae family after apples worldwide. Basically, most of pear cultivars come from species *Pyrus communis* L., *Pyrus pyratser* (L.) Du Roi, *Pyrus bretschneiderii* Redher, *Pyrus pyrifolia* (Burm.) Nakai, *Pyrus ussuriensis* Maxim. Conducted research was focused on observation and evaluation of vegetative and fruit traits of four pear botanical species - *Pyrus betulaefolia* Bunge, *Pyrus nivalis* Jacq., *Pyrus salicifolia* Pall., *Pyrus ussuriensis* Maxim., one subspecies of pear - *Pyrus communis* L. subsp. *Caucasica* (Fed.) Browicz and one intergeneric hybrid of *Pyrus communis* L. and *Sorbus aria* Os. - *Sorbopyrus auricularis* (Knoop) C. K. Schneid. Selected species met the pre-selection assumptions of growth and fertility and were incorporated into the genebank collection of pear cultivars consisted of more than 130 fruit cultivars in natural conditions. *Pyrus communis* L. seedling was used as a rootstock. Considerable differences in vegetative and pomological traits of studied species were determined. The results showed generally high and regular fertility and in particular very good phytosanitary status of selected botanical species of pear, especially regarding resistance to pear rust. Interspecific hybrid showed vigorous growth and very late start of flowering, which was after main flowering time of pear cultivars. *Pyrus nivalis* Jacq. and *Pyrus ussuriensis* Maxim. excelled in fruit firmness and resistance to bruising. Fruits of *Pyrus nivalis* Jacq., *Pyrus salicifolia* Pall. and *Sorbopyrus auricularis* (Knoop) K. C. Schneid. were without russeting of fruit skin. One of the positive features of *Pyrus ussuriensis* is low premature fruit fall.

P313

Evaluation of Potential of Botanical Apple Species for their Use in Fruit Cultivar Breeding

Jiri Kaplan, Ales Matejcek, Jitka Matejickova, Jiri Sedlak, Frantisek Paprstein
Research and Breeding Institute of Pomology Holovousy Ltd., Holovousy, Horice, Czech Republic

Genus *Malus* Mill. is used traditionally for production of fruit. Apples have a big share in the world fruit production. Thirteen botanical species were selected for research purposes from this extensive genus in RBIP Holovousy. Only few of these species contributed to the creation of apple cultivars and rootstocks. Regarding current needs of apple breeding, these botanical species can provide many valuable parental traits. The plantation was included into the germplasm apple collection established on M9 rootstock. All trees of studied species grow vigorously and produce fruit in conditions of experimental locality. Totally, there are more than 1100 apple cultivars in the germplasm. Vegetative (including phenology) and fruit characters were observed and evaluated in all studied species. Obtained common characteristics and determined differences were compared in relation to studied species and cultivars. Differences were proved in the beginning and length of flowering. For example, *Malus sikkimensis* (Wenz.) Koehne flowered as the latest with the longest flowering period also in the comparison with apple cultivars. *Malus platycarpa* Rehder exceeded in flesh firmness. *Malus rockii* Rehder and *Malus transitoria* (Batalin) C. K. Schneid were characterised by long storability in cold store. *Malus kirgisorum* (Al. Fed. & Fed.) is also interesting due to its purple violet colour and fruit size. Obtained results proved a considerable variability among studied species, which can be used as sources of needed characteristics for future breeding programmes in relation to soil and climatic conditions and grower requirements in the Czech Republic.

P314

Low Copy gypsy Retrotransposons Are Associated with Genes and Show Patchy Distribution in Carrot (*Daucus carota* L.)

Alicja Macko-Podgórn¹, Katarzyna Stelmach¹, Douglas Senalik², Philipp W. Simon², Dariusz Grzebelus¹

¹*Institute of Plant Biology and Biotechnology, University of Agriculture in Krakow, Krakow, Poland,*

²*USDA-ARS Vegetable Crops Research Unit and Department of Horticulture, University of Wisconsin-Madison,, Madison, WI 53706, USA*

Long Terminal Repeat (LTR) retrotransposons are mobile DNA segments transposing via a 'copy and paste' mechanism utilizing an RNA intermediate. Each transposition event leads to an increase in their copy number and results in polymorphism at the insertion site. Moreover, LTR-retrotransposons inserted in genic regions may affect expression of adjacent genes.

We identified a gypsy retrotransposon, named DcG1, represented by a single copy in the carrot reference genome. The insertion was localized inbetween two genes; a gene coding for a Hsp70-like heat shock protein and a gene coding for a protein carrying EF-hand, calcium binding motif. Amplification of the insertion site in 88 cultivated and wild accessions showed presence of DcG1 in six accessions of cultivated carrot, most of the remaining accessions carried homozygous empty site and only rarely other variants were observed, e.g. a solo-LTR of DcG1, insertion of an unrelated LTR retrotransposon or its solo-LTR. Distribution of DcG1 was further analyzed by PCR amplification of its internal regions, i.e. domains encoding RT and gag proteins. DcG1-specific amplicons were detected in 21 of 88 cultivated and wild accessions of carrot, indicating its patchy distribution within the species. We also investigated positions of DcG1 copies in other accessions and identified four other insertion sites. Interestingly, three of them were adjacent to genes and in each case the DcG1 insertion site was unique to the accession in which it was originally identified, suggesting a very recent mobilization and preferential insertion into genic regions.

P315

Fingerprinting of the Highbush Blueberry Cultivars (*Vaccinium corymbosum* L.) Using EST-SSR Markers

Malgorzata Czernicka¹, Jaroslaw Plawiak¹, Monika Bieniasz²

¹*Institute of Plant Biology and Biotechnology, University of Agriculture in Krakow, Krakow, Poland,*

²*Department of Pomology and Apiculture, University of Agriculture in Krakow, Krakow, Poland*

Taking into account the increasing demands for blueberry fruit, it is necessary to have reliable and quick method for genotyping and genetic traceability of the commercially grown cultivars. Microsatellites (SSRs) markers are very powerful tools for identification of cultivars due to their ubiquitous distribution among the eukaryotic genomes, their high level of polymorphism, their codominant inheritance and their high discrimination power. The objective of this work was to select a set of EST-SSR markers for genotyping of 12 cultivars of *V. corymbosum* grown in Poland and 2 wild taxa i.e. *V. stamineum* 'Deerberry' and *V. darrowi*. Genomic DNA was extracted from leaf tissue according to Rogers and Bendich method (1985). DNA was amplified with 13 EST-SSR primers and PCR products were separated using CEQ 8000 genetic analyzer (Beckman Coulter) in order to obtain set of alleles for each locus. The results allowed to indicate the most appropriate alleles to distinguish all examined blueberry cultivars. The microsatellite markers showed excellent promise for further use in germplasm identification, in genetic studies of different *Vaccinium* L. populations.

Rogers S.O., Bendich A.J. 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol. Biol.* 5: 69–76.

The study has been supported by the Polish Ministry of Science and Higher Education (grant no. N N310 441838).

P316

Intron-Flanking EST Markers in the Genetic Relationship Analysis of Rhododendron Hybrids and Their Parental Taxa

Jaroslaw Plawiak, Malgorzata Czernicka

Institute of Plant Biology and Biotechnology, University of Agriculture in Krakow, Krakow, Poland

The aim of the research was to evaluate the genetic relationship and the inheritance of frost resistance of rhododendrons. The plant materials were three Asiatic species i.e. *R. aureum*, *R. brachycarpum*, *R. purdomii*, six cultivars of 'Catawbiense Hybridum' and 'Yakushmanum Hybridum' groups and 67 specimens among 7 populations of interspecific hybrids. The genomic DNA was isolated using DNeasy Plant Mini Kit (Fermentas). DNA was amplified with 24 EST-PCR primers. As results 25 EST-PCR markers were identified that were applied for the construction of dendrogram and analysis of the genetic relationship of tested plants. The dendrogram illustrated the division of parental plants into 3 groups groups. The largest one was constituted by cultivars of *R. catawbiense*. Second group was created by *R. brachycarpum*, *R. purdomii* and *R. yakushmanum* 'Koichiro Wada'. The smallest group was constituted by plants of *R. aureum*. The studies confirmed the hybrid character of 32 plants among 7 F1 hybrid populations.

P317

Evaluation of Total Anthocyanins Content and Antioxidant Activity in Purple Waxy Corn Inbred Lines to Develop Antioxidant Rich Varieties

Jin Seok Lee, Seong Hyu Sin, Sang Gon Kim, Jung Tae Kim, Hwan Hee Bae, Beom Young Son, Seong Bum Baek, Young Up Kwon

National Institute of Crop Science, Rural Development Administration, Suwon, Republic of Korea

This study was carried out to evaluate changes of total anthocyanins content and antioxidant activity according to ripening days after silking in purple waxy corn inbred lines. Purple waxy corn inbred lines have pericarps and aleurone layer containing anthocyanins showing antioxidant activity. Fifteen purple waxy corn inbred lines were planted and their seeds were harvested at 19, 21, 23, 25, 27, 45 days after pollination (DAP) in Korea, 2011. Total anthocyanins content and free radical scavenging activity were determined, respectively. Total anthocyanins content in seeds of these inbred lines gradually increased by 45 DAP, which is harvest time when the total anthocyanins content the highest. Total anthocyanins content was various among inbred lines. Antioxidant activities increased along with total anthocyanins content and also different among inbred lines, resulting in Correlation between total anthocyanins content and antioxidant activities was positive and their correlation coefficient was 0.529. Two of fifteen inbreds were found to have more than 100mg/100g of total anthocyanins with high antioxidant activity, suggesting that those inbreds might be used for breeding materials for antioxidant rich waxy corn varieties.

P318

Comparative Proteome Analysis of Mature Kernels in 3 Korean Waxy Corn Cultivars

Sang Gon Kim, Jin-Seok Lee, Seong-Hyu Sin, Jung-Tae Kim, Hwan Hee Bae, Beom-Young Son, Seong-Bum Baek, Young-Up Kwon

National Institute of Crop Science, Rural Development Administration, Suwon, Republic of Korea

Maize (*Zea mays* L.) is the most important crop in the world for human and animal. Although the new varieties of maize have been extensively developed in Korea, little is known about the changes of proteome in maize mature kernels for biological understanding of the complex mechanism among 3 Korean waxy corn cultivars (Ilmichal, Eolrukchal 1, and Heukjinjuchal). A total protein content of Ilmichal was higher than those of Eolrukchal 1 and Heukjinjuchal cultivars, while contents of starch and fatty acid were similar among 3 Korean waxy corns. Proteins were extracted from 3 different waxy corn kernels using phenol method, and separated by 2-DE. After staining of 2-DE gels with colloidal Coomassie brilliant blue (CBB), 37 spots were differentially expressed and were identified using MALDI-TOF/TOF mass spectrometry. They were major involved in 37.8% storage proteins, 18.9% stress related proteins, and 18.9% metabolism. Storage proteins (globulin-2) and stress related proteins (heat shock protein and general stress protein) were highly expressed in Ilmichal or Heukjinjuchal. Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of corresponding genes of five randomly selected proteins, including glyoxalase family protein (accession number, B6SGF3), globulin-2 (Q7M1Z8), heat shock protein 1 and 3 (B6TGQ2 and B6TDB5), and vicilin-like embryo storage protein (Q03865), showed that just as protein abundance, these proteins are also regulated at the transcript level. Taken together, these results may provide a better understanding of the characterization between Korean waxy corn cultivars and support further molecular breeding.

P319

Pre-Harvest Factors Affect Sweet Cherry Tree Physiology and Yield

Berta Gonçalves¹, José Moutinho Pereira¹, Carlos Correia¹, Eunice Bacelar¹, Helena Ferreira¹, Filipa Queirós², Ana Paula Silva¹, Ana Barros¹, Alberto Santos¹

¹CITAB, Universidade de Trás-os-Montes e Alto Douro, UTAD, Vila Real, Portugal, ²Universidade de Trás-os-Montes e Alto Douro, UTAD, Vila Real, Portugal

Sweet cherry (*Prunus avium* L.) has traditionally been grown in Portugal where it has considerable economic and social importance. Although the most frequently grown cultivars are well adapted to the local environmental conditions, few field experiments have been conducted to study orchard management practices in order to optimize the tree growth and fruit set. Therefore, variations in morpho-anatomical, biochemical and physiological parameters and yield were studied in six-year old drip-irrigated *Prunus avium* L. Skeena and Sweetheart cultivars grafted on Edabriz, under three plant spacing (0.7, 1.4 and 2.8 x 4.5 m) in Carrazedo de Montenegro region, north of Portugal. At higher plant density, cherry trees showed significantly lower specific leaf weight, as well as lower photosynthetic rate, stomatal conductance and intrinsic water use efficiency and lower concentration of total chlorophyll. However, there were no significant differences in transpiration rate between cultivars and plant spacing. A strongly decrease in the production of cherry at 0.7 x 4.5 m plant spacing was also observed. Therefore, these results suggest that Skeena and Sweetheart cultivars benefit from management practices, e.g., a best plant spacing that optimize the canopy light exposition and, consequently, increase fruit production.

Acknowledgements: This work was supported by Project INNOFOOD - INNOvation in the FOOD sector through the valorization of food and agro-food by-products - NORTE-07-0124-FEDER-0000029, financed by the North Portugal Regional Operational Programme (ON.2 - O Novo Norte) under the National Strategic Reference Framework (QREN), through FEDER, as well as by PIDDAC through FCT/MEC.

P320

Terpenoids of *Arabidopsis thaliana* - Statistical Analysis

Katarzyna Gawarecka, Jaroslaw Poznanski, Ewa Swiezewska
Institute of Biochemistry and Biophysics PAS, Warsaw, Poland

Arabidopsis thaliana has adapted to different habitats and this resulted in numerous natural variants (ecotypes). For experiment 123 ecotypes were used to collect data on the content of selected terpenoids. Terpenoids representing the most diverse group of natural compounds fulfil various important functions in cells. Although data on the synthesis of terpenoids are available, still information on the possible mechanisms regulating this process are scarce.

Content of terpenoids was measured using HPLC, GC and spectrophotometric methods and obtained data were analysed statistically. Preliminary analysis showed that distribution of concentration of all examined compounds cannot be regarded as Gaussian, so all further analyses were performed using nonparametric approach. Thus, Spearman's rank correlation coefficients showed statistically significant correlation between carotenoids and chlorophylls ($p < 5\%$, $R^2 = 0.97$). Visible or significant correlations were also observed within prenols, dolichols, phytosterols and plastoquinone ($R^2 > 0.6$).

Principal Component Analysis (PCA) demonstrated that three out of seven principal components explain 89.4% of the total variance. Almost equal contributions of prenols, dolichols, phytosterols and plastoquinone predominate in the first principal component (50.7% of variance). Carotenoids and chlorophylls give the highest impact to the second component (26.4%), while the third one is dominated by tocopherols concentration (12.3%).

Obtained results suggest the occurrence of the common cellular mechanisms jointly regulating the biosynthesis and/or accumulation of prenols, dolichols, phytosterols and plastoquinone. Biosynthesis/accumulation of carotenoids and chlorophylls seems to be co-regulated independently to the aforementioned terpenoids while regulation of tocopherol biosynthesis seems not to be coupled with the other analysed terpenoids.

P321

Differences in Leaf Surface Compounds among Finnish Silver Birch Genotypes and Provenances

Sarita Keski-Saari, Maya Deepak, Jenna Lihavainen, Jennifer Dumont, Sari Kontunen-Soppela, Lars Granlund, Ilkka Porali, Markku Hauta-Kasari, Elina Oksanen, Markku Keinänen
University of Eastern Finland (UEF), Joensuu, Finland

Leaf surface layer of terrestrial plants is the first line of defense to face different abiotic stress conditions, such as high light. Therefore, natural variation in surface compounds is expected to be high and dependent on the light environment in which the plant is adapted. We studied the variation in leaf surface compounds in 12 silver birch (*Betula pendula*) genotypes, originating from three provenances from northern, central and southern Finland (Loppi 60°N, Vehmersalmi 63°N and Rovaniemi 66°N). The genotypes were grown in a common garden experiment in central Finland (Joensuu 63°N). The leaves were dipped in dichloromethane for 30 s, evaporated to dryness and stored at -70 °C until analyses. Phenolics and triterpenoids were analysed by APCI-HPLC-MS from acetonitrile dissolved extracts. The compound profiles differed between the genotypes, with both qualitative and quantitative differences. There was some difference between the provenances as well, which implies that there is some adaptation of leaf surface components to different light and/or climatic conditions in Finland.

Acknowledgement: This study is part of the European Union Structural Funds project "Spectral imaging and analysis in environmental and industrial applications" funded by the Finnish Funding Agency for Technology and Innovation (Tekes), filing number 70005/13.

P322

***In Vivo* Digestibility of Four Perennial Ryegrass Cultivars**

Brian Garry^{1,2}, Michael O'Donovan¹, Tommy Boland², Eva Lewis¹

¹Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co.Cork, Ireland, ²School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

Perennial ryegrass cultivars differ in their digestibility and *in vivo* digestibility studies are used to measure this difference. The *in vivo* dry matter digestibility (DMD) of four perennial ryegrass cultivars (Glenroyal (GR), Delphin (DE), Tyrella (TY) and Astonenergy (AS)) was measured using twelve Texel wether sheep housed in digestibility stalls. Cultivars GR and TY were diploid while AS and DE were tetraploid. The experiment was conducted using an incomplete Latin square design (four cultivars × two periods) which was repeated twice. Each period of the Latin square consisted of 12 days (6 days diet adaptation and 6 days collection of faeces). Latin square one (LS1) was from 13th May to 7th June and LS2 was from 19th August to 13th September 2013. The leaf, pseudostem, true stem and dead proportions of the sward were measured. There was a cultivar × LS interaction for DMD. In LS1, TY (845 g/kg) had a greater DMD than DE (796 g/kg) ($P < 0.05$). A reduction occurred in DMD from LS1 to LS2 for all cultivars ($P < 0.001$). This was associated with an increased amount of dead material in the sward in LS2 ($P < 0.001$). In LS2, there was no significant difference between cultivars in DMD but TY (19.1%) and GR (21.9%) had a greater proportion of dead material than DE (14.9%) and AS (15.9%) ($P < 0.001$). These results indicate that there are small differences between perennial ryegrass cultivars in DMD but large effects of season on perennial ryegrass cultivar DMD.

P323

Perennial Ryegrass Morphology and Effects on Organic Matter Digestibility

Marion Beecher^{1,2}, Deirdre Hennessy¹, Tommy Boland², Norann Galvin¹, Christina Fleming¹, Mary McEvoy¹, Michael O'Donovan¹, Eva Lewis¹

¹Teagasc, & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co.Cork, Ireland,

²School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

The proportions of the morphological components leaf (L), pseudostem (PS) and true stem (TS) in perennial ryegrass differ throughout the growing season. These components vary in digestibility and can impact on overall sward digestibility. The aim of the study was to measure the change in L, PS, TS and dead (D) proportions and to measure the organic matter digestibility (OMD) of these components throughout the growing season in four perennial ryegrass cultivars.

The cultivars were Bealey, Astonenergy, Spelga and Abermagic, each sown as a monoculture grazing sward. On 10 occasions prior to each grazing (rotations), between May 2011 and March 2012, the L, PS, TS and D proportions (> 4cm) in each cultivar were determined. Dry matter (DM) of each morphological component was determined. In vitro OMD was determined using the neutral detergent cellulase method.

There was an interaction between cultivar and rotation for the DM proportions of L, PS, TS and D. In May and June Astonenergy had a higher L proportion and lower TS than all other cultivars ($P < 0.05$). From July onwards, there were no differences between cultivars in L or TS proportion. Differences in the OMD of the individual components of all cultivars was in the order $L > PS > TS > D$.

The proportion of the morphological components of cultivars changes throughout the growing season. Differences in the proportions of plant components between cultivars are evident predominantly during the reproductive period.

P324

Evaluation of Biochemical, Genotoxic and Microbiological Features of Different Viburnum Genotypes

Algimantas Paulauskas¹, Judita Zukauskienė¹, Eugenija Kupcinskiene¹, Laima Cesoniene¹, Remigijus Daubaras¹, Juozas Lazutka², Grazina Slapsyte², Asta Stapulionyte², Algimantas Paskevicius³
¹*Faculty of Nature Sciences of Vytautas Magnus University, Kaunas, Lithuania,* ²*Faculty of Natural Sciences of Vilnius University, Vilnius, Lithuania,* ³*Laboratory of Biodeterioration Research of Nature Research Centre, Vilnius, Lithuania*

The aim of this work was to evaluate 10 different genotypes of *Viburnum* using SSR (Simple Sequence Repeat) markers and carry out analyses of specific biochemical, microbiological and genotoxic traits. Following other *Viburnum* genus studies, eleven SSR primer pairs tested. Among them only 8 have generated fragments. Totally 97 SSR fragments were obtained. For separate SSR loci number of DNA fragments was varying from 2 to 10, and their length from 80 to 150 base pairs (bp). Examination of different genotypes according to biochemistry of fruits revealed a considerable variation among accessions. The content of anthocyanins and phenolic compounds differed significantly among genotypes. The genotoxic potential of lyophilized juices of *Viburnum opulus* and *Viburnum sargentii* fruits was evaluated using the Ames Salmonella/microsome mutagenicity test, alkaline single-cell gel electrophoresis assay (Comet assay) and cytokinesis-block micronucleus test in human lymphocytes in vitro. The results demonstrated that lyophilized juices of *V. opulus* and *V. sargentii* fruits are not genotoxic under the conditions of the present study. The antimicrobial properties of the juice of *Viburnum* fruits were evaluated using the agar diffusion method. The results of the bioassays showed bacteriocidal and bacteriostatic activity of the juice of *Viburnum* fruits against 8 tested bacteria. The juice of *Viburnum* fruits strongly inhibited the growth of *Bacillus macerans* and *Micrococcus* sp. The juice of *Viburnum* fruits showed no inhibitory effect on the growth of *Candida parapsilosis*.

P325

Molecular Diversity of Lithuania Populations of Common Juniper (*Juniperus communis* L.)

Ramunas Vilcinskas, Lina Zybartaite, Algimantas Paulauskas, Eugenija Kupcinskiene
Vytautas Magnus University, Department of Biology, Kaunas, Lithuania

Juniperus communis L. (var. *communis* L.) is 1 out of 3 conifer species naturally growing around the Baltic Sea. In Eastern Baltic region studies of this species are mainly concentrated in radial growth parameters and profiles of essential oils. Till now studies about genetic diversity of Lithuania populations of *Juniperus communis* are missing. For junipers Random Amplified Polymorphic DNA (RAPD) method have been applied in wider scope compared to inter simple sequence repeat (ISSR) markers, despite ISSR are less prone to laboratory conditions. Present study aimed at evaluation of genetic diversity of *J. communis* populations occurring in the range of habitats and climatic conditions of Lithuania. Fourteen locations of *Juniperus communis* L. were selected in the way to cover all the territory of Lithuania and represent various climatic zones, types of habitats. Total genomic DNA was isolated using modified CTAB method. The ISSR markers were selected and analyses performed as it is described by Adams et al. (2003). For ISSR analysis 15 oligonucleotid primers were used. Each ISSR primer generated from 61 to 80 polymorphic bands. For evaluation of molecular diversity within each population, percentage of polymorphic loci, Nei's gene diversity, Shannon's information index for genetic variation, coefficient of genetic differentiation and gene flow were calculated. For comparison of populations principal component analyses, Analyses of Molecular Variance were applied. Between populations the percentage of polymorphic loci ranged from 59.8 % to 71.1 %. The total molecular variation was much higher (90 %) among populations, compared to interpopulation variability.

P326

Is Chromoplast a New Bioenergetic Organelle?

Marta Renato^{1,2}, Albert Boronat², Joaquín Azcón-Bieto¹

¹University of Barcelona (Department of Plant Biology), Barcelona, Spain, ²Center for Research in Agricultural Genomics, Bellaterra, Barcelona, Spain

Chromoplasts are plastids specialized in the synthesis and accumulation of carotenoids. During tomato (*Solanum lycopersicum*) fruit ripening, chloroplasts differentiate into chromoplasts losing their photosynthetic machinery and synthesizing large amounts of carotenoids. Chromoplasts present an electron transport chain ending in O₂ uptake activity, known as chromorespiration, a process which is not well understood. In this work, respiratory assays with chromoplasts isolated from tomato fruit were conducted. Results show that chromoplast O₂ uptake activity is stimulated by the electron donors NADH and NADPH, and is sensitive to octyl-gallate, inhibitor of the plastidial terminal oxidase (PTOX). It also responds to the uncoupler carbonylcyanide m-chlorophenylhydrazone (CCCP), indicating that a membrane proton gradient develops in chromoplasts. The ATP synthesis rate of isolated chromoplasts was quantified, and results indicate that it is dependent on NAD(P)H, is inhibited by octyl-gallate and CCCP, and is also sensitive to 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), inhibitor of cytochrome b₆f complex. To analyze the significance of chromoplast respiration in tomato tissue, we studied the effect of octyl-gallate on oxygen uptake and ATP levels of tomato pericarp at different ripening stages. Data suggest that chromorespiration increases over the course of ripening, is responsible of about 26% of total oxygen consumption in ripe tomato tissue and represents a relevant contribution to ATP content in red fruit. Preliminary molecular studies are being conducted to identify the components which participate in chromorespiration, like a type-II NAD(P)H dehydrogenase and the cytochrome b₆f. Also, the localization of these components inside the chromoplast is being analyzed through electron microscopy.

P327

Light Regulates the Splicing of the *petB* and *petD* Introns of the *psbB* Operon in *Populus deltoides*

Sandhya Mehrotra¹, Prabodh Kumar Trivedi²

¹Birla Institute of Technology and Sciences, Pilani, Rajasthan, India, ²National Botanical Research Institute, Lucknow, UP, India

The chloroplastic *psbB* operon in higher plants encodes five thylakoid polypeptides viz. the 51 kDa chl a apoprotein (*psbB*), a 10 kDa phosphoprotein (*psbH*), a protein required for maintaining optimal PSII activity under adverse growth conditions (*psbT*), all associated with photosystem II, as well as cytochrome b6 (*petB*), subunit IV (*petD*) of the cytochrome b/f complex as well as the divergently transcribed *psbN* gene. The *psbB* operon is initially transcribed as a large polycistronic transcript of 5.5 kb and subsequently processed into oligocistronic transcripts of 4.7 and 3.8 kb one after the other. The 4.7 kb transcript arises after one of the two introns has been spliced out and the 3.8 kb transcript arises after both the introns have been spliced out. We report that the accumulation of the 5.5 kb, 4.7 kb and 3.8 kb transcripts showed differential accumulation under 24 hour day night cycle and also that the *petD* and *petB* intron splicing is regulated differentially in varying light dark conditions. While the *petD* intron is spliced primarily in light, the *petB* intron is spliced away during the dark conditions. A distinct pattern of accumulation of the primary and the two secondary transcripts under natural and induced light dark conditions exists. We present data on the northern blot analyses as obtained by hybridization of total cellular RNA of *Populus deltoides* with the probes of *psbB* and *petD* introns as a proxy to intron splicing under the 24 hour day-night cycle as well under induced light dark conditions.

P328

An Evolutionary Model for Protein Body Formation in the Endoplasmic Reticulum of Cereal Endosperm Cells

Davide Mainieri, Francesca Morandini, Marie Maitrejean, Andrea Sacconi, Emanuela Pedrazzini, Alessandro Vitale

Istituto di Biologia e Biotecnologia Agraria, CNR, Milano, Italy

The seed storage proteins present in all plants accumulate in storage vacuoles. Prolamins, which are the major seed storage proteins in cereals and are present only in these plants, instead accumulate within the endoplasmic reticulum (ER) lumen as very large insoluble polymers held by disulfide bonds, termed protein bodies. The model prolamin gamma-zein of maize contains seven cysteine residues involved in interchain bonds. We show that progressive substitution of these amino acids with serine residues leads to similarly progressive increase in solubility and availability to traffic from the ER along the secretory pathway. Total substitution results in very efficient secretion, whereas the presence of a single cysteine is sufficient to promote partial sorting to the vacuole via a pathway that is sensitive to brefeldin A and wortmannin, similarly to the normal traffic pathway of vacuolar storage proteins. We propose that the mechanism leading to accumulation of prolamins in the ER is a further evolutionary step of the one responsible for accumulation in storage vacuoles. Supported by the FILAGRO Project of CNR-Regione Lombardia

P329

Genetic Control of the Chloroplast Antioxidant Capacity

Wei Guo, Radoslaw Rudnik, Margarete Baier

Dahlem Center of Plant Science - FU Berlin - Plant Physiology, Berlin, Germany

Chloroplasts provide cells with energy and a wide range of metabolites. The efficiency is limited by photooxidative generation of reactive oxygen species (ROS), which inactivate enzymes and destroy metabolites. Peroxiredoxins and ascorbate peroxidases protect chloroplasts against peroxide accumulation in the chloroplast stroma and at the thylakoid membrane. Like all other chloroplast antioxidant enzymes they are nuclear encoded and post-translationally targeted to their site of action (1). Expression is induced during acclimation phases and post-stress resetting. Analysis of antisense and knock-out lines demonstrated that stromal ascorbate peroxidase, thylakoid-bound ascorbate peroxidase and 2-Cys peroxiredoxin A and B form a redox-regulated compensatory network (2, 3). ERF1 transcription factors with highly conserved DNA-binding motifs, but variable regulatory domains, orchestrate the relative expression levels. Specific support is provided by upstream modulation. In young leaves, RIMB1 supports activation of peroxiredoxin genes and genes for chloroplast ascorbate peroxidases via protein-protein-interaction with the transcription factors. In contrast, the peroxiredoxin regulating factor PRRF1 promotes only 2-Cys peroxiredoxin expression, but not chloroplast APx expression. It is expressed in the Arabidopsis variants Col-0 and C24, but not in various other accessions and may contribute to natural variation in gene expression regulation (4). We conclude that the expression of the most prominent chloroplast peroxidases is controlled by the combination of specificity and redundancy in the activity regulation of paralogous transcription factors.

Literature cited: (1) BMC Plant Biology 10, 133; (2) Biochem. J. 412, 275; (3) Plant Physiol. 124, 823; (4) Physiol. Plant. 146, 53

P330

H⁺-Pyrophosphatase Is Necessary for Normal Growth of *Arabidopsis thaliana*

Mariko Asaoka¹, Shoji Segami¹, Chihiro Sato¹, Ali Ferjani², Masayoshi Maeshima¹
¹Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, ²Tokyo Gakugei University, Tokyo, Japan

Vacuolar membrane H⁺-pyrophosphatase (H⁺-PPase) uses energy from the PPi hydrolysis to pump protons into vacuoles. The enzyme accounts for 10% in weight of vacuolar membrane proteins. The loss-of-function mutants of H⁺-PPase showed changes in cotyledon development and the phenotype was recovered by expression of soluble PPase, indicating physiological importance of the PPi scavenger function of H⁺-PPase. We further examined physiological meanings of the proton pump function by analyzing phenotypes of over-expression and loss-of-function mutants. When plants were grown in agar plates supplemented with 1% sucrose for 10 days and then cultivated on rockwool without sucrose, the loss-of-function mutants showed no phenotype at early stage and severe growth defect in 3-week-old plants. We prepared three lines of overexpressors of H⁺-PPase, which have 1.5 to 3-fold activity and protein content of H⁺-PPase of Col-0 plants. The fresh weight of plants with the highest H⁺-PPase activity was more than 120% of that of Col-0. The loss-of-function plants complemented with H⁺-PPase recovered their fresh weight to the same level of Col-0. The results suggest that H⁺-PPase is necessary for normal growth not only in germination stage but also in late growth stage. We also performed analysis of leaf areas, totally biomass, cell size, and cell number in the early developmental stage and total sucrose content of these plants. We will discuss how the H⁺-PPase activity affects the growth of plants.

P331

Physiological Role of Plastid Membrane Transporters Involved in Transport of Intermediates of Starch Biosynthesis in Rice

Ryosuke Satoh^{1,3}, Syota Shiraishi³, Thomas Okita², Hikaru Satoh³

¹Nagoya University, Nagoya-shi, Aichi-ken, Japan, ²Washington State University, Pullman, Washington State, USA, ³Kyushu University, Hukuoka-shi, Hukuoka-ken, Japan

Starch is the end-product of photosynthesis and stored as energy and carbon source in the sink organ. Amyloplasts in endosperm is the major sink organelles in rice. Starch is composed of the linear polyglucan amylose and the branched α -polyglucan amylopectin. Both amylose and amylopectin are elongated by addition of glucose moiety provided as ADP-glucose (ADP-Glc), which is synthesized from glucose-1-P (G1P) by ADP-Glc pyrophosphorylase (AGPase). ADP-Glc synthesized in the cytosol is transported into amyloplast by ADP-Glc transporter (Nakamura et al. 2005). Synthesis and transport of ADP-Glc are key steps in the starch biosynthesis and starch granule formation, however, the molecular bases are not clear. Here we focused our attention to a rice mutant shrunken endosperm 3 (*shr3*), which has a mutation in ADP-Glc transporter (BT1-1) (Shiraishi. 2011). We characterized genetic and biochemical properties of this mutant rice (BT1-1, *shr3*), especially seeds and their starch granules. This study revealed the following two points. Firstly, approximately 80% of substrate for starch biosynthesis in endosperms was transported by BT1-1 at middle and late stages of starch formation. Indeed, *shr3* had immature starch grains. Secondary, another transporter glucose 6-phosphate transporter 1 (GPT1) transports the cytosol substrate into plastids during early stage of starch formation. The starch content in EM482, which is an amino acid substitution mutant of GPT1, decreased by 20%. We discuss physiological regulation of transport of intermediate of starch formation through *shr3* and EM482 mutants.

P332

Mitochondrial Transcriptome Regulation and Cross-Talk in Plants

Adnan Khan Niazi¹, Romain Val¹, Etienne Delannoy², Marta Gabryelska³, Eliza Wyszko³, Frederique Weber-Lotfi¹, Maciej Szymanski³, Jan Barciszewski³, Andre Dietrich¹

¹*Institut de Biologie Moleculaire des Plantes, CNRS and UdS, Strasbourg, France,* ²*Unite de Recherche en Genomique Vegetale, INRA-Universite d'Evry Val d'Essonne, Evry, France,* ³*Institute of Bioorganic Chemistry, PAN, Poznan, Poland*

Biogenesis of the oxidative phosphorylation (OXPHOS) complexes needs the assembly of mitochondrion-encoded and nuclear-encoded subunits, implying cross-talk between the two compartments. Regulation of mitochondrial gene expression and inter-compartment genome coordination is still little understood. We addressed this question through a novel genetic approach, circumventing the absence of mitochondrial transgenic methodologies for multicellular eukaryotes. We developed an RNA targeting strategy based on the natural pathway of tRNA import from the cytosol into mitochondria in plants. We established that a tRNA mimic can function as a shuttle to import into mitochondria passenger RNAs expressed from nuclear transgenes. Taking trans-cleaving ribozymes as passenger sequences allowed to knock down individual RNAs in the mitochondria of plant transformants. The resulting regulation response was analyzed at the whole transcriptome level. Knockdown of individual mitochondrial mRNAs encoding subunits of OXPHOS complexes triggered a decrease in the level of most other organelle mRNAs. Such an effect was not observed when importing into mitochondria a ribozyme having no specific target or when down-regulating a mitochondrial transcript carrying no known function. Contrary to the current view of mitochondrial regulation processes in plants, our results imply that mitochondrial mRNAs encoding subunits of OXPHOS complexes are coordinated. Further high throughput analyses showed that knockdown of a mitochondrial mRNA encoding an OXPHOS complex subunit triggered a retrograde response resulting in up- or down-regulation of numerous nuclear or plastid mRNAs. Remarkably, these included nuclear mRNAs for pentatricopeptide (PPR) proteins, a family of factors involved in most aspects of RNA metabolism in plant mitochondria.

P333

Zinc Iodide-Osmium Tetroxide (ZIO) Reactivity in Rubiaceae Colleters: Ultrastructural and Functional Aspects of the Epithelial Cells

Silvia Machado, Tatiane Rodrigues, Fernanda Tresmondi
Univ. Estadual Paulista/UNESP, Botucatu, Sao Paulo, Brazil

Conventional techniques in transmission electron microscopy (TEM) combined with ultracytochemical markers facilitate the observation and identification of the endomembrane system involved in secretory processes in plants. One of these procedures is the zinc iodide-osmium tetroxide (ZIO) method. In this study we employed the ZIO method in order to investigate the cell machinery involved with the mucilage and resin production in colleters on the vegetative apex of *Alibertia concolor*, a member of Rubiaceae. Samples were fixed with 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1M phosphate buffer pH 7.3, for 12 h, and incubated at 108°C in a solution containing Zn, I, TRISaminomethane and OsO_4 for 22 h; then, the samples were processed according to TEM conventional techniques. Colleters are constituted by a parenchyma axis covered by a secretory epithelium. Mitochondria, endoplasmic reticulum (ER), Golgi bodies and vesicles reacted positively to ZIO in epithelial cells at the secretory phase. In a same epithelium, adjacent secretory cells showed difference in the abundance of ER and Golgi bodies, and in the intensity of staining with ZIO. The use of ZIO method improved the observation and identification of Golgi apparatus and ER profiles. Based on the ultrastructural peculiarities of the endomembrane system, we suggest the specialization of neighboring epithelial cells in the production of hydrophilic or lipophilic substances.

P334

Chloroplast Transformation with Synthetic Lactoferricin B Fused SmGFP in *Nicotiana tabacum*

Seung-bum Lee, Seong-han Sohn, Seok-cheol Seo, Yeon-hee Lee
National Academy of Agricultural Science, Suwon-si, Republic of Korea

The chloroplast transformation was performed to produce high-value pharmaceutical protein at low cost. Lactoferricin B gene is from bovine and it is a multifunctional protein with several functions such as anti-bacterial, anti-cancer and anti-viral. The chloroplast transformation is beneficial when highly expressing foreign genes because of many copies of genome in the chloroplast organelle. Lactoferricin B protein is composed of twenty-four amino acids and it was artificially synthesized. At the same time, codon optimization to chloroplast gene was done to improve the expression when the gene was transformed into tobacco chloroplast. In order to easily detect the protein after expression, smGFP fusion and 6xHis attachment to lactoferricin B was carried out together. GFP fused Lactoferricin B was recombined with each chloroplast transformation vectors, pBL-CtV and pLD-CtV, and the vectors pBL-utrGFP6xhisLF and pLD-utrGFP6xhisLF were constructed for tobacco chloroplast transformation. The gene gun was used as the method for chloroplast transformation. Several transgenic plants were obtained and some of them were analyzed. As the result of PCR using specific primer combination, insertion of GFP fused Lactoferricin B gene into the chloroplast genome was confirmed. Southern hybridization also represented the same result. In T1 generation of the transgenic plant, the expression of the introduced fusion gene of each transgenic plant was detected through immunoblot analysis using GFP antibody. And the expression quantity of GFP fused lactoferricin B among transgenic plant was measured as the average of 1.67%

P335

Heterologous Expression and Purification of Barley Isoamylase

Alexander Striebeck¹, Lyann Sim¹, Monica M. Palcic², Mats Hansson¹

¹Carlsberg Laboratory, Copenhagen, Denmark, ²University of Copenhagen, Copenhagen, Denmark

Starch is a polysaccharide made of countless glucose molecules joined by glycosidic bonds. The glucose chains form two super-structures, amylose and amylopectin, both of which consist of α -1,4-linked residues. Amylopectin contains additional α -1,6-links between the linear chains. These dispersed branches are the basis for the crystalline structure of starch and therefore important for the behaviour of starch in industrial applications.

Amylopectin is synthesised by a vast number of starch synthases, starch branching and debranching enzymes (DBEs) in a well-orchestrated manner. Results indicate that the activity of DBEs is necessary for the formation of the crystalline structure of starch. The molecular basis and the control of this debranching process, however, are not well understood.

Isoamylase 1 and 2 (ISA1/2) belong to family 13 of glycoside hydrolases with the potential to cleave α -1,6-glycosidic bonds present in amylopectin. Despite being classified as a hydrolase, ISA2 is thought to be inactive due to the change of amino acid residues in the putative catalytic site. However, studies in *Arabidopsis* and various crops have shown that ISA1 and ISA2 can form heteromeric complexes. Hence, ISA2 may work as a regulatory subunit for ISA1.

Here, we present the heterologous expression, purification and preliminary characterisation of barley (*Hordeum vulgare*) ISA1 and ISA2. This work forms the foundation for further studies to characterise the interaction between barley ISA1 and ISA2 and will act as a primer for enzyme kinetic as well as protein crystallography studies to determine the molecular mechanism of starch debranching in higher plants.

P336

Arabidopsis Lon1 and Lon4 Proteases Evolved Complementary Subsets of Regulatory Components for Dual Targeting to Mitochondria and Chloroplasts

Dikran Tsitsekian, Gerasimos Daras, Anastasios Alatzas, Natasa Kanali, Margarita Thomopoulou, Stamatis Rigas, Polydefkis Hatzopoulos
Department of Biotechnology, Agricultural University of Athens, Athens, Greece

Cellular homeostasis relies on components of protein quality control including chaperones and proteases. In bacteria and eukaryotic organelles, Lon protease plays critical role in removing irreparably damaged proteins and thereby preventing the accumulation of deleterious degradation-resistant aggregates. *Arabidopsis thaliana* has a small family of four nuclear genes with distinct features regarding the gene expression profile, subcellular targeting and substrate recognition motifs. Since plant organelles are semi-autonomous and Lons have diverse substrate recognition motifs, it is tempting to suggest that AtLon genes are paralogs. Adaptive evolution through repetitive gene duplication events of a single archaic gene could lead into present-day AtLon members with complementing sets of subfunctions providing rapid adaptability for canonical development under different environmental conditions. Two protein isoforms are produced by alternative transcription and translation initiation of AtLon1 gene and targeted by twin presequences to mitochondria and chloroplasts. However, AtLon4, has an ambiguous presequence generating a single dual-targeted protein isoform. The targeting mechanism of AtLon4 may be evolved from the twin presequences of an ancestral Lon1-like gene. We postulate that AtLon1 could represent a genetic "fossil" of the primordial Lon gene. In the *Arabidopsis* genome a handful of genes have been recognized to possess AtLon1 twin presequence configuration as opposed to genes with ambiguous dual targeting signatures corroborating the genetic "fossil" concept. Lon in association with other nuclear encoded ATP-dependent proteases builds up an elegant nevertheless, tight interconnected circuit that properly and accurately channels proteostasis and protein quality among the distinct subcellular compartments.

P337

Studies on Subcellular Localization of *Arabidopsis thaliana* Cis-Prenyltransferases

Przemyslaw Surowiecki, Liliana Surmacz, Ewa Swiezewska
Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

cis-Prenyltransferases (CPTs) are enzymes that catalyze sequential cis-condensation of isopentenyl diphosphate to allylic diphosphate. As the result a family of prenyl diphosphates of variable chain-length are formed by eukaryotic CPTs in contrast to prokaryotic ones which produce a single prenyl diphosphate. In bacteria, polyprenyl diphosphates are involved in peptidoglycan biosynthesis while in eukaryotic cells they serve as cofactors of protein glycosylation.

Several genes encoding CPTs have been cloned from bacteria, yeast, plants and animals. *Saccharomyces cerevisiae* have two active cis-prenyltransferases with different cellular localization, namely RER2 is localized in ER whereas SRT1 in lipid bodies. Data on subcellular localization of plant CPTs are limited. Only one of the seven tomato CPTs has been found in the cytoplasm/ER (SICPT3) while six remaining SICPTs have been localized in plastids. *Arabidopsis thaliana* have a family of nine CPTs - AtCPT1 to AtCPT9, however the localization of only one of them, AtCPT6 - in ER has been reported. In silico analysis also indicates ER/Golgi localization of AtCPT1 and AtCPT8, while plastidial or nuclear localization is predicted for the remaining AtCPTs.

Studies on subcellular localization of AtCPTs have been initiated recently. To this end protoplasts isolated from the *Arabidopsis* leaves were transformed with respective constructs encoding tagged AtCPTs, AtCPT-ECFP. Co-localization of the tagged proteins with YFP was followed in the protoplasts isolated from *Arabidopsis* lines expressing this fluorescent organelle marker as indicator of the ER, Golgi or plasma membrane, respectively.

This research was supported by grant funded by the National Science Centre [DEC-2011/03/B/NZ1/00568].

P338

Formation of Convolutated Plasma Membrane Domains in Chara Requires Photosynthesis but not Close Contact with Chloroplasts

Ilse Foissner, Margit Hoeflberger, Annela Sommer
University of Salzburg, Cell Biology/Plant Physiology, Salzburg, Austria

The characean green algae are a convenient system to study membrane areas with enhanced transport activities. Their cells form highly convoluted plasma membrane domains called charasomes. Numerous studies indicate that they are involved in the photosynthesis-dependent acidification of the environment consistent with the accumulation of H⁺-ATPases (Schmölzer et al. 2011). The charasome-rich regions alternate with charasome-poor areas which correlate with the pattern of acid and alkaline bands at the cell surface. The signals leading to localized plasma membrane deposition remain largely unknown. Charasomes have so far only been described from internodal cells and in close proximity to the stationary chloroplasts. Here we demonstrate that charasomes are also present in nodal cells and even in protonemata and rhizoids when exposed to light. We show further that charasomes may form in chloroplast-free areas created by local UV irradiation and that this treatment does not affect the pH banding pattern of the internodal cells. Charasomes are absent from plasmodesmata-containing cross walls between adjacent living cells but they form after cutting the neighbor cell so that the cross wall is exposed to the environment. Our study proves that charasome formation requires photosynthetic activity but not close contact with chloroplasts. Our data also suggest that functional plasmodesmata inhibit the formation of charasomes and/or that charasomes form only beneath cell walls which are in direct contact with the surrounding medium.

Schmölzer et al. (2011) *Plant Cell Physiol* 52: 1274-1288

P339

Two Fluorescent Protein Fusions as Plasma Membrane and Vacuole Markers in Live Cell Imaging

Sara Bettencourt^{1,2}, Inês Carqueijeiro^{1,2}, Patrícia Duarte¹, Mariana Sottomayor^{1,2}

¹*IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal,*

²*Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Porto, Portugal*

Nowadays, one of the major challenges for biologists is to assign functions to the countless number of candidate genes identified by genome and transcriptome projects. A central determinant of protein function is the subcellular localization, which can be deeply investigated by live cell imaging. Thus, the fusion with fluorescent proteins (FP) has become an extremely valuable tool for determining directly and non-invasively where a protein resides within the plant cell. Furthermore, when investigating the localization of newly characterized proteins within the cell, it is desirable to confirm the data by co-expressing with known fluorescent subcellular markers where possible. In this way, as plant cells have a variety of endomembrane compartments, an important factor for the success of localization studies is the availability and diversity of specific organellar markers.

In this work, FP fusions with an arabinogalactan protein and a class III peroxidase from *Arabidopsis* were produced and are shown to work as species independent subcellular markers for the plasma membrane and the vacuole, respectively. These fusions were successfully tested by protoplast transient expression in four plant models, *Arabidopsis thaliana*, *Nicotiana tabacum*, *Catharanthus roseus* and *Vitis vinifera*. The respective FP fusions in a binary vector were also successfully tested by agroinfiltration of *N. tabacum* leaves. In conclusion, these two fusions are available for plant biologists to be used as subcellular markers for plasma membrane and vacuole in any plant species.

P340

Shredding the Signal: Systems for Targeting Peptides Degradation in Mitochondria and Chloroplasts

Beata Kmiec¹, Pedro F. Teixeira¹, Monika W. Murcha², Rui Branca³, Janne Lehtiö³, James Whelan^{2,4}, Elzbieta Glaser¹

¹Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, ²ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, Perth, Australia, ³Clinical Proteomics Mass Spectrometry, Department of Oncology-Pathology, Science for Life Laboratory and Karolinska Institutet, Stockholm, Sweden, ⁴Department of Botany, AgriBio, the Centre for AgriBioscience, LaTrobe University, Melbourne, Australia

In plant cells, both mitochondria and chloroplasts contain a multitude of proteolytic systems participating either in targeted proteolysis related to preprotein maturation upon their import into these organelles or in a general degradation of damaged proteins. A consequence of these reactions is the production of presequences, transit peptides and other short peptides that due to their toxic effects must be either exported or degraded. Up to now only the Presequence Protease, PreP, was shown to degrade targeting peptides in the endosymbiotic organelles.

We have recently identified a new component of the organellar proteolytic system in *Arabidopsis thaliana*, the Organellar Oligopeptidase, OOP. OOP is dually targeted to the mitochondrial matrix and the chloroplastic stroma where it degrades short mitochondrial and chloroplastic targeting peptides or their fragments ranging between 8 and 23 a.a in length (partially overlapping with PreP, which cleaves peptides 10-65 a.a long). Functional overlap between PreP and OOP suggests that these two systems might cooperate and complement each other constituting a targeting peptide degradation pathway. In agreement with this prep oop crosses in *Arabidopsis thaliana* display more severe phenotype than prep and oop knockouts alone. Further analysis of the prep oop knockouts might give clues about the role of the organellar peptide degradation in the plant development.

P341

Structural Constrains of Targeting Peptide Degradation by the Mitochondrial and Chloroplactic Organellar OligoPeptidase - OOP

Pedro F. Teixeira¹, Beata Kmiec¹, Rui Branca³, Janne Lehtiö³, Ronnie Berntsson¹, Monika W. Murcha², Pål Stenmark¹, James Whelan^{2,4}, Elzbieta Glaser¹

¹Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden, ²ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, Perth, Australia, ³Clinical Proteomics Mass Spectrometry, Department of Oncology-Pathology, Science for Life Laboratory and Karolinska Institutet, Stockholm, Sweden, ⁴Department of Botany, AgriBio, the Centre for AgriBioscience, LaTrobe University, Melbourne, Australia

Despite containing their own genome, more than 99 % of proteins localized in the mitochondria or the chloroplasts are encoded in the nucleus and synthesized in cytosolic ribosomes. To ensure correct targeting, most proteins destined to the mitochondrial matrix or the chloroplast stroma contain an amino-terminal extension designated targeting sequence. Upon reaching the correct destination these targeting peptides are cleaved by the mitochondrial or stromal processing peptidases, generating free intraorganellar peptides that can potentially impair organellar function. We have recently identified and characterized a component of the targeting peptide degradation pathway in *Arabidopsis thaliana*, the organellar oligopeptidase, OOP (At5g65620). OOP belongs to the M3A family of metalloproteases and is dually localized to mitochondria and chloroplasts. Analysis of peptide degradation by OOP revealed substrate size ranging from 8 to 23 aa residues. Mass spectrometry analysis showed that OOP does not exhibit a strict cleavage pattern but shows a weak preference for hydrophobic residues (F/L) at the P1 position. The crystal structures of OOP, at 1.8–1.9 Å, exhibit an ellipsoidal shape consisting of two major domains enclosing a catalytic cavity of 3,000 Å³. The structural and biochemical data suggest that the protein undergoes conformational changes to allow peptide binding and proteolysis. Our results demonstrate OOP is a member of the targeting-peptide degradation pathway in mitochondria and chloroplasts.

P342

Phenotyping and Beyond: Modelling the Relationships between Traits

Denis Vile¹, Justine Bresson¹, Maryline Lièvre¹, François Vasseur², Crispulo Balsera¹, Alexis Bédiée¹,
Gaëlle Rolland¹, Myriam Dauzat¹, Bertrand Muller¹, Christine Granier¹
¹INRA, Montpellier, France, ²MPI, Tuebingen, Germany

Plant phenotyping technology has become more advanced with the capacity to measure many morphological and physiological traits on a given individual. With increasing automation, getting access to various traits on a high number of genotypes over time raises the need to develop systems for data storage and analyses, all congregating into plant phenotyping pipelines. Here, we highlight several studies that illustrate the latest advances in plant multi-trait phenotyping and discuss future needs to ensure the best use of all these quantitative data. We assert that the next challenge is to disentangle how plant traits are embedded in networks of dependencies (and independencies) by modelling the relationships between them and how these are affected by genetics and environment.

P343

Thermal and Hyperspectral Imaging Techniques as Fast and Non-Destructive Tools for Forest Tree Seeds Screening

Jennifer Dumont¹, Maxime Mistretta¹, Lars Granlund¹, Hirvonen Tapani¹, Markku Nygren², Jouni Hiltunen¹, Porali Ilkka¹, Sarita Keski-Saari¹, Elina Oksanen¹, Markku Hauta-Kasari¹, Markku Keinänen¹

¹University of Eastern Finland, Joensuu, Finland, ²Finnish Forest Research Institute, Suonenjoki, Finland

Seed quality is of prime importance for agriculture and forestry, since it is tightly linked to the resistance to biotic and abiotic stress, the germination rate and the plant performance. Therefore, the international seed industry needs high throughput screening methods. X-ray technique is routinely used as a diagnostic method of tree seed analysis to detect empty seeds, mechanical damage and abnormally developed internal seed structures and to assess the seed viability. Nevertheless, X-ray can cause radiation damages in biological samples and represent a time consuming and costly method. Thermal and hyperspectral imaging are fast, sensitive and non-destructive tools and therefore suitable techniques to rapidly assess seed characteristics and viability. Our study conducted on Scots pine and Norway spruce seeds, shows that thermal and hyperspectral imaging can be considered as an alternative technique for conventional methods in collecting accurate high throughput data on forest tree seeds. These techniques provide a wide range of information that can be linked to the seed biochemical composition and external traits. They may allow simultaneous determination of distribution patterns of several compounds, such as defensive compounds, thanks to their specific absorption patterns in near and middle infrared region.

Acknowledgement: This study is part of the European Union Structural Funds project "Spectral imaging and analysis in environmental and industrial applications" funded by the Finnish Funding Agency for Technology and Innovation (Tekes), filing number 70005/13.

P344

UAV Thermal Imagery Contribution to High Throughput Field Phenotyping of Apple Tree Hybrid Population and Characterization of Genotypic Response to Water Stress

David Gómez-Candón¹, Nicolas Virlet¹, Evelyne Costes², Audrey Jolivot³, Sébastien Martinez², Sylvain Labbé⁴, Jean Luc Regnard¹

¹UMR AGAP, Montpellier SupAgro, Montpellier, France, ²UMR AGAP, INRA, Montpellier, France,

³CIRAD, UMR TETIS, Montpellier, France, ⁴IRSTEA, UMR TETIS, Montpellier, France

High-throughput field phenotyping can be performed by using high resolution multispectral imagery. This presentation focuses on Unmanned Aerial Vehicle (UAV) sensed thermal imagery used to assess the response of an apple population to drought and analyze the genotypic variability of stomatal behavior. Studies were performed in an experimental apple orchard located in Southern France submitted to different water regimes. For remote image acquisition, UAV flights were performed at different dates, hours and altitudes with a thermal camera on board (0.10-0.27m spatial resolution). Temperature of different reference ground targets (hot, cold, wet and dry bare soil) was continuously measured by thermo-radiometers for image radiometric calibration. To assess the effect of image resolution and that of vegetation cover fraction, a sample of 18 apple trees was chosen and the mean canopy temperature (Ts) in tree central zone and its variability were measured. As distortions were revealed in Ts, it seemed advisable to separate mixed pixels (including shaded leaves and soil) from well-illuminated vegetation. Considering the effect of altitude, standard deviation of Ts increased according to the image resolution, and this was particularly true where resolution was close to leaf average size (0.10m²). Thanks to the ultra-high resolution of remote images obtained, and beyond capacity of the approach to delineate efficiently each individual tree within the whole trial, it was possible to analyze inter- and intra-canopy thermal variations. By using image-based vegetation and stress indices as phenotypic variables, genetic dissection of the traits is currently undertaken and first results will be presented.

P345

Hit-Me: High Throughput Metabolic Phenotyping in PHENOME

Camille Benard^{1,2}, Yves Gibon^{1,2}, Duyen Prodhomme^{1,2}, Patricia Ballias^{1,2}

¹*Institut National de la Recherche Agronomique (INRA), Villenave d'Ornon, France,* ²*Plateforme Métabolome du Centre de Génomique Fonctionnelle Bordeaux (CGFB métabolome), Villenave d'Ornon, France*

PHENOME is a French national project co-funded by ANR aimed at providing an up-to-date, versatile and high throughput infrastructure as well as a suite of methods allowing the characterisation of panels of genotypes of different species under scenarios associated with climate changes. The project objectives are to:

- Build or upgrade highly instrumented platforms in seven French sites to deal with the most important crops, biological questions and environmental stresses
- Develop new sensors, methods, data analysis and databases able to cope with millions of data points
- Disseminate techniques and methods towards the phenotyping community

An originality of PHENOME is the possibility to range from the field down to the cellular level. Indeed, there is increasing evidence that metabolic traits could be used as powerful tools in plant breeding. The Hit-Me node located at INRA-Bordeaux is a nearly unique facility enabling the quantification of metabolites and enzyme activities in very large numbers of samples thanks to the use of laboratory automation. New facilities for sample storage, grinding and distribution are being implemented. A new robotized pipetting station allows fully automated sample processing, from extraction to data reading. Improvements include automated capping/decapping of tubes, centrifugation and spectrophotometry reading. At the time Hit-Me has a capacity of 300,000 analyses per year, the aim is to reach 106 within two years. The next steps will be the implementation of a barcoding system and the enhancement of the present version of the laboratory information management system, to improve sample traceability, data handling and ultimately data mining.

P346

Lessons from the Moss *Physcomitrella patens* on Mechanism and Evolution of Protection Mechanisms from Excess Illumination

Caterina Gerotto, Cinzia Franchin, Giorgio Arrigoni, Tomas Morosinotto
Università di Padova, Padova, Italy

Sunlight provides energy supporting life of photosynthetic organisms but, when in excess, it also leads to the formation of harmful reactive oxygen species. One main mechanism to avoid this stress is the so called Non Photochemical Quenching (NPQ) which allows the safe dissipation of excess energy as heat. Although widespread among oxygenic photosynthetic organisms, NPQ in land plants depends on the presence of PsbS, while a different protein, called LHCSR, is required in algae. We demonstrated that in the moss *Physcomitrella patens* both proteins are present and active in NPQ. We also investigated Charophyta, the algae which later diverged from plants and found that only the closest to plants also show PSBS-dependent NPQ, while the other relies on LHCSR. These results suggest that upon land colonization photosynthetic organisms evolved a new PSBS-dependent mechanism for NPQ before losing the ancestral one found in algae, also suggesting a point during plants evolution where this might have occurred.

This work also provided new information on NPQ mechanism. Analysis of *P.patens* plants depleted/overexpressing PSBS/LHCSR showed in fact that they work independently and that their molecular mechanisms are different. While LHCSR is active directly on NPQ, PSBS works indirectly by inducing a conformational change in antenna proteins that are the final responsible of energy dissipation. We also purified PSBS from *P.patens* plants overexpressing the protein. Interestingly other polypeptides were co-purified with which were identified by mass spectrometry as specific antenna subunits which are thus most likely the responsible of energy dissipation.

P347

Physiological Responses of *Fagus sylvatica* and *Quercus robur* Seedlings to Light Intensity

Ignacio Sevillano^{1,2}, Ian Short¹, Conor O'Reilly²

¹Teagasc (Forestry Development Department), Dublin, Ireland, ²University College Dublin (UCD Forestry), Dublin, Ireland

Broadleaf planting has become increasingly important in Ireland over the recent years and light is recognised as one of the main environmental factors affecting stand development, but to date there has been little research on broadleaf responses to light intensity, particularly in Ireland. Continuous cover forestry (CCF) is increasing as an alternative to clear-cutting and uses the control of light to produce plantation benefits. The physiological responses of beech (*Fagus sylvatica*) and oak (*Quercus robur*) seedlings to four different shade environments (100%, 62%, 51% and 28% of incident photosynthetically active radiation, PAR) were studied in a shadehouse experiment during the summer of 2013.

Light-response curves (only measured in the extreme treatments) differed between beech seedlings of the Control treatment (PAR=100%) and those of the heavy shade treatment (PAR=28%) when $PAR > 100 \mu\text{mol m}^{-2} \text{s}^{-1}$, while there was little difference for oak between both treatments. Light-response curves generally showed maximum photosynthetic rates (A_{max}) at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ in both treatments, and this PAR value was used while measuring stomatal conductance (g_s) and water use efficiency (WUE). Significantly higher values of A_{max} and g_s were found in oak than in beech in the 62%, 51% and 28% treatments. There were no significant differences between oak and beech for A_{max} and g_s in the Control treatment or for WUE in the four treatments.

P348

Overexpression of Chloroplast NADPH-Dependent Thioredoxin Reductase Enhances Leaf Growth in Arabidopsis

Jouni Toivola¹, Lauri Nikkanen¹, Käthe M. Dahlström², Tiina A. Salminen², Anna Lepistö¹, Florence Vignols³, Eevi Rintamäki¹

¹University of Turku, Turku, Finland, ²Åbo Akademi University, Turku, Finland, ³UMR186 IRD-University of Montpellier2-CIRAD, Montpellier, France

Thioredoxins are crucial components of the regulatory redox networks in all living cells. Plant chloroplasts have versatile thioredoxin systems including two thioredoxin reductases and multiple types of thioredoxins. Plastid-localized NADPH-dependent thioredoxin reductase (NTRC) forms a complete thioredoxin system including both reductase (NTRd) and thioredoxin (TRXd) domains in a single polypeptide. To study the action of NTRC and NTRC domains *in vivo*, we have complemented the *ntrc* knockout line of Arabidopsis with the wild type and full-length *NTRC* genes, in which 2-Cys motifs either in NTRd, or in TRXd were inactivated. Overexpression of wild-type *NTRC* (OE-NTRC) promoted plant growth by increasing the number and size of leaves. Increase in growth light intensity promoted biomass production in OE-NTRC lines. The dry weight of the OE-NTRC rosettes varied from 120 % to 190 % of wild type rosette dry weight in the independent OE-NTRC lines grown under moderate light intensity. Overexpression of NTRC increased starch accumulation in leaves. Complementation of the *ntrc* line by the full-length *NTRC* gene with active reductase but inactive thioredoxin, or vice versa, partly recovered rosette biomass yield, suggesting that the NTRC domains are capable of interacting with ferredoxin-dependent thioredoxin system. We propose that overexpression of NTRC promotes plant biomass yield both by stimulation of chloroplast biosynthetic and protected pathways controlled by NTRC. Our data indicate that overexpression of chloroplast thiol redox-regulator has a potential to increase biofuel yield in plant species suitable for sustainable bioenergy production.

P349

Exogenous H₂O₂ Could Prevent the Paraquat Mediated Oxidative Stress on Energy Distribution and Integrity of Thylakoid Membranes

Svetozar Stoichev¹, Iren Tzonova¹, Irina Moskova², Sashka Krumova¹, Vera Alexsieva², Mira Busheva¹

¹*Institute of biophysics and biomedical engineering, Sofia, Bulgaria,* ²*Institute of plant physiology and genetics, Sofia, Bulgaria*

The current investigation provides evidences that PQ treatment lowered the photosynthetic efficiency deducing PSII quantum yield (as suggested by 77K fluorescence) and reduced peripheral antennae of the PSII supercomplex (as suggested by CD spectroscopy) changing the supercomplex assembly. Considering the recovery of pigment amount, scarcity of lipid peroxidation and enhanced activity of antioxidant enzymes, we could assume that the low concentrations of H₂O₂ prevent to some extent the LHC assign contribution to fluorescence, depressed by PQ toxicity, and therefore normalized the membrane integrity and energy supply to photosystems. Combine with previous investigations we further deduced, that pretreatment of pea plants with exogenous H₂O₂ alleviate the PQ mediated oxidative stress on light reactions in photosystems in the level of thylakoid membranes. This study was supported by the National Science Fund of the Bulgarian Ministry of Education and Science, Project DMU 03-60 /12-15.

P350

Gas Exchange Regulation by Whole Plant Hydraulic Architecture in *Pinus radiata* Clones

Juan Rodríguez-Gamir¹, Jianming Xue¹, Jean-Christophe Domec², Michael J. Clearwater³, Peter Clinton¹

¹SCION, Christchurch, New Zealand, ²Duke University, Durham, USA, ³University of Waikato, Hamilton, New Zealand

Plant hydraulic conductance exerts a strong influence on many aspects of plant physiology, namely: gas exchange, growth, productivity or stress response. Studies suggest that hydraulic conductance of different plant organs such as leaves, stems or root can regulate the gas exchange parameters. However, there is a lack of understanding about the integration of whole woody plant hydraulics and its implication in water relations and gas exchange. Here we tested the hypothesis that whole plant hydraulic conductance modulates plant transpiration and photosynthesis using nine different clones of 2-years old *Pinus radiata* D. Don. Root and shoot hydraulic conductance was measured with a hydraulic conductance flow meter (HCFM) (Dynamax, Houston, TX, USA). With them, the whole plant hydraulic conductance was calculated. Gas exchange parameters were measured with a LI-6400 Portable Photosynthesis System (Li-COR, Lincoln, NE, USA) and whole plant transpiration was gravimetrically determined.

Significant differences were found among clones in root, shoot and whole plant hydraulic conductance values and in the normalized values of these parameters that were scaled by dividing by root or leaf biomass. Additionally, the contribution of root or shoot hydraulic conductance to whole plant hydraulic conductance differed significantly among the clones. Our results showed that whole plant transpiration was highly dependent on whole plant hydraulic conductance and clones with more efficient whole plant hydraulic architecture and more efficient root system had higher net photosynthesis in leaves.

P351

Identification of New Phosphorylation Sites in Key C4 Maize Enzymes - A Proteomics Approach

Bruno Alexandre¹, Ana Paula Farinha¹, Nelson J. M. Saibo^{1,2}, M. Margarida Oliveira^{1,2}, Isabel A. Abreu^{1,2}

¹*Instituto Biologia Experimental e Tecnológica (iBET), Oeiras, Portugal,* ²*Instituto de Tecnologia Química e Biológica (ITQB) da Universidade Nova de Lisboa, Oeiras, Portugal*

Phosphoenolpyruvate carboxylase (PEPC), NADP-malic enzyme (NADP-ME), and pyruvate orthophosphate dikinase (PPDK) are key enzymes in the photosynthesis of C4 plants. Distinct isoforms of PEPC, PPDK and NADP-ME were detected by 2-DE/Western-blot followed by MALDI-TOF-TOF analysis in maize leaves (3rd leaf) from 12-day-old seedlings, collected along several time points during the 16h light/8h dark photoperiod. Specific staining of phosphoproteins (Pro-Q Diamond) followed by staining with Sypro Ruby was performed to assess differences in the phosphorylation level of these enzymes. For PEPC, a higher level of phosphorylation was detected at 4 hours of light period, decreasing until 12 hours and increasing during the pre-dawn period (30 min before light). A positional shift towards the basic end of the gel was observed by 2-DE/Western-blot in the 4h light sample after alkaline phosphatase treatment compared with untreated sample, suggesting intense phosphorylation at this time point. For NADP-ME, five distinct isoforms were detected, with pH ranging between 4.8 and 5.2. PPDK isoforms were also detected in a pH range from 5.0 to 5.5. In order to assess the nature of the different isoforms, we performed phosphoproteomics analyses of 1DE gel slices containing our target-enzymes in a LIT Orbitrap Spectrometer and we were able to detect several new putative phosphorylation sites. We are now validating these putative phosphorylation sites and assessing their functional role.

Acknowledgements:

3to4 (FP7-KBBE-2011-5, Collaborative Project n.289582), Pest-OE/EBQ/LA0004/2011, IAA(SFRH/BPD/78314/2011), NJMS(Programa-Ciência and FCT Investigator (POPH)), Sheffield University Mass Spectrometry Facility.

P352

Soluble Carbohydrates and Chlorophyll Contents in *Brassicaceae* Microgreens as Affected by Light Intensity and Spectrum

Ausra Brazaityte¹, Giedre Samuoliene¹, Jule Jankauskiene¹, Sandra Sakalauskiene¹, Akvile Virsile¹, Ramunas Sirtautas¹, Algirdas Novickovas², Jurga Miliauskiene¹, Viktorija Vastakaite¹, Laurynas Dabasinskas², Pavelas Duchovskis¹

¹*Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Kaunas district, Lithuania,* ²*Institute of Applied Research, Vilnius University, Vilnius, Lithuania*

The objective of our studies was to evaluate the effect of light intensity and spectrum produced by light-emitting diodes on changes of soluble carbohydrates and chlorophyll content in *Brassicaceae* microgreens. Mustard, red pak choi and tatsoi were grown in phytotron chambers to harvest time. Day/night temperature 21/17°C, 16 h photoperiod and ~70% relative air humidity were maintained. Five lighting modules with the basal 455, 638, 665 and 731 nm LEDs were used in the experiments. Two experiments were carried out: 1st – evaluation of illumination intensity of 545, 440, 330, 220 and 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 2nd – evaluation of the effects of 520, 595, or 622 nm LEDs supplemental for the basal set of LEDs. PPFD level in this experiment was maintained at 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Our results revealed that the highest content of soluble carbohydrates in mustard and red pak choi was under light intensity of 440 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and in tatsoi - under 330 $\mu\text{mol m}^{-2} \text{s}^{-1}$. 110–120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD lighting was unfavorable for their synthesis. The highest chlorophyll content was determined under light intensity of 440–545 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Supplemental light of various wavelengths had no effect on chlorophyll content and uneven impact on carbohydrates in microgreens. All supplemental wavelengths increased carbohydrates content in tatsoi, but had no effect on their content in red pak choi. Carbohydrates content significantly increased in tatsoi under supplemental 622 nm light.

Acknowledgements. This research was funded by a grant (No. SVE-03/2011) from the Research Council of Lithuania.

P353

The Impact of Light-Emitting Diodes and High-Pressure Sodium Lamps Lighting on the Photosynthesis Parameters of Tomato Transplants

Ausra Brazaityte, Aiste Bagdonaviciene, Jule Jankauskiene, Giedre Samuoliene, Akvile Virsile, Sandra Sakalauskiene, Ramunas Sirtautas, Jurga Miliauskiene, Pavelas Duchovskis
Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Kaunas district, Lithuania

The aim of this study was to assess the effect of supplementary blue (455, 470 nm), cyan (505 nm) and green (530 nm) light-emitting diodes (LEDs) to high pressure sodium (HPS) lamps on photosynthesis parameters of tomato hybrid 'Magnus' F₁ transplants. Experiment was performed in greenhouse of Institute of Horticulture, LCAFS. The transplants were grown 30 days in peat:vermiculite (1:1) substrate under 16 h photoperiod, the day/night temperature was 19-25°C/14-16°C and relative air humidity - 50-60%. Four types of solid-state lamps were designed using high-power AlInGaN LEDs: 455, 470, 505 and 530 nm. The generated photosynthetic photon flux density (PPFD) of each type of solid-state modules was ~15 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and of HPS lamps was ~180 $\mu\text{mol m}^{-2}\text{s}^{-1}$. As reference transplants were growth under HPS, PPFD ~200 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Measurements of gas exchange parameters, chlorophylls content, leaf area, fresh weight and development stage were evaluated in the end of experiment. The results revealed that both supplemental blue and green LEDs decreased photosynthetic and transpiration rate, reduced water use efficiency compared to the reference treatment. HPS lamps without LEDs had positive effect on photosynthesis rate, but transplants were elongated and delayed in the development. The increase of photosynthetic pigments was determined under supplemental blue 455 nm and cyan 505 nm lighting and stomatal area and frequency, cells size, leaf area and fresh weight under supplemental blue 455 nm. Thereby HPS lamps supplemented with 455 nm LEDs was useful for normal functioning of photosynthetic processes and transplants growth.

P354

A Two-Dimensional Micro-Scale Model of Gas Exchange during Photosynthesis in Maize (*Zea mays* L.) Leaves

Moges Ashagrie Retta¹, Quang Tri Ho¹, Xinyou Yin², Pieter Verboven¹, Herman N.C. Berghuijs², Bart M. Nicolai¹, Paul C. Struik²

¹*KU Leuven, Leuven, Belgium,* ²*Wageningen University, Wageningen, The Netherlands*

Gas exchange in leaves of C4 plants was investigated using a microscale model of combined gas diffusion and C4 photosynthesis kinetics. Maize (*Zea mays* L.) leaf was chosen as the model system. The actual 2-D microstructures of a leaves were incorporated into the microscale model. The uptake of CO₂ is facilitated by the carbonic anhydrase enzyme that converts it to bicarbonate ions. This step is taken into account in the model by rewriting the kinetic equation for CO₂ limited rate of phosphoenolpyruvate carboxylation using concentration of bicarbonate ions instead of CO₂ concentration. The model was validated using combined gas exchange and chlorophyll fluorescence measurements on young, full-grown leaves and old leaves from plants grown under high or low nitrogen levels. In general, there was good agreement between model predictions of photosynthesis and experimental data. The model provided detailed insight into CO₂ exchange and photosynthesis. In addition, the effect of nitrogen supply on leaf microstructure and, hence, gas exchange characteristics such as bundle sheath conductance was quantified.

Key words: C4 photosynthesis, gas exchange, bundle sheath conductance, CO₂ concentration mechanism, leaf microstructure

P355

Cold Storage to Overcome Dormancy Affects the Carbohydrate Status and Photosynthetic Capacity of *Rhododendron simsii*

Annelies Christiaens^{1,2}, Peter Lootens³, Bruno Gobin², Marie-Christine Van Labeke¹

¹Department of Plant Production, Ghent University, Ghent, Belgium, ²PCS Ornamental Plant Research, Destelbergen, Belgium, ³Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit, Melle, Belgium

Global warming leads to increasing irregular and unexpected warm spells during autumn, and therefore natural chilling requirements to break dormancy are at risk. Controlled cold treatment can provide an answer to this problem. Nevertheless, artificial cold treatment will have its consequences on carbon reserves and photosynthesis. We have looked at the effect of dark cold storage at 7°C to break flower bud dormancy in the evergreen *Rhododendron simsii*. Carbohydrate and starch content in leaves of an early ('Nordlicht'), and late ('Thesla') flowering cultivar showed that carbon loss due to respiration lead to a serious reduction in starch reserves at the end of cold treatment. Photosynthetic measurements showed that during cold treatment dark respiration decreased by 58% and 63% for 'Nordlicht' and 'Thesla', respectively. Immediately after cold treatment dark respiration increased and stabilized after 3 days. The light compensation point followed the same trend as dark respiration. The quantum efficiency showed no significant changes during the first days after cold treatment, but was significantly higher than in plants with dormant flower buds, at the start of cold treatment. In conclusion, photosynthesis stabilized 3 days after cold treatment and was improved compared to the level before cold treatment.

P356

Changing Sink Demand Affects Photosynthesis and Carbohydrate Partitioning in Olive (*Olea europaea* L.)

Afef Haouari^{1,3}, Marie-Christine Van Labeke¹, Mohamed Braham², Mohamed Chaieb³
¹Department of Plant Production, Ghent University, Ghent, Belgium, ²Institute of the Olive Tree, Sousse, Tunisia, ³Laboratory of Plant Biology, Faculty of Sciences of Sfax, Sfax, Tunisia

Regulation of the balance between vegetative and reproductive growth determines the yield of olive trees. To better understand the interaction between vegetative growth and fruiting, we manipulated the sink demand by girdling, decapitation and two leaf-to-fruit ratios and studied their effects on photosynthesis and carbohydrate partitioning between source and sink organs. At pre-flowering six trees of the olive cultivar 'Picholine' were selected, three trees with decapitation of the young shoots while the other trees remained intact. On each tree two source-sink ratios 1 inflorescence/1 leaf (1/1) and 1 inflorescence/3 leaves (1/3) were imposed on 1-year-old shoots this respectively for girdled and ungirdled branches.

Our results showed that soluble sugars and starch content of leaves was significantly influenced by decapitation and girdling. However, no difference in carbohydrate content was found between the two source-sink ratios. Fruit sugar contents were not significantly affected by the different treatments (girdling, decapitation and source-sink ratio). The large availability of assimilates by girdling and decapitation decreased net photosynthesis, stomatal conductance and intercellular CO₂ concentration. In ungirdled branches, the decapitation decreased net photosynthesis by 14% on average for the two ratios and by 27% in girdled branches. The effect of girdling was more pronounced for decapitated shoots than the control. The data give valuable information to optimize cultural practices.

P357

Internal and External Factors Affecting Pigment Composition in Plants: A Meta-Analytic Approach

Raquel Esteban¹, Beatriz Fernandez-Marin², Jose Ignacio Garcia-Plazaola², Antonio Hernandez², Unai Artetxe², Oihana Barrutia²

¹*Agrobiotechnology Institute, IdAB-CSICUPNA-Gobierno de Navarra, Pamplona, Spain,* ²*Department of Plant Biology and Ecology, University of Basque Country (UPV/EHU), Bilbao, Spain*

Photosynthetic pigment composition has been a major target of study in plant ecophysiology during the last three decades. The development of highly resolutive HPLC techniques has allowed the generation of a vast amount of information, but a complete compilation of the existent literature is still lacking. In the present study we aim to fulfill this gap, and specifically we wonder whether pigment composition: (i), is the same for all unstressed plants; (ii), fits the expected from the thylakoidal polypeptide composition and (iii), is constrained/modulated by environmental/phylogenetic factors. Searching on the "ISI Web of Science", 2306 articles describing pigment composition were initially selected. After the application of several filters and inclusion criteria, 526 articles were finally selected and a database containing data for 809 species was constructed. Results show that pigment composition is universal, however, there are important quantitative differences between green algae and plants. Among non-stressed plants, pigment contents were remarkably consistent with biochemical literature on thylakoidal protein composition. When stress responses were reviewed, xanthophyll cycle (VAZ) pool was the only to increase dramatically. Present results highlight the existence of narrow limits for some pigment ratios and also support the presence of a pool of free "unbound" VAZ. Values reported here could be considered as references for future studies on pigment composition.

P358

Regulation of Photosynthetic Activity by Light in the Unicellular Acidophilic Red Alga *Cyanidioschyzon merolae*

Ilona Baclawska, Eugeniusz Parys, Elzbieta Romanowska

Department of Molecular Plant Physiology, Warsaw University, Warsaw, Poland

Cyanidioschyzon merolae is unicellular, ultrasmall eukaryotic and autotrophic red alga living in very acidic sulfate-rich hot springs. Photosystem I (PSI) contains phycobilisomes and Lhcr as the light-harvesting antennae while photosystem II (PSII) contains only phycobilisomes. In this study synchronized and nonsynchronized *C. merolae* cells were grown in artificial medium (pH 2.5, 42°C) at various light conditions. We examined photosynthesis rate of cells, activity of photosystems (PSI and PSII) in isolated thylakoids. Also absorption spectra at room temperature and at 77K fluorescence spectra of thylakoids, algae grown under LL, ML and HL (35, 80 and 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively), were measured.

Rate of photosynthesis (CO_2 uptake and O_2 evolution) was higher in synchronized than nonsynchronized cultures what correlated with higher PSI and PSII activity in thylakoids. Light intensity during growth did not change PSI activity, while the PSII activity was higher under low light intensity in comparison with high light. Interestingly photosynthesis rate was not inhibited by oxygen in cells grown under low CO_2 , which was present under high CO_2 concentration.

Our results indicate that synchronized and nonsynchronized cells have different amount of phycobiliproteins. Maximum fluorescence of PSI at 77 K was higher in synchronized cultures compared to nonsynchronized. We found, that the *C. merolae* does not possess CO_2 -concentrating mechanism (CCM) and PSI play crucial role to maintain photosynthetic activity.

P359

Presence of PsaA Protein in Dark Grown Seedlings of Pea and Bean

Lucja Rudowska, Justyna Obrebska, Radoslaw Mazur, Piotr Kozlowski, Joanna Trzcinska-Danielewicz, Agnieszka Mostowska
University of Warsaw, Faculty of Biology, Warsaw, Poland

Chloroplast biogenesis is a complex process that requires coordination of light induced changes at various levels of cell organization from gene expression to the ultrastructure of the developing chloroplast.

For our experiments we selected two species which differ in low temperature tolerance: Bean (*Phaseolus coccineus*), a chilling sensitive plant (CS) and Pea (*Pisum sativum*) a chilling tolerant plant, both from a temperate climate.

Our scotomorphogenetic (etiolated) model of growth represents the initial seedling growth occurring beneath the soil surface where etioplast with a paracrystalline prolamellar body (PLB) differentiates into mature chloroplast with thylakoid arrangements.

In this study we focused on changes in the presence of main photosynthetic proteins during the chloroplast biogenesis process in optimal temperature and in chilling-stress conditions.

We observed that the profiles of the examined protein appearance differed in stressed and control plants. For further analyses we selected PsaA protein, its presence was surprisingly detected in dark grown seedlings. Using the Western-blot technique we localized PsaA in pea protein extracts of etiolated control and chilled plants while in bean material the presence of PsaA was detected in chilled plants only. By Northern blot analyses we localized *psaa* gene products in all examined RNA extracts from the light and dark grown plants.

We excluded the role of green light used in all experiments in the darkness, in the regulation of PsaA translation. We also checked the levels of the PsaA protein and the corresponding RNA during the etiolation process in control and chilling conditions.

P360

Novel Transcription Factors Binding to ZmNADP-ME and ZmPEPC1 Gene Promoters

Ana Rita Borba^{1,2}, Tânia Serra^{1,2}, Alicja Górska^{1,2}, Maria Margarida Oliveira^{1,2}, Nelson Saibo^{1,2}
¹IBET, Oeiras, Portugal, ²ITQB, Oeiras, Portugal

C4 plants are more productive than C3 plants, due to a higher CO₂ assimilation rate associated with improved water and nitrogen use efficiency. A key feature of the C4 metabolism is compartmentalization of photosynthetic reactions between mesophyll (M) and bundle sheath (BS). In maize, CO₂ is fixed by PEPC in the M, where the resulting C4 acid is subsequently converted into malate. Then, malate is transported to the BS cells where it is decarboxylated by the NADP-ME in the chloroplasts to release CO₂ near RuBisCO. The compartmentalization of photosynthetic enzymes requires sophisticated transcriptional regulatory mechanisms. This work aims to identify and functionally characterize novel transcription factors (TFs) regulating ZmNADP-ME and ZmPEPC1 gene expression. Several TFs were already identified by Y1H, using the promoters as baits to screen maize leaf cDNA expression library. Different bHLH TFs were found to interact with the ZmNADP-ME gene promoter, being candidates to regulate its cell-specific gene expression. Among the proteins identified as binding to ZmPEPC1 promoter, we have TFs from different families: HB, CTT, and CPP. The DNA-protein interactions identified in yeast will be further validated by EMSA. The novel TFs will also be characterized regarding their protein-protein interactions (Y2H and BiFC) and transcriptional activity. To assess cell localization (M and/or BS), GFP fusion constructs will be bombard into maize leaves. A mutant/transgenic approach will also be used to further characterize the biological function of the identified TFs.

Acknowledgments: Work supported by EU project 3to4 and Pest-OE/EBQ/LA0004/2011 (FCT). AG (SFRH/BD/89743/2012) and NS (FCT-Investigator) funded by FCT

P361

Foreign Protein Accumulation in Chloroplast Using Thylakoid Targeted Transit Peptides

Sang Hoon Ma, Ah Young Kim, Se Hee Park, Hyun Min Kim, Ji Sun Park, Seo Young Park, Mi Jin Jeon, Young Hee Joung*

Chonnam National University, Gwang-ju, Republic of Korea

Oxygen-evolving protein is one of the chloroplast thylakoid lumen targeted protein and it has transit peptide for chloroplast targeting. Four different transit peptides (TP1-4) were isolated from tobacco oxygen-evolving protein genes and fused to gfp gene. The TP fused gfp constructs were transformed into tobacco plant *Nicotiana tabacum* cv. Xanthi NC. The transit peptide fused gfp transgenic tobacco plants showed obviously increased GFP expression level compared to without transit peptide gfp transgenic plants. Especially TP1 fused gfp transgenic plants showed 50 folds higher expression level of GFP than without transit peptide. We observed that the transit peptide fused GFP was localized into chloroplast thylakoid and the transit peptide were removed from GFP after translocation. Several genes of plant enzymes, such as glycosyltransferase and P450s, were fused to TP1 and transiently expressed in tobacco leaves using Agro-infiltration. The glycosyltransferase and P450 enzyme were highly expressed in chloroplast using TP1 fused constructs, while the enzymes were not detected using without TP1.

P362

Red, Orange, and Green Narrow-Band LED Lighting Impairs Photosynthesis and Growth in Barley Seedlings

Olga Avercheva, Elizaveta Bassarskaya, Galina Kochetova, Tatiana Zhigalova
Lomonosov Moscow State University, Moscow, Russia

Light spectral quality determines plant growth and morphology through the photoreceptor system. It also determines the development and function of the photosynthetic apparatus, which supplies the plant with organic compounds needed for growth. We studied morphological and photosynthetic parameters in 9-day-old barley (*Hordeum vulgare* L.) seedlings grown with 60-70 $\mu\text{mol PAR}/(\text{m}^2 \text{ s})$ and LED light sources with narrow-band light of different spectrum: red (emission maximum 660 nm), orange (590 nm), green (535 nm), and blue (450 nm). Plants grown with fluorescent lamps were used as a control. Plants grown with blue light showed higher photosynthetic parameters than control plants, such as photophosphorylation rate in isolated thylakoids and qP parameter of chlorophyll fluorescence, but did not show any increase in biomass. Plants grown with red, orange, and green light showed lower photosynthetic parameters characterizing photophosphorylation rate and electron transport chain functioning. They also showed lower shoot dry weight or lower weight of the second leaf. Before 9 days, plants get most of their nutrition from the seed; however, photosynthesis may already play a role as a source of organic compounds. Our results show that lower mass accumulation and second leaf growth, observed in plants grown with red, orange, and green light, may be due to impaired photosynthesis. The reason for this may be photomorphogenic effects of narrow-band light which do not allow the photosynthetic apparatus to develop properly. However, higher photosynthetic parameters do not lead to increased biomass and faster growth, as seen from plants grown with blue light.

P363

Regulation of Light-(In)dependent Chlorophyll Biosynthesis in Response to Low Temperature in Norway Spruce (*Picea abies* Karst.)

Tibor Stolarik¹, Andrej Pavlovic^{1,2}, Petr Ilík¹

¹Department of Biophysics, Centre of the region Haná for Agricultural and Biotechnological Research, Palacký University in Olomouc, Olomouc, Czech Republic, ²Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia

Chlorophyll biosynthesis is strictly light-dependent in angiosperm plants at the photoreduction step of protochlorophyllide (Pchl_{id}) to chlorophyllide (Chl_{id}). This reaction is catalysed by the nuclear encoded light-dependent protochlorophyllide oxidoreductase (LPOR). On the other hand, seedlings of many gymnosperms are able to synthesize chlorophyll also in the dark by the catalytic activity of the light-independent protochlorophyllide oxidoreductase (DPOR), which is composed of three subunits (Chl_{IN}, Chl_{IB}, Chl_{IN}) encoded by plastid genome. Whereas regulation of light-dependent pathway is now well recognized, the regulation of light-independent pathway is not well known. For example, it was found that low temperature (~ 8°C) strongly inhibits chlorophyll biosynthesis in pine seedlings in the dark (Muramatsu et al., 2001). In this study, we focused on the key enzymes in the Chl biosynthetic pathway: glutamyl-t-RNA reductase (a key enzyme in aminolevulinic acid synthesis), DPOR and LPOR to reveal regulatory mechanisms responsible for etiolation of Norway spruce seedlings at low temperature in the dark. We also investigated the pigment-protein complexes organization by measurements of 77K fluorescence and Western blots. The photoactivation of latent photosystem II developed in dark at different temperatures was evaluated by fast chlorophyll a fluorescence transient.

References: Muramatsu S., Kojima K., Igasaki T, Azumi Y, Shinohara K. (2001) Plant Cell Physiol 42: 868-872.

P364

The Control over Assimilate Allocation to Activate Root Growth and Increase Nitrogen Assimilation from the Atmosphere

Vladimir Chikov, Svetlana Batasheva, Guzel Akhtyamova
Kazan Institute of Biochemistry and Biophysics, Kazan, Russia

It was shown under model conditions that acidification of atmosphere around the plant with HCl vapours or alkalization with NH₃ vapours resulted in opposite changes of photosynthesis and ¹⁴C-assimilate contents in the apoplast of leaves. On the basis of lower apoplastic invertase activity in alkaline environments we used complex compounds of ammonia with zinc and cuprum (ammoniates) with the common formula {[Cu Zn (NH₃)_n]⁺²• A⁻²}, where A is carbonate or malate anion, to reduce the apoplastic pH. Adsorption of ammoniate metal ions on cell wall polymers results in increased ratio of ¹⁴C-sucrose to ¹⁴C-hexoses and enhanced relative synthesis of ¹⁴C-carbohydrates during photosynthesis in ¹⁴CO₂, making the glycolate pathway more closed to Calvin cycle and thus optimizing photosynthesis. Spraying of attached leaves with ammoniate solutions with concentrations of 10⁻⁵ to 10⁻⁶ M intensifies sugar export from leaves to sink organs (including roots), thus increasing the sink organ growth. The effect lasts as long as the sprayed leaves are functionally active. This effect of ammoniates was observed in different species (sugar beet, potato, flax, spring and winter wheat) and increased with decrease in nitrogen fertilizer contents in soil. The analysis of nitrogen balance in the plant-soil system revealed appearance of additional nitrogen in soil following the plant treatment with ammoniates, which indicates the enhanced nitrogen assimilation by soil microorganisms. Thus, using ammoniates to decrease apoplastic invertase activity it is possible to retain high productivity of plants and accumulate organic nitrogen in soil on the background of lower use of mineral nitrogen fertilizers.

P365

Loci for the Photosynthetic Capacity and Transpiration Efficiency on Wt10245×Wt11238 and Carneval×MP1401 Pea (*Pisum sativum* L.) Maps

Magdalena Gawłowska, Andrzej Gorny, Dominika Ratajczak, Wojciech Swiecicki
1Institute of Plant Genetics Polish Academy of Sciences, Poznan, Poland

Photosynthetic capacity of plant foliage is crucial for biomass production. The major objective of our study was to examine the genotypic variation of components, correlations between them and QTLs of the leaf photosynthesis and seasonal transpiration efficiency (TE; water use efficiency). The materials consist two mapping populations: Wt10245×Wt11238 (conventional×acacia) and Carneval×MP1401 (afila×afila). The plants were grown in a phytotronic chamber under optimal conditions. Major photosynthetic indices were measured using the Licor4600X system, while the seasonal water transpiration was determined using the gravimetric method.

Afila leaves of the Carneval×MP1401 population exhibit a smaller leaf area (64% less), and lower photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (g_s), carboxylation efficiency (P_n/C_i) (17-46% less) than physiological parameters in the second Wt10245×Wt11238 population. One QTL was found for photosynthetic activity and two QTLs for the seasonal TE in the Carneval×MP1401 population. Four QTLs were detected in 2011 and 14 QTLs in 2012 for the Wt10245×Wt11238. Some of photosynthetic capacity loci were overlapped with each other. One locus on the Carneval×MP1401 map was connected with dry matter percentage in the VB linkage group, near the SSR-marker AB83. QTLs responsible for the leaf chlorophyll content, CO_2 assimilation in the Wt10245×Wt11238 map were detected at the similar interval. QTLs connected with stem parameters in the Wt10245×Wt11238 map were localised at the similar interval (te-Pis_GEN_9_3_1). Overlapping of QTLs for photosynthetic and stem properties could be explained by the energy coming from photosynthesis and diverted into the stabilization of plant canopy and leaf tigmotrophic function.

P366

The Protective Role of PsbS Protein in Photo-Oxidative Damage in Photosystem II of Higher Plants

Ravindra Kale, Pavel Pospíšil

Department of Biophysics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Olomouc, Czech Republic

PsbS protein in higher plants play important role in the protection of photosystem II (PSII) from the adverse effects of intensive high light. The essential function of collection and dissipation of light energy in PSII is regulated by the light harvesting complex (LHC). It is well established that PsbS protein has a role in organization of LHCII and PSII core proteins in thylakoid membrane. The protective role of Psbs protein in the photo-oxidative stress was studied in *Arabidopsis thaliana* wild type (ecotype Columbia) and mutants which lack PsbS (npq4) protein and over expressing PsbS protein (OePsbS). Light-induced singlet oxygen (1O_2) formation was measured by 1) electron paramagnetic resonance (EPR) spin-trapping spectroscopy in PSII membrane isolated from *Arabidopsis* leaves and 2) imaging of SOSG fluorescence using laser confocal microscopy. The spatial distribution of photo-oxidative damage in *Arabidopsis* leaves was monitored by two-dimensional ultra-weak photon emission measured using CCD camera. The protective effect of PsbS protein in oxidative damage of PSII protein was studied by 2D-CN/SDS-PAGE.

P367

The Role of Alternative Oxidase Pathway in Arbuscular Mycorrhizal Infection in *Arundo donax*, a New Bioenergy Crop

Nestor Fernandez Del Saz, Antonia Romero-Munar, Elena Baraza, Javier Gulias, Miquel Ribas-Carbo
Universitat de les Illes Balears, Palma de Mallorca, Spain

Giant reed (*Arundo donax* L.) is a perennial rhizomatous grass considered an interesting crop for bioenergy production under Mediterranean conditions. Giant reed is associated with root-colonizing arbuscular mycorrhizal fungi (AM). The global predominance of AM associations, their impact on plant nutrient uptake and their key role in soil carbon (C) input means that mycorrhizal respiration represents an important deficiency in our understanding of plant, fungal and soil physiology. The objectives of the present study were: how photosynthesis and respiration change between AM plants and non AM plants in leaves; and whether there was a role for alternative oxidase pathway (AP) in optimizing symbiosis maintenance in leaves and roots. The study was performed with commercial clone of *A. donax*, one half was infected with commercial AM inoculum at the beginning while the other half was kept uninfected. Plantlets were grown under greenhouse conditions in Mallorca, West Mediterranean Basin. Gas exchange and in vivo respiration were measured in plantlets. The mitochondrial electron partitioning between the cytochrome (COX) and alternative (AOX) pathways and their actual activities were measured by the oxygen-isotope-fractionation technique. Higher net assimilation rate in AM plantlets has been observed while in vivo respirations show no differences in leaves. On the other had, in roots, AM infection caused an increase of the activity of AOX and a decrease of COX, although total respiration rates were similar.

This work was financed by the Spanish Ministry of Science and Innovation (MICINN) - project BFU2011-23294 and OPTIMA EU-FP7 - 289642

P368

Identification of Morphological and Phenological Traits Underlying Sorghum Sweetness Variability and Its Aptitude for Multipurpose (Grain, Sugar, Fodder) in West Africa: Role of Photoperiod Sensitivity

Klanvi Thierry Tovignan¹, Delphine Luquet³, Daniel Fonceka¹, Gilles Trouche³, Ibrahima Ndoye², Ndiaga Cisse¹

¹Centre d'Etude Regional pour l'Amélioration de l'Adaptation à la Sécheresse, CERAAS, Thiès, Senegal, ²Département de Biologie Végétale, Université Cheikh Anta DIOP, UCAD, Dakar, Senegal, ³CIRAD, UMR AGAP, Montpellier, France

Sweet sorghum is highly coveted to contribute to take up food and energy challenges. A collection of 84 accessions of West Africa landraces was screened to identify genotypes and trait combination of interest for multipurpose (sugar/grain/biomass), with a particular focus on the role of photoperiod-sensitivity. Two sowing dates were used (July, August 2012) and highlighted a generally high but variable photoperiod-sensitivity across accessions. Late sowing resulted in shortened vegetative phase and a significant decrease of variables related to plant size, stem sugar, biomass and grain productions. Broad sense heritability and genetic advance were high for most of growth, biomass and sugar related traits, suggesting their interest for breeding. Surprisingly, all traits related to plant size were positively correlated to plant sugar production excepted plant height. A cluster analysis identified three groups, contrasting in their ability to combine sugar, grain or biomass production. No significant difference in stay-green was found among clusters. All clusters gathered tall genotypes, but the two sweet clusters (II and III) were characterized by shortest plants for which tallness was explained by internode number, by contrast with Cluster I, characterized by the longest, less sweet internodes. Cluster II was the most suitable for multipurpose, with 26 genotypes, mainly Caudatum, with the longest cycle, the largest sugar production, due to high sugar concentration and plant biomass, and grain yield. Studying the post-anthesis drought stress of these genotypes will provide further insight on their ability to combine multipurpose and drought tolerance .

Key-words: Sweet sorghum, landraces, photoperiod-sensitivity, stem sugar accumulation, multi-purpose

P369

Transcriptional Response of Sugarcane during Meristem Culture

Thaya Ganzke¹, Joseph Lamendola¹, Meghan Hickey¹, Corban Rivera¹, Beatty Mary², Gina Zastrow-Hayes², Kevin Hayes², [David Hallahan](#)¹

¹DuPont Central Research & Development, Wilmington, DE, USA, ²DuPont Pioneer, Johnston, IA, USA

Sugarcane mericlone may be conducted on semi-solid or in liquid medium. We have employed next-generation sequencing techniques to better understand the response of sugarcane in culture to these different culture regimes. Firstly, RNA prepared from cane stalk apices was subjected to Illumina sequencing to generate a BLAST-searchable transcriptome database. 170×10^6 reads (average length 88bp) were assembled into 100,828 contigs (40.9% > 1kbp) using the Oases short-read assembler. BLAST analysis identified several sugarcane orthologs of known meristem-specific genes. Illumina RNA-seq analysis of transcripts in different tissues from different tissue culture regimes was conducted, and the data aligned to the transcriptome assembly. Analysis of these data using Arrayviewer allowed identification of genes differentially expressed 1) between meristematic and non-meristematic tissue, 2) between different tissue culture regimes (semi-solid and liquid media) and 3) over time in culture in the two different regimes. Expression profiling of key meristematic genes and the general utility of transcriptomic analysis in tissue culture will be discussed.

P370

Plants for Improved Biomass Utilization

Holger Klose^{1,2}, Markus Günl³, Rainer Fischer^{2,4}, Björn Usadel^{2,3}, Ulrich Commandeur²
¹*Institute of Biology I, RWTH Aachen University, Aachen, Germany,* ²*Institute for Molecular Biotechnology (Biology VII), RWTH Aachen University, Aachen, Germany,* ³*Institute of Bio- and Geosciences, IBG-2: Plant Sciences, Forschungszentrum Jülich, Aachen, Germany,* ⁴*Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Aachen, Germany*

Plant biomass is a promising alternative to conventional, non-renewable sources of energy. However, the conversion process required for cellulosic materials has much greater complexity. One bottleneck is the production costs of biomass-degrading enzymes, such as cellulases. A potential approach to solve this obstacle is via a comparably cost efficient production of cellulolytic enzymes in plants. However, as cellulases and other glucanases break down plant cell walls it is important to analyse the impact of heterologously expressed cellulases on normal plant growth and development.

Herein, we explore the impact of different recombinant cellulase expression strategies in tobacco plants, including differential protein targeting and inducible protein expression. We analysed the in planta production of cellulases by detailed phenotypical analyses, such as biochemical characterization of plant cell walls. Constitutive high level expression of a mesophilic cellulase led to detrimental changes in the plant phenotype, including growth and development. The retardation of the enzyme inside the endoplasmic reticulum could not completely avoid this impact on the plant development. The implementation of an alcohol inducible promoter system for cellulase production led to enzyme expression without any significant phenotypical effects on the plant. This system can be used to produce significant amounts of cellulase in plant leaves without detrimental effects on plant development. Additionally the possibility of controlled application and an economically priced inducer underline the potential of this approach for biomass degradation.

P371

Mycorrhizal Inoculation Increases Shoot and Root Biomass in *Oryzopsis miliacea* Seedlings under Water Deficit Conditions

Chayla Achir, Antònia Romero, Elena Baraza, Josep Cifre, Javier Gulías
University of the Balearic Islands, Palma de Mallorca (Islas Baleares), Spain

Several reports suggest that VAM (vesiculo arbuscular mycorrhiza) inoculation can improve plant performance under drought and low nutrient conditions, what can be a key point in sustainable crop bioenergy production. *Oryzopsis miliacea* is a forage species traditionally used in semi-arid regions that has also been used for land restoration and biomass production in Mediterranean marginal lands. The objective of this study was to evaluate the effects of VAM on biomass accumulation and gas exchange of *Oryzopsis miliacea* seedlings under water-deficit conditions. The experiment was carried out under greenhouse conditions during the first growth cycle. Plants were grown in 4L-pots filled with a sterile low-nutrient substrate. Half of the plants were inoculated by using a commercial inoculum containing: *Glomus mosseae* and *Glomus intraradices*. Four treatments were applied: mycorrhizal well-watered and water-deficit plants (Vww, Vwd) and non-mycorrhizal well-watered and water-deficit plants (NVww, NVwd). Biomass accumulation and gas exchange parameters were determined. Inoculated (VAM) plants showed a significantly higher percentage of VAM mycelia in their roots than non-inoculated ones. Shoot and root biomass of VAM plants were significantly higher, up to 7-times in root biomass, than non-VAM ones. As a consequence, the root to shoot ratio was significantly increased in VAM plants. These differences were greater in water-deficit plants than in well-watered ones. Similarly, gas exchange rates were higher in VAM than in non-VAM plants. As a conclusion, mycorrhizal inoculation significantly increased root and shoot biomass, what can increase seedling survival and productivity under low water availability.

P372

OPTimising MISCanthus (OPTIMISC) Biomass Quality for Biobased Applications

R.T van der Weijde¹, A Kiesel², H Muylle³, Y Iqbal², M Mos⁴, O Kalinina², K Farrar⁵, I Donnison⁵, I Roldán-Ruiz³, O Dolstra², I Lewandowski¹, Luisa Trindade¹

¹Wageningen University, Wageningen UR Plant Breeding, Wageningen, The Netherlands, ²University of Hohenheim, Hohenheim, Germany, ³Institute for Agricultural and Fisheries Research, Melle, Belgium, ⁴Blankney Estates, Blankney, UK, ⁵Aberystwyth University, Aberystwyth, UK

With the advent of biorefinery technologies enabling plant biomass to be processed into biobased products, many researchers set out to study and improve candidate biomass crops. One of these candidates is the undomesticated perennial energy grass miscanthus, characterized by a high productivity and resource use efficiency. Within OPTIMISC, an EU-FP7 project, we aim to characterize and optimize biomass quality in different miscanthus species and inter-specific hybrids. The main goal of this project is to identify high-yielding miscanthus genotypes giving rise to biomass of excellent quality for different added value uses and improved feedstocks for multiple plant-derived bioproducts, such as bioethanol, biomass to liquid (BtL) biofuels, heat and power, fibre boards and building materials. The chemical composition and structure of the cell wall plays a major role in biomass quality. Lignin content is in particular of great importance: in general high lignin content results in a higher calorific value for burning but also makes the cellulosic portion recalcitrant to fermentation. In order to optimise the crop composition and quality different experiments are being conducted. These include i) evaluation of the influence of agro-ecological conditions on the composition of miscanthus biomass, by means of a multisite trial; ii) in depth analysis of biomass quality for parameters relevant to different value chains, including evaluation of biomass quality under different harvest regimes of contrasting genotypes and iii) evaluation of the impact of different abiotic stresses on the quality of biomass from miscanthus genotypes with contrasting tolerance to drought, salt, chilling and frost.

P373

Genotyping by Sequencing of a Collection of *Miscanthus* spp. Accessions

Manfred Klaas¹, Paul Cormican², Thibault Michel¹, Mike Jones³, Trevor Hodkinson³, Susanne Barth¹
¹Teagasc Crops Environment and Land Use Programme, Oak Park Crop Research Centre Carlow, Ireland, ²Teagasc Animal and Bioscience Research Department, Animal & Grassland Research and Innovation Centre, Ireland, ³Trinity College Dublin, Trinity Centre for Biodiversity Research, Trinity College, Dublin D2, Ireland

A genotyping by sequencing (GBS) experiment to genotype our living *Miscanthus* spp. collection was initiated. This collection includes ~170 accessions of *M. sinensis*, *M. sachariflorus*, *M. x giganteus* and ~100 hybrids. In addition we analyzed DNAs of several closely related species. Our procedure follows with modifications the protocol from Elshire et al. 2011 (PLoS ONE 6(5): e19379. doi:10.1371). Illumina HiSeq2000 next generation sequencing was applied to generate large numbers of SNPs from sequencing genomic DNA. The SNPs are used as molecular markers to determine the genetic distances between the accessions. To reduce the complexity of the results, the genomic DNA was fragmented by the restriction enzyme PstI. We used a set of 96 adapters with different barcodes and sequenced in parallel 96 samples. After sequencing on the Illumina HiSeq 2000 platform 139 million reads were obtained. We present the analysis of population genetics data after coming through the SNP discovery pipeline.

P374

Effect of Short-Term Chilling Stress on the Photosynthetic Light Reactions of *Miscanthus sacchariflorus* Genotypes

Peter Lootens, Simon Fonteyne, Tom De Swaef, Hilde Muylle, Isabel Roldán-Ruiz
ILVO, Melle, Belgium

Miscanthus is a C4 rhizomatous grass species, originating from Eastern Asia. The genus has become a leading candidate crop for production of lignocellulosic feedstock thanks to high yield of some genotypes. Currently, a single clone, *M. x giganteus*, is used in Europe at commercial scale but its growing area is limited to temperate areas. One of the objectives of the EU-project OPTIMISC is to develop miscanthus varieties that can be grown over a wider range of environments in Europe and preferably on marginal land to overcome competition with feed and food production. We are testing *M. sacchariflorus*, *M. sinensis* and hybrid genotypes of diverse geographical origins for tolerance to chilling temperatures. This would allow expanding the miscanthus cultivation area to colder regions potentially resulting in earlier light capture, a longer growing season and a higher yield. Here we present the reaction of 5 *M. sacchariflorus* genotypes to exposure to chilling temperatures. Chlorophyll fluorescence measurements of plants exposed to 14°C during two days were compared to those of plants grown at 28°C. The maximum quantum efficiency of PSII (Fv/Fm) at 14°C was significantly lower for all genotypes than at 28°C due to a decrease in the fraction of PSII centres able of photochemistry and a sustained down-regulation. During the subsequent nights, the stressed plants were not able to fully recover. The genotypes OPM3 and OPM25 showed the least depression of Fv/Fm after two days of chilling stress and are probably the better adapted to growth at low temperatures.

P375

Environmental Characterisation of Miscanthus in Trial Locations in Europe and Turkey

Christopher Nunn¹, Olena Kalinina², Mensure Ozguven⁴, Heinrich Schüle⁶, Tim Van der Weijde³, Ivan Tarakanov⁵, Astley Hastings⁷, Iris Lewandowski², John Clifton-Brown¹
¹*Aberystwyth University, Aberystwyth, UK*, ²*University of Hohenheim, Hohenheim, Germany*,
³*Wageningen University, Wageningen, The Netherlands*, ⁴*University of Cukurova, Adana, Turkey*,
⁵*Russian State Agrarian University, Moscow, Russia*, ⁶*German Agrarian Centre in Ukraine, Potash, Ukraine*, ⁷*University of Aberdeen, Aberdeen, UK*

The perennial rhizomatous C4 grass, *Miscanthus*, is a potentially high-yielding biofuel crop. The most widely used and studied genotype is the sterile hybrid, *Miscanthus x giganteus*. Reliance on this one clone on a commercial scale incurs risks from biotic and abiotic stresses. As the climate changes, these stresses are likely to increase. This paper is a study of the impact of environment on different *Miscanthus* genotypes with the aim to improve crop germplasm selection for the growing conditions across Europe. Fifteen genotypes were selected, based on high performance in previous trials. These selections included *sinensis*, *sacchariflorus* and eight elite hybrids. Six locations - Turkey, Germany, Ukraine, the Netherlands, Wales and Russia – were planted with plot trials of forty nine plants at 2 m⁻² planting density. To map the changing soil texture and depth across each site, a near time zero soil core was taken from every plot. The average soil depth ranges from 99.5cm ±0.2cm in Ukraine to 38.5cm ±1.2cm in Wales. The German site had significant variation, ranging in depth from 21cm to >1m. At the end of the second growing season average plant height ranged from 170cm in Germany and the Netherlands to 70cm in Wales. The impact of the soil depth and texture on water holding capacity and from this plant growth will be discussed. Further study shows the impact of soil depth and texture on mature crop growth. Yields following the second growth season will be discussed.

P376

Study of Perennial Bioenergy Grasses under Stress Conditions on Mediterranean Areas

Elena Sánchez, Gladys Lino, Claudia Arias, Salvador Nogués
Universitat de Barcelona, Barcelona, Catalonia, Spain

During recent years biofuels have been produced from food crops (1st generation). Biofuel production from these crops is criticized by the deflection away from uses in the food and animal food chain that has increased the interest in developing biofuels produced from non-food biomass (2nd generation). Some perennial grasses (eg. *Arundo donax* L. and *Panicum virgatum* L.) growing in marginal lands avoid to create competition with food production and agricultural land use. These crops are able to survive over prolonged dry periods. Also its high biomass productivity has been observed reducing crop inputs (fertilization, water availability and plant density) and this high yield is furthermore stable in the long-term.

Characteristic stresses of marginal lands (drought (Well watered (field capacity) and water stress (25% field capacity), salinity (S- (Hoagland sol.) and S+ (Hoagland sol. + 16mScm⁻¹NaCl)) were applied to both species during two months. Physiological (Relative water content), photosynthetic and fluorescence parameters (A_{inst} , g_s , F_v/F_m , qP) and growth development (height, leaf number, shoot/root ratio, SLA, LAI, LMA) were measured.

Differences in RWC and decreases in photosynthesis parameters are observed in stress treatments. Significant differences are observed in growth parameters between treatments.

Panicum virgatum L. seems to be more tolerant to stresses separately although *Arundo donax* L, seems to endure better both stresses. The electron transport of photosystem in the thylakoid membrane appear to be very tolerant while the carboxylation process in the Calvin Cycle is most affected.

P377

Differences in Leaf Photosynthesis, Specific Leaf Area and Cold Tolerance for 14 Miscanthus Genotypes

Xiurong Jiao¹, Kirsten Kørup Sorensen¹, Mathias Neumann Andersen¹, Poul Erik Lærke¹, Uffe Jørgensen¹, Thomas Prade², Stanisław Jeżowski³, Szymon Ornatowski³

¹Aarhus University, Department of Agroecology, Foulum, Denmark, ²Swedish University of Agricultural Sciences, Alnarp, Sweden, ³Institute of Plant Genetics of the Polish Academy of Sciences, Poznań, Poland

Miscanthus species, which are C4 perennial grasses, are considered potential bioenergy crops with high production of biomass, low inputs, and low emissions to the environment. However, the productivity is challenged by inhibition of the photosynthetic capacity at temperatures below 15°C. In this study, we tried to identify differences in cold tolerance in order to select genotypes with high photosynthetic capacity in a cool temperate climate.

Fourteen genotypes of *M. sacchariflorus*, *M. sinensis*, *M. tinctorius*, *M. giganteus* and *M. sinensis* × *M. sacchariflorus* were grown under warm (24°C) and cold (14°C) conditions in a controlled environment. Measurements of photosynthetic light response curves, specific leaf area (SLA) and net photosynthetic rate (A) changes over time were made at the two temperatures. Daily shoot growth rates were measured in field trials. Apparent quantum yield (AQY), dark respiration (DR), A_{1500} (A at PAR of 1500 $\mu\text{mol}^{-1} \text{m}^{-2} \text{s}^{-1}$) and A_{sat} (light saturated A) were calculated from the light response curves.

When the growth temperature was decreased from 24°C to 14°C, AQY, DR, A_{1500} and A_{sat} were significantly reduced for all the genotypes, though at different rates. SLA showed a positive linear relationship with A_{1500} at 14°C. After decreasing the temperature to 14°C, A_{1000} declined sharply for all genotypes on the first day, and this trend continued until the sixth day where after it stabilised for most genotypes. The results suggest that SLA and shoot growth rate may be useful parameters for genotype selection for high productivity.

P378

Ouricuri (*Syagrus coronata*) Productivity in Brazil's Semi-Arid Region: Is It Possible to Reconcile Traditional Use and Biofuel Production?

Renato Soares Vanderlei^{1,2}, Flávia de Barros Prado Moura¹, Bárbara Resende de Moraes¹
¹Federal University of Alagoas, Maceió, Alagoas, Brazil, ²Trinity College Dublin, Dublin, Ireland

The Ouricuri (*Syagrus coronata*) is an endemic species of Brazil's northeastern semi-arid region that provides food and shelter for wildlife and resources for low-income populations. This palm tree stand out for its widespread use. It is estimated 60% of oil in each fruit, which may be used for cosmetics, food or biofuel production, and its leaves are used for making brooms, baskets, and roofs of homes. Nevertheless, there is a lack of studies examining the impact of intense leaves extraction on the production of this palm tree's fruits. To analyze the impact of this activity, 30 Ouricuri trees were selected in the Federal University of Alagoas campus. This is an area where leaves are intensively collected by the local population. Data was collected for one year, every 15 days, and quantified by the number of leaves present. Inflorescences, infructescences and mature fruits were also collected if present. All fruits were weighed and measured using a precision scale and digital caliper. Linear regression was performed to analyze the data. A strong correlation between the number of leaves and total number of inflorescence was observed ($y: -4.8122x + 1.2969, p = 0.00002$ and $R^2 = 0.4812$). The withdrawal of Ouricuri leaves results in decreased productivity of inflorescences and, consequently, the number of fruits. Planting Ouricuri in large scale may be an alternative for biofuel production, once the tree has high hardiness and suitability to sandy and silty soils, and can be used to recover highly degraded areas undergoing desertification, contributing for the development of the region with the worst HDI of the country.

P379

Novel Seed Based Miscanthus Hybrids as a Step Forward in Developing Bioenergy Sector

Michal Mos^{1,2}, Chris Ashman², Graham Harding¹, Richard Flavell³, Iain Donnison², John Clifton-Brown²

¹Blankney Estates Limited, Lincoln, UK, ²IBERS, Aberystwyth University, Aberystwyth, UK, ³Ceres Inc., California, USA

Second generation lignocellulose biomass crops such as Miscanthus have many attractive characteristics resulting in high output-input ratios using land more efficiently than first generation bioenergy crops.

Although clone based Miscanthus hybrids have been key to recognizing Miscanthus' biomass potential, poor establishment of Miscanthus x giganteus and high planting costs have created barriers to wide scale crop adoption.

Novel Miscanthus Hybrids are expected to improve biomass quality traits and allow trait selection based on end use. Miscanthus hybrid seed would enable rapid scale up of the crop and will reduce establishment costs, once hybrids are proven and seed-based agronomies are ready to be deployed.

The Miscanthus breeding program at IBERS, Aberystwyth University, UK has produced novel interspecific hybrids. Phenotypic and genotypic characterisation of wild and hybrid germplasm at multiple locations is used to guide selections and cross combinations. Trials in the OPTIMISC (EU-FP7) and GIANT LINK (DEFRA-BBSRC) programmes are testing traits of selections from the Miscanthus breeding program and develop agronomic methods to increase establishment rate and reduce risk.

The existing agronomic techniques have been implemented to reduce the cost and risk of establishment, using to plant a semi-automated planter (common in vegetable production) with a modular plant.

P380

Developing New Hybrids of Rice to Boost Food Security in Egypt

Galal Anis, Ahmed El-Sherif

*Rice Research & Training Center, Field Crops Research Institute, Agricultural Research Center,
33717 Sakha - Kafr El-Sheikh, Egypt., Kafr El-Sheikh, Egypt*

Rice (*Oryza sativa* L.) is the second largest crop grown in the world in terms of both area and production. Hybrid rice covers about 50% or 15 million ha of the total rice area each year in China. The yearly increased of grain yield by hybrid rice can feed more than 70 million people every year. Using hybrid rice with 15-20% yield advantage over improved inbred varieties is the best way to increase rice production. In this investigation we studied twenty F_1 hybrids produced by crossing the rice cytoplasmic male sterile (CMS) lines IR69625A and G46A with ten elite rice genotypes during three seasons of 2011, 2012 and 2013 in Egypt. Pollen fertility % and spikelet fertility % identified that GZ6296, GZ9057 and GZ9399 were the good restorers and could be utilized for development of new rice hybrids. The most desirable values were detected by the hybrid combinations; G46A/GZ6296, G46A/GZ9057 and G46A/GZ9399 for yield and its component traits against two commercial Egyptian rice hybrids. The hybrid combination G46A/GZ6296 recorded the best values for grain yield (6.430 t/f) followed by G46A/GZ9057 (6.390 t/f). Also, these new hybrids were tested for grain quality traits and recorded 71.12 % and 72.10 % for milling trait for the hybrids G46A/GZ6296 and G46A/GZ9057, respectively. So, we can use these new hybrid rice combinations as a commercial hybrids and releasing it for the farmers in Egypt.

P381

The Influence of Cut Date and Phenology on the Regrowth Profile of Perennial Ryegrass under Silage Production

Gerard Hoppe^{1,2}, Trevor Gilliland^{3,2}

¹Agri-Food & Biosciences Institute, Loughgall, County Armagh, UK, ²Queens University, Belfast, County Down, UK, ³Agri-Food & Biosciences Institute, Belfast, County Down, UK

Perennial ryegrass (*Lolium perenne* L.) is the most important pasture grass in agriculture in the UK, Ireland and Western Europe, providing the major source of feed for ruminant livestock. The importance of ryegrass is in part due to its ability to regrow rapidly after grazing or cutting, an event which causes major changes to the plants physiological and nutritional composition. The phenological stage at defoliation can potentially affect regrowth and herbage quality. A field study was undertaken to determine the response of five diploid ryegrass cultivars grown under silage management when cut 2 weeks early or later than the average (15 May) first silage cut date. The split plot design with 3 replicates allowed main (cut date) and split-plots (cultivars - Lilora, Merbo, Aberdart, Premium & Lilora) effects and CD x V interactions to be assessed in 2010, 2011 and 2012. All swards were cut to a residual stubble height of 5cm; dry matter production, surface sward height in addition to tiller number and type were measured on a weekly basis to determine regrowth profiles. ANOVA results reveal significant differences ($P > 0.05$) for CD and V varied within and between years. Herbage production (determined by SSH) increased for all cultivars with delayed cutting; rates differed within and between treatments and years. Analysis of quadratic relationships for SSH regrowth profiles for cultivars differed within and between years. Regrowth patterns were strongly influenced by phenology and prevailing climatic variables prior to and during regrowth. Results have implications for breeders and official testing systems.

P382

Genotype and Water Availability Effects on Chickpea Seed Yield and Bioactive Protein Content

Isa C. Ribeiro¹, Céline Leclercq², Sébastien Planchon², Nuno Simões³, António Toureiro³, Isabel Duarte³, J. Miguel Costa¹, João B. Freire⁴, Manuela Chaves^{1,4}, Carla Pinheiro¹

¹Instituto de Tecnologia Química e Biológica (ITQB-UNL), Oeiras, Portugal, ²Centre de Recherche Public Gabriel Lippmann, Belvaux, Luxembourg, ³Instituto Nacional de Recursos Biológicos, IP/INIA, Elvas, Portugal, ⁴Instituto Superior de Agronomia, Lisboa, Portugal

Grain legume seeds are crucial staple foods, particularly in underdeveloped countries due to their balanced and rich nutritional composition, constituting essential sources of protein, calories, minerals and vitamins. Chickpea (*Cicer arietinum* L.) has one of the best nutritional compositions and ranks 3rd in world legume production. Limited water availability during seed development was proven to be deleterious to seed yield and composition, yet its effect on seed nutritional quality remains unknown. Chickpea contains several bioactive substances with potential health-beneficial effects, still, it is considered one major food allergen in Mediterranean countries and India. Our aim is to evaluate the effect of the genotype and of growing conditions (water availability) on chickpea seed nutritional quality, particularly on the presence of bioactive proteins - proteins that have the potential to modulate health after ingestion. To fulfill this objective we focused on proteins resistant to cooking and to simulated digestion that might influence consumer's health. Our results show that, after cooking and simulated human digestion, about 36% of the initial material and 22% of the initial protein amount remained undigested, and we detected 116 polypeptides resistant to cooking and digestion. The identification of the proteins resistant to digestion was achieved using a combination of different MS techniques (LC-MS/MS and MALDI-TOF-TOF) and different proteases (Trypsin and AspN). We also report our progresses in clarifying the role of GxE interactions on seed yield and on the seed content in digestion resistant protein and their potential impact on human health.

P383

Detoxification of Chloroacetamide Herbicide Metazachlor and Its Relation with Short- and Long-Term Trade-Off in Yield and Quality of *Brassica napus*

Hanne Vercampt, Tony Remans, Jaco Vangronsveld, Ann Cuypers
Hasselt University, Diepenbeek, Belgium

With the growing world population, the agricultural sector is increasingly relying on pesticide-use to ensure food production. However, the short- and long-term effects of herbicides on crop quality are not yet fully understood. Herbicides contain active ingredients, which act specifically on plant physiology, inhibiting weed growth. In the current study, the short-term phytotoxic effects of the chloroacetamide herbicide, metazachlor, are linked to the long-term life history traits of the crop *Brassica napus* (rapeseed). The extent of phytotoxicity is determined by the efficiency of detoxification on the one hand, leading to direct removal of metazachlor and its metabolites, and activities of antioxidant enzymes on the other hand, which are responsible for removal of reactive oxygen species (ROS). Glutathione-S-transferases (GSTs) play a crucial role in phase II detoxification reactions, conjugating xenobiotic metabolites to glutathione (GSH), preceding compartmentalisation in the vacuole. Under controlled conditions, pre-emergent application of metazachlor in microcosm experiments resulted in growth reduction of *B. napus* 14 and 28 days after treatment. In order to unravel the mechanisms of metazachlor detoxification and its changes in the cellular redox state, samples were taken 24 and 72 hours after application. Results regarding quantitative H_2O_2 assessment, glutathione redox state, GST capacity, antioxidant enzyme capacity and life history traits are presented. The results of this study can contribute to select a set of early response plant parameters, which reflect long-term effects on crop quality and which are consequently useful for herbicide development and selection of crop varieties.

P384

Naturally Transgenic Plants. Millions Years in the Biosphere.

Olga Pavlova, Tatiana Matveeva, Denis Bogomaz, Ludmila Lutova
Saint-Petersburg State University, Saint-Petersburg, Russia

Agrobacterial transformation is widely spread technique for obtaining genetically modified organisms, especially, plants. Agrotransformation is based on the integration of agrobacterial plasmid part (T-DNA ("transferred DNA") into the plant genome. Usually, agroinfection results in developing diseases "crown gall"/"hairy root". As a rule, infected plant dies. However, under natural conditions several of infected plants could survived and propagated. In fact, descendants of such plants are naturally genetically-modified organisms. Sequence homologous to *Agrobacterium* were detected in genomes of *Nicotiana* spp. and *Linaria vulgaris* L. (White et al., 1983, Intrieri, Buiatti, 2001, Matveeva et al., 2012). So, these genetically-modified organisms have been used by people for centuries in medicine (toadflax) and for smoking (tobacco plants). We have revealed *Agrobacterium rhizogenes* T-DNA-like sequences in *Linaria dalmatica* (P.) Mill. genome (oncogenes homologues: *rolB*, *rolC*, ORF13, ORF14 and *mis* gene encoding mikimopine-synthase). Some of the identified sequences have accumulated multiple mutations, and the others remained unchanged. It means agrotransformation occurred in history of genus *Linaria*, infected plant could survive and T-DNA insertion was fixed in plant genome. Perhaps, T-DNA was maintained by natural selection because it gave some advantages for plant. Search and study of T-DNA-like sequences in plants could provide understanding of plants and bacteria co-evolution. Also, naturally transgenic plants can be used as a counterargument in disputes about safety of genetically modified crops used in modern agriculture, medicine, veterinary medicine.

Work was supported by RFBR №14-04-01480, Interdisciplinary project SPSU №0.37.526.2013, Research Resource Center for molecular and cell technologies

P385

Development of New Amaranth Variety through Mutagenesis

Andrea Hricová¹, Alena Gajdosova¹, Gabriela Libiakova¹, Jozef Fejer²

¹*Institute of Plant Genetics and Biotechnology Slovak Academy of Sciences, Nitra, Slovakia,*

²*Department of Ecology, Faculty of Human and Natural Sciences, Presov University, Presov, Slovakia*

Introduction of new crops and re-introduction of traditional crops of regional importance can markedly contribute to the protection of the agricultural environment and to sustaining its biodiversity. These species commonly called „neglected“, „underutilized“ or „minor“ are often characterized as the range of species with under-exploited potential for contributing to food security, health, income generation, and environmental services. Underutilized species have only marginally been the subject of research and consequently, they cannot compete with improved cultivars of major crops on which the global food production is based. Therefore, there is a necessity to focus research on germplasm collection, evaluation and conservation, risk assessments/food safety with respect to the value of the products for human consumption, genetic improvement for increasing quality and tolerance to changing climate conditions.

Our research was oriented towards improvement of the quality and quantity of amaranth production through radiation-induced mutagenesis. We have characterized collection of putative amaranth (*Amaranthus cruentus* L.) mutant genotypes on molecular and biochemical level. Based on a long-term significantly increased yield parameter (seed weight) and improved nutritional quality we have passed on one genotype in which the effect of γ radiation was most remarkable to state variety trials for the conduct of tests for distinctness, uniformity and stability of tested new plant variety. Successful DUS trials led to the registration of the tested genotype as a new amaranth variety „Pribina“.

Acknowledgement

This research was co-funded by project no.2/0066/13: Exploitation of modern biotechnologies in amaranth breeding programme and European Community project no.26220220180: Building Research Centre „Agrobiotech“.

P386

Taxonomic Diversity and Utilization of Local Vegetables in Southwest Nigeria

Olaf Thomas Bouman

Cape Breton University, Sydney, Nova Scotia, Canada

The issue of food security has often been perceived as the need to produce increasing quantities of food for a growing world population while the need for a greening of the green revolution is still emerging. Quantitative estimates of long-term global food consumption and production have been grossly divergent and of limited relevance for analyzing the issue of food security at the national and local level in Africa. Statistics by the Food and Agriculture Organization (FAO) showed for Nigeria an increase of root crop production by more than 50 % and a concomitant decrease of the under-nourished proportion in the Nigerian population by approximately 50 % during a 20-year period from 1991 to 2010. This apparent improvement of quantitative food security does however, not address the inherent nutritional limitation of starchy root crops. This paper will present an analysis of food consumption and production based on a survey of 870 rural households across four densely populated states of Southwest Nigeria in 2011. The survey revealed marked taxonomic diversity of local vegetable species. Interestingly, there were more households consuming these vegetable species than the number of households cultivating them. The presentation will discuss the potential of locally identified vegetables for improving the quality of regional food security and their agronomic potential. The research was conducted in conjunction with an agronomic capacity building project supported by the Canadian Food Security Research Fund.

P387

Seed Conservation of Plant Genetic Resources for Food Security - Genetic and Biochemical Backgrounds of Viability Loss during Long-Term Storage of Barley

Manuela Nagel¹, Ilse Kranner², Steven P. C. Groot³, Jan Kodde³, Andreas Börner¹

¹IPK Gatersleben, Stadt Seeland, Germany, ²University of Innsbruck, Innsbruck, Austria,

³Wageningen University and Research Centre, Wageningen, The Netherlands

The Global Strategy for Plant Conservation of the Convention on Biological Diversity, illustrates that our plant genetic resources have been given a priority status and need to be conserved by *in situ* and *ex situ* approaches. Due to lower cost and better control especially seed conservation is mainly used for most of the 7.4 Mio worldwide stored genebank accessions. However, the ability of seeds to survive a certain period of time, termed seed longevity, is strongly dependent on the growth conditions of the mother plant, pre-storage and storage conditions and the genetic background. Strong differences in the shelf life of seeds exist among species. The present study on barley accessions also shows differences appearing between single genotypes. After a storage period of 34 years at 0°C most accessions maintained high germination rates (> 90%) but in some cases depletions below 50% was observed. A follow up association mapping study using genebank accessions confirmed the genetic control of seed longevity and linked it to a variety of abiotic and biotic stress reactions occurring during different developmental stages. Reactive oxygen species and antioxidants are assumed to be majorly involved in this reaction. To understand abiotic stress reaction occurring during storage we investigate the relationships between the antioxidants tocopherols, glutathione (γ-glutamyl-cysteinyl-glycine) and long-term stored and artificially aged barley accessions. To assess abiotic stress the half-cell reduction potential of was measured. Viability loss concurred with a shift towards more oxidizing intracellular conditions, suggesting that oxidative stress contributes to seed deterioration irrespective of ageing treatment.

P388

VALORAM: A Case Study for the Selection of Putative Biocontrol Agents which Can Be Used as Part of an Integrated Pest Management System

Barbara Doyle Prestwich, Siva Velivelli
University College Cork, Cork, Ireland

The most pressing issues facing the human race today is that of global food security, ever more vulnerable in the wake of major disturbances e.g climate change. Agricultural practices, globally, have to become more sustainable. Legislative changes in relation to the control and use of agrichemicals (especially in Europe) are dictating a more integrated approach for disease management. The use of biological control methods is now recognised as part of a viable holistic management strategy. The VALORAM project (<http://valoram.ucc.ie>), funded under FP7, examined the role of Andean microbial communities in crop production and protection. One outcome of this research was a greater understanding of the microbial diversity of the rhizosphere of the potato plants in the Central Andean region coupled with the knowledge of how the application of certain rhizobacterial isolates can both enhance plant growth and offer greater disease protection. Using a streamlined *in vitro* screening system, it was possible to examine the performance of hundreds of isolates at the laboratory level, in relation to their growth-promotion capacity and in their disease suppression capability in relation to both *Phytophthora infestans* and *Rhizoctonia solani*. Additionally, a detailed knowledge of signature volatile compounds emitted by a number of bacterial isolates was determined. Many of these have previously been identified in the literature as having an effect on growth-promotion and disease suppression. The strategy followed in the VALORAM project, may offer a template for the future isolation and determination of putative biocontrol agents, useful as part of any integrated pest management system.

P389

Influence of HPPD-Inhibiting Herbicide on Injury, Growth and Yield of Indica Japonica Rice (*Oryza sativa* L.)

Sang-Yeol Kim¹, Sang-Ik Han², Jong-Hee Lee², Ji-Yoon Lee², Jun-Hyeon Cho², Seong-Hwan Oh², Yeong-Bo Son², No-Bong Park¹, You-Chun Song², Min-Hee Nam²

¹Yeongdeog Substation, National Institute of Crop Science, RDA, Yeongdeog 766-851, Republic of Korea, ²Department of Functional Crop, National Institute of Crop Science, RDA, Miryang 627-803, Republic of Korea

A field experiment was conducted to evaluate the effect of *p*-Hydroxyphenylpyruvate dioxygenase (HPPD) inhibiting herbicides such as benzobicyclon and mesotrione+pretilachlor (MP) on plant injury, growth and yield of three Indica Japonica rice cultivars (cv. Hanareumbyeo, Hanareum2, Dasanbyeo) in 2012~2013, at National Institute of Crop Science, Miryang, Korea. The herbicides, benzobicyclon and MP, were applied as preemergence treatment at the rate of 350g, 90g a.i. ha⁻¹ (1x) and 750g, 180g a.i. ha⁻¹ (2x), respectively. Three rice cultivars were extremely sensitive to benzobicyclon and MP herbicides even at the 1x rate as reflected by high visual injury of leaf chlorosis. The percentage of foliar chlorosis of rice plant at the 1x rate was 34~64% in benzobicyclon and 63~75% in MP while plant injury was more severe at the 2x rate of benzobicyclon (76~83%) and MP (83~89%) herbicides. Although leaf chlorosis injury of the three rice cultivars induced by the 1x rate of benzobicyclon and MP was recovered after 30 DAT, the high dose (2x rate) of benzobicyclon and MP caused 6.5~11.2% and 2.1~3.3% of plant death, respectively. The heading date of the injured rice plant was delayed and panicle number per square meter, ripened grain ratio and 1,000-brown rice weight were decreased due to the injury of rice plant by the herbicide treatments. Rice yield of the Indica Japonica rice treated with benzobicyclon and MP was significantly reduced by 6~10% even at 1x rate and 15~39% at 2x rate per ha, respectively. The result suggests that treatment of HPPD inhibiting herbicides to control weed should be harmful to Indica Japonica rice cultivation.

P390

Co-Expression of Rice Nicotianamine Synthase and Barley Nicotianamine Amino Transferase Increases the Level of Iron and Zinc in Rice Endosperm

Raviraj Banakar¹, Teresa Capell¹, Paul Christou^{1,2}

¹*Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida-Agrtotecnio Center Lleida, Lleida, Catalunya, Spain,* ²*Institutio Catalana de Recerca i Estudis Avancats, Barcelona, Spain*

Low iron and zinc levels in staple diets constitute a severe public health challenge particularly among impoverished populations in developing countries. In rice growing countries in SE Asia and other regions where rice is a staple, this is the main reason for the prevalence of iron deficiency anaemia and zinc deficiency. The low level of iron and zinc in rice endosperm has been attributed to the limited capacity of rice to take up iron and zinc from the soil. Nicotianamine (NA) and 2'-deoxy mugenic acid (DMA) are ligand molecules involved in the phytosiderophore cascade. DMA is responsible for the uptake of iron and zinc in the form of iron (III)-DMA and Zinc-DMA complex, respectively. Both NA and DMA are important for the internal mobilisation and seed loading of iron and zinc. Biosynthesis of NA and DMA is controlled by the enzymes nicotianamine synthase (NAS) and nicotianamine amino transferase (NAAT), respectively. We wished to test the hypothesis that simultaneous up-regulation of these two enzymes in rice plants might boost levels of NA and DMA and this in turn might translate to higher levels of seed iron and zinc. We determined that plants with enhanced levels of NA and DMA accumulated significantly higher levels of iron and zinc in seeds compared to wild type. Feeding these plants with exogenous iron did not result in any further increases in seed iron suggesting a threshold for the accumulation of iron in rice seeds.

P391

Physiological Response of Sunflower Plants to the Herbicide Imazamox

Dobrinka Balabanova, Jaco Vangronsveld, Ann Cuypers, Tony Remans, Andon Vassilev
Hasselt university, Hasselt, Belgium

Sunflower (*Helianthus annuus*) is an important agricultural crop. In Europe, annually about 16 million ha of farmland are sown with sunflower. Broadleaf weeds cause yield losses to sunflower production, which inevitably leads to the use of herbicides. Resistance to imidazolinone herbicides was found in a population of wild sunflower, and imidazolinone-tolerant sunflower crops were developed by conventional breeding methods and are commercialized as Clearfield* crops. Imidazolinone herbicides control weeds by inhibiting the enzyme acetohydroxyacid synthase (AHAS), which is the first common enzyme in the biosynthetic pathway of branched-chain amino acids in plants.

The Clearfield production system is a good solution to weed problems, but Clearfield sunflower hybrids are not 100% resistant to AHAS inhibitors. Although these hybrids carry the haplotype 5 of the AHAS1 gene, which is associated with imidazolinone resistance, we determined that the herbicide Imazamox (trade name Pulsar 40®) caused a growth reduction leading to yield losses. Addition of branched-chain amino acids (BCAA) helped to overcome this effect of imazamox toxicity. Our results showed improvement in the growth of sunflower plants treated with imazamox and addition of BCAA. At the physiological level the photosynthetic analyses showed slight changes in CO₂ exchange rate. For detoxification, increased activities of glutathione-S-transferases were observed. The level of oxidative stress was determined by investigating plant antioxidative enzymes and antioxidant metabolites, which increased in Imazamox-treated plants. Analyses of AHAS activity and gene expression of AHAS transcripts are still on-going.

In conclusion, Clearfield hybrids overcome the initial imazamox inhibition on biomass, when BCAA are added.

P392

Peroxydase Isoforms and Polyphenol Contents in Fruits of Landraces of Sweet Cherry Germoplasm of Campania Region (Italy)

Antonio Mirto, Federica Iannuzzi, Pasqualina Woodrow, Petronia Carillo, Amodio Fuggi
Second University of Naples, Caserta, Italy

Peroxidases are involved in many oxidative reactions in plants. They occurred in many isoforms that have been also used as genetic markers in the identification of cultivars. Such enzymes are of main concern along the food supply chain. They, in fact, cause a deterioration of organoleptic properties (color, flavor) of vegetable foods, and decrease their nutritional and nutraceutic qualities, by oxidizing phenolics even during fruit and vegetable cold storage. The blanching process was not effective, due to their high resistance to denaturation and to their ability to renature even in cold storage. In the aim of conservation and valorization of the agrobiodiversity 20 landraces of the sweet cherry germoplasm of Campania Region were analysed. Ripe fruits were used to characterise peroxidases, as well as to determine their polyphenol and antocyanin contents. Partial purified extracts from the fleshy part of fruit evidenced soluble and bound peroxidases. Acidic, neutral and basic isoforms were found: the basic ones showed the highest activity, while the neutral ones the lowest. The basic isoforms differed also in molecular weight. Kinetic analyses evidenced optimum pH and apparent K_m depending on the phenolic substrate. Significant denaturation rate in acetate buffer at pH 5 occurred at temperature higher than 67 °C ($K_d = 0,05 \text{ min}^{-1}$). The fruit samples were also used to extract and characterize polyphenol and antocianin contents and distribution among the ecotypes. Data were discussed comparatively.

Financial support was obtained by "Regione Campania (Italy), PSR 2007/2013, Misura 214, Azione f2, progetto Agrigenet".

P393

Effect of Harvest Year on Physical Properties and Chemical Composition in Highbush Goldtraube Cultivar (*Vaccinium corymbosum*) Grown in Portugal

Ana Silva¹, Berta Gonçalves¹, Alfredo Aires¹, Carlos Ribeiro³, Sofia Correia¹, Hortense Fernandes³, Luís Ferreira⁴, Valdemar Carnide², Ana Barros¹

¹Centre for the Research and Technology of Agro-Environmental and Biological Sciences-CITAB, University of Trás-os-Montes e Alto Douro, UTAD, 5000-801 Vila Real, Portugal, ²Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology. University of Trás-os-Montes e Alto Douro, UTAD, 5000-801 Vila Real, Portugal, ³Agronomy Department, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal, ⁴The Animal and Veterinary research Centre, CECAV, University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

The increase in consumption of small fruits represents a new tendency in Portugal. Therefore, the knowledge about the best quality indices, as chemical composition and structure of fruit, is quite important to satisfy the market demand and consumer requirements. The aim of this study was to evaluate the influence of harvest year on biometric parameters, structural properties, cellular structure, firmness and chemical composition of Goldtraube cultivar produced in Midwest of Portugal. Significant differences were found on several analyzed parameters between years (2011-2013), except on some cellular structure components. In spite of a rainfall amount in March and July 2013 higher than in the same months of the other two years, fruits were less weighted and smaller, but more spherical. The content on cellulose and hemicellulose was lower in the driest year (2012), but Goldtraube fruits were firmer than the others from 2011 and 2013, with higher compression force. Citric acid was the main organic acid and fructose the most abundant sugar. Total organic acids content and titratable acidity were lower in the year 2012 contrasting with the higher content in total free sugars and refractometric index. In 2013, the organic acids content was higher but the free sugars content was lesser, leading to more acidic blueberries. This work confirms the effect of harvest year in productivity and fruit quality of blueberries.

Acknowledgements: This work was supported by Project INNOFOOD - NORTE-07-0124-FEDER-0000029, financed by ON.2 - O Novo Norte under QREN, through FEDER, as well as by PIDDAC through FCT/MEC.

P394

Improving Wheat Photosynthesis to Improve Yields using Genetic Manipulation Approach

Sager Alotaibi

The University of Essex, Colchester/Essex, UK

The process of photosynthesis provides an unexploited research opportunity in the area of increasing crop yields. This research study is about manipulating wheat photosynthesis in order to improve yields. A number of different molecular approaches have been taken. First, we are trying to identify promoters in order to drive expression into the mesophyll tissues of dicots and wheat using a transient expression assay. Second, genetic manipulations of GDC complex in wheat have been taken by making two subunits expression constructs. The results of the transient expression analyses of *N. benthamiana* leaves demonstrated that *Brachypodium* SBPase and FBPaldolase promoters (monocots) have driven clear and strong GUS and eYFP expression in the mesophyll cells of *N. benthamiana* leaves (dicots). Therefore, it is worthwhile to test these promoters in wheat (monocots) in order to analyse their ability to drive expression in the mesophyll cells. Consequently, the SBPase::GUS and FBPaldolase::GUS were constructed, and the next steps are now underway and transient expression will be tested over the next month. The GDC-H gene was constructed successfully and is currently under transformation at Rothamsted Research to be over-expressed into wheat leaves. Additional molecular analyses including RT-PCR, q-PCR analysis, western blotting and physiological studies will be conducted on transgenic plants with increased levels of GDC-H, SBPase, *ictB* and decreased levels of RbcS and GDC-P, in order to explore the effects of these manipulations on photosynthetic CO₂ assimilation and wheat growth under elevated CO₂.

P395

Effect of Optical Wavelengths in Sprout Controlling of Potato (*Solanum tuberosum*. L)

Jin Yong-Ik, Chang Dong-Chil, Cho Ji-Hong, Cho Kwang-Soo, Im Ju-Sung, Yoo Hong-Seob, Sohn Hwang-Bae, Mekapogu Manjulatha, Jeong Jin-Choel
RDA, Pyeongchang, Republic of Korea

CIPC(Chloropham), a sprout inhibitor is known for effective inhibition of sprouting. Treating seed potato with sprout inhibitor breaks the dormancy thereby increasing the efficiency of its storage and availability. In the present study, it was investigated that the break of dormancy depends on the timing of applying CIPC after harvest and storage conditions after treatment. CIPC was applied to potato after two months of harvest with temperature maintained at 20°C. Here we showed the variation in the sprout rate by several light conditions and CIPC treatment along with light exposure followed by one month storage of potatoes. The light qualities used were UV(Ultra Violet), red and yellow. Sprout rate, number of sprouts, length and diameters of sprout were recorded after the treatments. The sprout rate of control treatment without CIPC and light was 100%, whereas under UV light, it was 67% and red and yellow light were 78%. Further, sprout rate of control treated only with CIPC was 11%, whereas it was 44% under red and yellow lights with CIPC, it was 0% in UV light. These results show that light condition is an important factor which could break dormancy of potato, in addition to CIPC treatment. Yellow light showed higher sprout number than other treatments with higher length and diameter of sprout. Hence it can be concluded that yellow light was effective in breaking dormancy. This suggests that yellow and UV light treatment can be used for effective sprout controlling in potato

P396

The Expression Analysis of Anthocyanin-Biosynthetic Genes in Color Potatoes for Transcriptome Profiling to Identify the Genes Involved in Anthocyanin Biosynthesis

Hwangbae Sohn, Kwangsoo Cho, Yulho Kim, Suyoung Hong, Sujeong Kim, Junghwan Nam, Mekapogu Manjulatha, Yongik Jin
National Institute of Crop Science, Pyeongchang, Gangwon, Republic of Korea

The major objective was to identify genes involved in potato anthocyanin biosynthesis in color potatoes bred in Rural Development Administration (RDA). The structural genes chalcone synthase (StCHS), flavonone 3-hydroxylase (StF3H), flavonoid 3' 5'-hydroxylase (StF3'5'H), dihydroflavonol 4-reductase (StDRF), anthocyanin synthase (StANS) and UDP-glucose: flavonoid 3-O-glucosyltransferase (St3GT) and the transcription factors anthocyanin 2 (StAN2), basic helix-loop-helix (StbHLH) were more strongly expressed in red-fleshed potato cultivar Hongyoung and purple-fleshed Jayoung bred in RDA. In stems of purple potato cultivar Jayoung, expression of anthocyanin-biosynthetic genes was detected at all stages of development and in 'Jayoung' tubers, detected at stage 3 (tuber initiation), followed by a reduction in this expression at stage 4 (tuber bulking) to 6 (tuber maturation). In 'Jayoung' leaves, 3GT gene was expressed, while the other genes involved in the biosynthetic pathway were not expressed. The amount of anthocyanin increased by two-fold in 'Jayoung' tubers at stage 6 compared to those at stage 3, but was similar at all stages in stems. In sprouts of 'Jayoung' tubers, the expression level of anthocyanin-biosynthetic genes and anthocyanin content were highly expressed compared to the other tuber tissues. In sprouts of 'Hongyoung', expression of all of the genes, except F3'5'H, was detected. To identify the genes involved in anthocyanin biosynthesis, sprout samples collected from white-fleshed cultivar Atlantic, purple-fleshed Jayoung and red-fleshed Hongyoung were subjected to transcriptome profiling using Illumina Hiseq 2000. Currently, the transcriptome data has been being analyzed.

P397

The Effect of Different Nitrogen Sources on the Sulfur Metabolism and Growth of Brassica under Ambient and Elevated CO₂

Martin Reich¹, Michael Tausz², Luit J. De Kok^{1,2}

¹Laboratory of Plant Physiology, University of Groningen, Groningen, The Netherlands, ²Department of Forest and Ecosystem Science, The University of Melbourne, Creswick, Australia

Although sulfur is an essential macronutrient for plants and involved in numerous vital processes there is very little known about the effect of the anticipated rising levels of CO₂ on sulfate uptake and assimilation. The ongoing increase in global atmospheric CO₂ levels presumably will have - at least at sufficient nutrient supply - a positive effect on plant biomass production and water-use-efficiency. However, during episodic nutrient limitation, which might occur more often at elevated CO₂, a surplus of carbon assimilation may disturb nutrient allocation and plant performance. Experiments with Brassica species, which are characterized by relatively high tissue sulfur content and a high relative growth rate, showed a transient growth stimulation by elevated CO₂ and a decrease of tissue sulfur content, mainly caused by dilution by non-structural carbohydrates and growth. Additionally the specific leaf area was decreased. The combination of elevated CO₂ and sulfur deprivation had an additive effect on the accumulation of dry matter content and the decrease of specific leaf area. These results suggest that periodical sulfur deficiency might display an even greater constraint under higher levels of CO₂ because of the anticipated negative impact of an over-accumulation of non-structural carbohydrates and a decreased specific leaf area for future growth. Currently, the influence of different nitrogen sources on these processes is investigated, in order to reveal the impact of varying nutrient supply and elevated CO₂ on the regulatory aspects of uptake and metabolism of N and S and plant performance both under controlled laboratory conditions and field conditions.

P398

Sink-Source Relations in Barley: Temporary Sinks and Their Role in Reutilization of Assimilates

Irina Kiselyova

Ural Federal University, Ekaterinburg, Russia

In barley plants leaves, ear elements and culm are able to photosynthesis and donate photosynthates to the acceptors: young leaves, growing straw, ear, roots and tillers. Applying ^{14}C it was found that leaf blades use about 75% of total CO_2 fixed during plant development. According to the size, lifespan, functional activity leaves form 2 groups: the lower ones supply by assimilates the vegetative growth and early ear development; the upper ones are responsible for ear growth and maturation, culm and tiller growth. During kernel maturation about 50% of CO_2 are fixed by non-foliar organs, including ear elements (34%). The study of the utilization of ^{14}C -compounds during ontogeny indicated the possibility of temporary sinks to donate carbohydrates to ear when leaf photosynthesis declined. The significant portion of assimilates, formed in vegetative phase were temporarily stored as fructans, pectin and hemicellulose in culm mainly in lower internodes. They finished to growth in length before earing, but accumulate biomass after earing. In the period of wax ripeness when leaf photosynthesis significantly decreased straw biomass reduced. This is accompanied by the decline in polysaccharides content, especially fructans. During ear maturation the products of fructans hydrolysis in culm were reutilized in growing grains that allowed accumulating up to 20% of grain weight. The role of reutilization is not the same in different conditions. It was shown that it varied from 0% to 25% depending on weather conditions.

P399

The *Arabidopsis thaliana* Pentatricopeptide Repeat Protein (AtPPR) Contains a Domain that Functions as an Adenylate Cyclase

Oziniel Ruzvidzo¹, Bridget Dikobe¹, Patience Chatukuta¹, David Kawadza¹, Christopher Gehring²
¹North-West University, Mmabatho, South Africa, ²King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

Since adverse climatic changes are likely to continue, there is therefore an urgent need to use rational and system-based approaches to develop crop plants with increased tolerance to both biotic and abiotic stress factors. This need has to date, led to an impressive body of work in the area of plant biotechnology, and a realization that only an integrated and systems-based approach can possibly deliver effective solutions. Part of this approach involved the study of a special group of proteins termed adenylate cyclases (ACs) that are thought to systemically affect plant homeostasis. These enzymes are capable of converting ATP to the second messenger, cAMP. In animals and lower eukaryotes, both ACs and cAMP have firmly been established as important signaling molecules with important roles in several cellular signaling systems. However and in higher plants, the only currently annotated and experimentally confirmed AC is a *Zea mays* pollen protein responsible for pollen growth. Recently, a number of candidate AC-encoding genes in the *Arabidopsis* genome have been proposed based on functionally assigned amino acids in the catalytic centres of known eukaryotic nucleotide cyclases. Here, we detail the cloning and functional characterization of a candidate AC domain from *Arabidopsis thaliana* in the form of a pentatricopeptide repeat-containing protein (AtPPR-AC; At1g62590). Through a series of *in vitro* and/or *in vivo* tests and assays, the recombinant AtPPR-AC was thoroughly pre-screened and practically evaluated for its possible AC functions. Findings from this work indicated that the recombinant AtPPR-AC is indeed a *bona fide* higher plant AC with potential roles in cell signaling and transduction systems.

P400

Vernalization and Photoperiodic Control of Flowering Time in Canola Plants of Various Geographical Origin

Catherine Savelieva, Ivan Tarakanov
Russian State Agrarian University, Moscow, Russia

To properly time flowering and cope with low temperature stress plants regulate their development through adaptive mechanisms that are responsive to day-length and temperature. In controlled environment we studied canola plants (*Brassica napus* L.) responses to various photoperiodic and temperature conditions in winter and double response cultivars of diverse geographic origin. In the experiments with step-wise plant transfer from inductive environment to non-inductive and vice versa, we investigated the length of juvenile phase and the duration of cold or/and photoperiodic exposure required for flower induction and evocation.

Growing under short-day conditions following the low-temperature exposure accelerated the development of plants with both obligate and facultative vernalization requirement. Short-day exposure after 6-8 weeks of vernalization induced transition to generative development in winter canola cultivar. Under short days the number of plants transitioned to the bud formation increased from about 63 to 100 percent after 6 and 8 weeks of vernalization, respectively, and only from about 31 to 38 percent under long days.

For low-latitude genotypes that do not have an obligate vernalization requirement, eight-week vernalization before short-day exposure reduced flowering period duration due to evocation phase acceleration.

For low-latitude ecotypes short days also provided stimulating effect in the absence of vernalization. We found that cultivating under short days following 1-2 week of long-day exposure in the first weeks of vegetation significantly accelerated their development. It was shown that low-latitude samples can implement various life strategies depending on photoperiod and can evolve as long-day or long-short-day plants.

P401

Auxin Biosynthesis and Distribution during Microspore Embryogenesis and Effects of Transport and Activity Inhibitions on Development

Héctor Rodríguez-Sanz¹, María-Fernanda López², María Teresa Solís¹, Aurelio Gómez-Cadenas², María-Carmen Risueño¹, Pilar S. Testillano¹
¹CIB-CSIC, Madrid, Spain, ²Universidad Jaume I, Castellon, Spain

The auxin indol-acetic acid (IAA) is a major plant growth regulator involved in developmental processes. Nitrilase genes (NIT) are responsible of the conversion of indol-3-acetonitrile (IAN) to IAA in Brassicaceae. Immature pollen grains (microspores) can be reprogrammed *in vitro* towards an embryogenesis pathway. In isolated microspore cultures of *Brassica napus*, the embryogenic switch and embryo formation are induced by heat treatment in a culture media free of plant growth regulators.

In this work IAA levels and distribution, and *BnNIT2* gene expression were analyzed during microspore embryogenesis in *Brassica napus*. The effects of inhibition of IAA transport and activity were also analyzed by treatments with N-1-naphthylphthalamic acid (NPA) and α -(*p*-Chlorophenoxy)isobutyric acid (PCIB) respectively. Experimental approach included immunofluorescence using anti-IAA antibodies and confocal analysis, *BnNIT2* expression analysis by qPCR, and quantification of IAA levels by liquid chromatography linked to mass spectrometry through an electrospray interface.

Results indicated *de novo* synthesis of IAA at early stages of microspore embryogenesis, progressive IAA increase with microspore-embryo development, and differential distribution pattern in embryo regions at late developmental stages. *BnNIT2* gene was up-regulated during microspore embryogenesis. Both, the inhibition of the IAA transport by NPA and the inhibition of IAA action by PCIB negatively affected the normal microspore-embryo development and modified the IAA distribution pattern. Results indicated for the first time that auxin synthesis and transport would be involved in stress-induced microspore embryogenesis initiation and development.

Funding by Spanish MINECO (BFU2011-23752) and CSIC (PIE 201020E038). HRS is recipient of a predoctoral FPI grant (BES-2009-014245) of MINECO.

P402

***Festuca arundinacea* Shows Genetic Diversity in the Response to Temperature during Germination**

Lina Qadir Ahmed, Jean-Louis Durand, Abraham Escobar-Gutiérrez A.J
INRA (Institut National de la Recherche Agronomique), Lusignan, France

In the context of climate change, grasslands are considered, similar to forest, as an important sink for atmospheric carbon. In Europe, grasslands cover at least 30% of the 160 Mha Agricultural Surface Area. Tall fescue (*Festuca arundinacea*) is major grass species growing in this ecosystem. On the other hand, temperature is one of the major factors controlling plant development, in particular seed germination. Indirectly, this affects recruitment of individual and hence the genetic dynamics of the community. Breeding *F. arundinacea* cultivars adapted to new ranges of temperature could be necessary. Knowing the variability of responses to temperature by different accessions is an unavoidable first step towards such breeding. The objective of the work presented here was to analyse the genetic variability of this species in response to temperature during germination. Nine populations of *F. arundinacea* were evaluated. Five of them are wild populations collected in different places in the Mediterranean area. Four are commercial cultivars in France. They were grown in chambers at constant temperature ranging from 5 to 40°C with 5°C intervals in the dark. Maximum germination percentage, lag to start germination and maximum germination velocity were estimated. It was observed that the responses of *F. arundinacea* populations showed statistically significant differences ($P < 0.05$). At least three groups of populations can be distinguished. The findings of this study suggest that high genetic variability exist within *F. arundinacea* for the response to temperature during germination. This variability could be exploited to breed new varieties adapted to the new environmental conditions induced by the global climate change.

Key words: Tall fescue, genotypes, climate change, response temperatures, germination.

P403

The Transformation of a Novel Model System

Adrian Dauphinee¹, Christian Lacroix², Arunika Gunawardena¹

¹Dalhousie University, Halifax, Nova Scotia, Canada, ²University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada

The lace plant (*Aponogeton madagascariensis*) is an aquatic monocot endemic to the rivers of Madagascar. The plant has a unique perforated leaf morphology that has made it popular as an aquarium ornamental for over a century. The holes that form between the longitudinal and transverse veins throughout the leaf lamina are created via developmentally regulated programmed cell death (PCD). PCD is critical for the development and survival of animals and plants, and the lace plant is an emerging model system for studying this process. This work aims to develop an efficient *Agrobacterium tumefaciens* mediated transformation protocol which will greatly enhance the potential of this model system. To achieve this aim, experiments were carried out utilizing various explants including leaf blades and sheaths from different developmental stages, as well as roots and callus tissues. Successful callus induction and plant regeneration protocols were established using corn tissues. Callus tissues were the most successful explant used in transformation experiments that employed *A. tumefaciens* vectors containing binary plasmids that conferred GFP fluorescence to transformed cells. Preliminary results indicate that the lace plant is amenable to *A. tumefaciens* mediated transformation and current efforts are directed toward the production of whole plant transformants expressing genes of interest that will aid in the elucidation of cellular dismantling pathways.

P404

Improved Agrobacterium-Mediated Transformation Method in *Brachypodium distachyon* for the Characterization of LOB-Domain Transcription Factors

Zoltán Zombori, Mária Szécsényi, Magdolna Gombos, János Györgyey
Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Brachypodium distachyon is considered to become the new model plant species for the agronomically most valuable monocotyledonous plant family, the *Poaceae*, because it has similar advantages like *Arabidopsis thaliana* has among dicots. The development of an efficient transformational method is also indispensable in the establishment of a model system due to its importance in several research fields. *Agrobacterium*-mediated methodology was applied during the development of the transformation process. The materials for the transformation were callus tissues originated from immatured embryos of *Brachypodium* Bd-21 ecotype. Various *Agrobacterium tumefaciens* strains, tissue culturing conditions, plant selection markers and hormonal conditions were tested for the improvement and optimization of the transformation method. During the regeneration, naphthalene acetic acid treatment was applied to foster the root development, resulted in high survival rate after planting. Our method allows us to characterize the plants of the T₁ progeny within one year.

LOB-domain transcription factors are plant-specific proteins involved in the formation of plant architecture. These genes are expressed in different plant tissues and organs, and they play important role in organ separation and lateral organ development. Their sequence contains a conserved LOB domain that harbors a 4-Cys and a leucine-zipper-like motif. Based on sequence alignments 24 LOB-domain transcription factors divided into two classes were identified in *Brachypodium*. Previous gene expression analysis revealed organ specific expression patterns of the *LBD13* and *LBD15* homologues. Altered expression of *LBD15* influenced germination, shoot and spike development, and the *LBD13* promoter drove root-specific GFP expression pattern in transgenic *Brachypodium* lines.

P405

The Plastid-Localized NAD-Dependent Malate Dehydrogenase is Crucial for Energy Homeostasis in Developing *Arabidopsis thaliana* Seeds

Jennifer Selinski¹, Nicolas König¹, Benedikt Wellmeyer¹, Guy T. Hanke¹, Vera Linke¹, H. Ekkehard Neuhaus², Renate Scheibe¹

¹University of Osnabrueck, Osnabrück, Germany, ²TU Kaiserslautern, Kaiserslautern, Germany

In the absence of photosynthesis, ATP is imported into chloroplasts and non-green plastids by ATP/ADP transporters or formed during glycolysis, the latter requiring continuous regeneration of NAD⁺, supplied by the plastidial isoform of NAD-MDH. During analysis of T-DNA insertion mutants of *A. thaliana* only heterozygous but no homozygous mutants could be identified. These heterozygous plants show higher transcript levels of an alternative NAD⁺-regenerating enzyme, NADH-GOGAT, and, most remarkably, improved growth compared to wild type when ammonium is the sole N-source. In-situ hybridization and GUS-histochemical staining revealed that pINAD-MDH was particularly abundant in male and female gametophytes. A knockout of pINAD-MDH has a strong effect on pollen tube growth. Knock-out pollen lacking pINAD-MDH do not germinate *in vitro*, but can fertilize the egg cell *in vivo*. Young siliques of selfed heterozygous plants contain both green and white seeds corresponding to wild-type/heterozygous (green) and homozygous knock-out (white) mutants in a (1:2):1 ratio. However, embryos of the homozygous knock-out seeds only reached the globular stage, did not green, and developed to tiny wrinkled seeds, suggesting that a blocked major physiological process in pINAD-MDH mutants stops both, embryo and endosperm development in order to avoid assimilate investment in compromised offspring (Selinski *et al.*, 2014).

P406

The FT/TFL Gene Family in *Chenopodium rubrum*, a Short-Day Plant with Unusual Mode of Activation of the Floral Promoter

Helena Storchová, Jana Drabesová
Inst Expt Bot AS CR, Prague, Czech Republic

Chenopodium rubrum is a short-day species capable of flower induction at seedling stage. Its floral promoter CrFTL1 is inhibited at night, but upregulated by light exposure following a period of darkness. This activation mode is unique among short-day plants. We assembled 454 and Illumina transcriptomes prepared from various tissues and organs and retrieved the sequences of flowering-related genes. We identified the genes homologous to the members of the FT/TFL gene family in *Arabidopsis thaliana*. The FT duplication occurred early in evolution of the family Amaranthaceae and resulted in two copies, CrFTL1 and CrFTL2. Whereas the former acts as a florigen, the latter has so far unknown function in *C. rubrum*. BvFT1, CrFTL2 homolog in sugar beet, acts as a floral inhibitor. Besides the ancient duplication, recent multiplications of FT genes owing to polyploidization take place in *Chenopodium*. The analyses of gene structure and expression provide important clues about gene function. However, the overexpression or silencing of the gene of interest are necessary to bring a final evidence. We improved floral dipping method to achieve a stable transformation of *C. rubrum* and observed phenotypic changes associated with CrFTL1 silencing. In conclusion, short-day *C. rubrum* becomes a suitable model for the studies of flowering to compare with long-day sugar beet in the same family Amaranthaceae.

P407

Combinatorial Interaction between AtbZIP44 and Other bZIP Transcription Factors in the Regulation of *Arabidopsis thaliana* Seed Maturation and Germination

Raquel Iglesias-Fernández, Victoria Llanos-Casado, Pilar Carbonero

Centro de Biotecnología y Genómica de Plantas (Universidad Politécnica de Madrid), Pozuelo de Alarcón (Madrid), Spain

During the seed maturation phase, seed storage proteins (SSPs) and lipids are deposited in the embryo of *Arabidopsis thaliana*, and these will be hydrolysed to be used as energy, C- and N-sources, for the seedling until photosynthesis is fully established. Upon seed imbibition, the dismantling of the endosperm cell walls (CWs) that are rich in mannan polymers, by endo- β -mannanases (MAN; EC. 3.2.1.78) and other hydrolytic enzymes are crucial for allowing germination *sensu stricto* to occur.

The transcriptional combinatorial network through which AtbZIP44 regulates gene expression during the two phases of seed development (maturation and germination) has been explored in the yeast 2-hybrid system, using as a bait bZIP44 and as a prey, an arrayed yeast library of *circa* 1,200 Transcription Factor Open Reading Frames (TF ORFs) from *Arabidopsis thaliana* (Castrillo *et al.*, 2011). The interaction between AtbZIP44 (S1-group) and other TFs of the C-group (AtbZIP9, AtbZIP10, AtbZIP25; Jakoby *et al.*, 2002) has been further established, and its physiological significance investigated by molecular techniques such as RT-qPCR analyses of putative regulated genes (*2SA1b*, *Cru3*, in the maturation phase; *AtMAN7* upon germination; Iglesias-Fernández *et al.*, 2013), transient trans-activation assays in tobacco leaves, mRNA Fluorescence *in situ* Hybridization (FISH) experiments, and by the analysis of seed maturation parameters and the germination kinetics of both over-expressor (oex) lines and T-DNA insertion mutants in the selected TF genes.

Castrillo *et al.* (2011) *PLos One* **6**:e21524.

Iglesias-Fernández *et al.* (2013) *Plant J.* **74**: 767-780.

Jakoby *et al.* (2002) *Trends Plant Sci.* **7**: 106-111.

P408

Viability and Programmed Cell Death in Isolated Cultured Endosperm of Cereals

Marzena Popielarska-Konieczna¹, Izabela Marcinska³, Malgorzata Kozieradzka-Kiszkurno², Dagmara Kwolek¹, Halina Slesak¹
¹Jagiellonian University, Cracow, Poland, ²University in Gdansk, Gdansk, Poland, ³Polish Academy of Sciences, Cracow, Poland

Endosperm plays an important role as a storage tissue for embryo and as a food for humankind. Experimental research concerning endosperm tissue could be convenient platform to manipulation of the development and study the molecular and biochemical mechanisms regulating seed storage accumulation in the endosperm. The object of our research are selected species of cereals. Its immature endosperm isolated at 8 - 10 days post anthesis (DPA) develops under in vitro conditions in similar way like in caryopsis *in vivo*. We conducted (Popielarska-Konieczna et al. 2013) histological studies on isolated endosperm of bread wheat (*Triticum aestivum*), durum wheat (*T. durum*) and triticale (*Triticosecale*). Endosperm development of cereals is strongly connected with the starch accumulation, endoreduplication and programmed cell death (PCD) (Sabelli 2012). In bread wheat and triticale induction of PCD starts around 16 DPA (Li et al. 2010). Our data (not published) revealed that cultured endosperm tissue of bread wheat, which accumulated starch granules under in vitro conditions, showed the viability during 2-3 months of the culture, what was confirmed using Evans blue staining procedure. On the other hand, the analysis of internucleosomal fragmentation of DNA revealed the induction of PCD in cultured endosperm starts faster than in vivo. DNA degradation was detected by the visualization of the ladder, which differs from DNA laddering specific for mature endosperm excised from caryopsis. Ultrastructural studies of starch grains and analysis of starch content are in progress.

The present works are financially supported by grant no. 2012/07/B/NZ9/01325 from The National Science Centre (Poland).

P409

Exploring the Genetic Regulation of Determinate Root Growth in Cactaceae

Svetlana Shishkova¹, Laura Las Peñas^{2,1}, Marta Matvienko³, Andrés Cuevas-Moreira¹, Gustavo Rodríguez-Alonso¹, Mayra López-Valle¹, Selene Napsucialy-Mendivil¹, Marcela Ramírez-Yarza¹, Joseph G. Dubrovsky¹

¹Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mor., Mexico,

²Instituto Multidisciplinario de Biología Vegetal, Córdoba, Argentina, ³CLC bio, Cambridge, MA, USA

Species of Cactaceae are well adapted to arid habitats. We have shown that most species from Cactoideae subfamily of the Cactaceae exhibit determinate growth of the primary root and that primary root growth in Cactoideae species matches their environment. Determinate root growth implies early root apical meristem (RAM) consumption and cell differentiation at the root tip. Our results also suggest that this type of growth became fixed after separation of the Cactioideae/Opuntioideae and Maihuenioideae /Pereskioideae lineages (Shishkova *et al.*, 2013).

To characterize genes involved in the RAM maintenance and determinate root growth in cardón *Pachycereus pringlei*, we employed mRNA-seq and smallRNA-seq, and used CLC GenomicWorkbench software for their analysis. mRNA-seq reads were *de novo* assembled and annotated by protein similarity. We analyzed differential gene expression in primary root tips in the initial growth phase (when RAM is still present), and in the terminal phase (when RAM is already exhausted). We also grouped and annotated small RNAs differentially expressed in the root tip. Besides conserved miRNAs, we identified hundreds of novel, species-specific smRNAs that show differential expression in the two growth phases.

Significant conservation was revealed for the amino acid sequence and RNA expression patterns of various proteins of *P. pringlei* and other plant species. Our results highlight the importance of hormone homeostasis and signaling in *P. pringlei* root development and suggest that the primary root tip after meristem exhaustion performs functions similar to those of the differentiation zone of *Arabidopsis* root. This work was supported by UNAM-DGAPA-PAPIIT (IN204912).

P410

Root Architecture during Drought Adaptation and the LOB-Domain Transcriptional Factor Family in *Brachypodium distachyon*

János Györgyey, Zoltán Zombori, Magdolna Gombos, Mária Szécsényi
Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Plant cells are more autonomous than animal cells and plant cells maintain totipotency, a capability to return from differentiated physiological state to meristem cell state which is the functional equivalent of the recently discovered stem cells of animals and human. This transition takes place in plant cells via reactivation of their cell cycle.

Brachypodium distachyon, a model plant for grass species including important crop plants as well, is used to understand how the question of "to divide or to differentiate" is regulated in plant cells. Root development is studied because a strong and efficient root system is also extremely important for the survival of our crop plants under stressful environmental conditions.

The characterization of the *Brachypodium* genotypes differing in drought tolerance was completed in terms of root growth and architecture and general growth parameters. Principal component analysis revealed that the primary component mainly derives from fresh and dry weight data and shoot length while the secondary component derives from primary root length and root/shoot length ratio out of more than twenty parameters measured.

LOB-domain transcription factors are plant specific proteins involved in diverse developmental processes from floral development to emergence of lateral roots. Eleven genes coding for these proteins as well as key cell cycle regulator cyclins were identified and characterized in details at transcript level. Various plant part/organ specificities were found, one of them possesses exceptionally high root-specificity. Ectopic expression of one of them in *Brachypodium* lines resulted in altered inflorescence development and reduced fertility.

P411

Developmental Programs of the Root Determinacy and Indeterminacy and Their Regulation

Joseph Dubrovsky¹, Blanca Jazmín Reyes-Hernández¹, Alejandra Hernández-Barrera^{1,2}, Héctor Hugo Torres-Martínez¹, Selene Napsucialy-Mendivil¹, Yamel Ugartechea-Chirino^{1,2}, Svetlana Shishkova¹

¹Departamento de Biología Molecular de Plantas, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Morelos, Mexico, ²Laboratorio de Genética Molecular, Desarrollo y Evolución de Plantas, Instituto de Ecología, UNAM, Mexico-City, Mexico

Roots with determinate growth commonly have indeterminate growth phase. However, it is not well understood how indeterminacy-to-determinacy switch (IDS) operates and whether root indeterminacy and determinacy represent different developmental programs. We have identified and characterized *Arabidopsis* mutants that show primary root determinate growth (an exhaustion pattern) and a very slow but indeterminate growth (a maintaining pattern). Root determinacy in the *moots koom2* ('short root' from Mayan, *mko2*) mutant is caused by a point mutation in *FOLYLPOLYGLUTAMATE SYNTHETASE1* (*FPGS1*). Alterations in folate metabolism cause the loss of stem cell activity and induce the quiescent center (QC) cell division. Folate-dependent pathway of the IDS operates independently of auxin gradients and regulatory modules participating in the root apical meristem (RAM) maintenance (*WOX5*, *PLETHORA*, and *SCR*). In a mutant with a maintaining pattern, such as *arabidopsis* homolog of *trithorax1* (*atx1*), the QC and stem cells maintain their activity, while the RAM could lose its organization but not consumed. Using these and other mutants, we conclude that a developmental program for maintenance of meristem is different from that for the maintenance of indeterminacy. When the former program is compromised, a smaller meristem is preserved, whereas when the latter program does not operate, the whole meristem becomes consumed. Therefore, the IDS represents a specific developmental pathway in regulation of whole RAM behavior and we propose that it is a different level of regulation of root meristem development in addition to meristem maintenance. The work was funded by UNAM-DGAPA-PAPIIT (IN204312 and IN204912) and CONACyT (206843).

P412

Myosins XI-K, XI-1, and XI-2 Are Required for Development of Different Epidermal Cells in *Arabidopsis*

Eve-Ly Ojangu, Krista Tanner, Pille Pata, Heiti Paves, [Erkki Truve](#)
Tallinn University of Technology, Tallinn, Estonia

The positioning and dynamics of vesicles and organelles, and thus the growth of plant cells, is mediated by the acto-myosin system. In *Arabidopsis* there are 13 class XI myosins which mediate vesicle and organelle transport in different cell types. So far the involvement of five class XI myosins in cell expansion during the shoot and root development has been shown, three of which, XI-1, XI-2, and XI-K, are essential for organelle transport.

Simultaneous depletion of *Arabidopsis* class XI myosins XI-K, XI-1, and XI-2 in double and triple mutant plants affected the growth of several types of epidermal cells. The size and shape of root hairs, trichomes, leaf pavement cells and the elongation of the stigmatic papillae of double and triple mutant plants were affected to different extent. Reduced cell size led to significant size reduction of shoot organs in the case of triple mutant, affecting bolt formation, flowering time and fertility. Phenotype analysis revealed that the reduced fertility of triple mutant plants was caused by delayed or insufficient development of pistils.

We conclude that the class XI myosins XI-K, XI-1 and XI-2 have partially redundant roles in the growth of epidermal cells. Myosin XI-K plays more important role whereas myosins XI-1 and XI-2 have minor roles in the determination of size and shape of epidermal cells, because the absence of these two myosins is compensated by XI-K. Co-operation between myosins XI-K and XI-2 appears to play an important role in these processes.

P413

Autophagy and ROS/RNS in Programmed Cell Death during Stress-Induced Pollen Embryogenesis and Pollen Development

María-Teresa Solís, Ivett Bárány, Vanesa Cano, María Rodríguez-Serrano, María C. Risueño, Pilar S. Testillano

Biological Research Center, CIB-CSIC, Madrid, Spain

Microspores develop to follow the gametophytic pathway for producing pollen and gametes. In vitro, microspores can be induced, under stress treatments to deviate their gametophytic development and to switch to embryogenesis, forming haploid embryos and homozygous breeding lines in a short time; the efficiency of the process is affected by cell death of microspores after the stress. At defined stages of microspore development, the tapetum nursing tissue undergoes developmentally regulated programmed cell death (PCD)

In this work we studied the existence and dynamics of autophagy compartments, markers and genes in relation to ROS/RNS production, caspase-like activity and ultrastructural rearrangements during two cell death processes: PCD of microspores in culture after embryogenesis induction by stress, and PCD of tapetum during pollen development, in *Brassica napus* and *Hordeum vulgare*.

In microspores cultures, ROS and NO production, cell death proportion and caspase 3-like activity, and *ATG8* gene expression increased after stress treatments. In tapetal cells at PCD initiation, nuclear condensation, cytochrome C release and high caspase 3-like activity were detected concomitantly with increase of vesicles, vacuoles and different endomembrane compartments in the cytoplasm, resembling structures of the autophagic pathway.

Results will be discussed on the light of the participation of autophagy in the PCD during the two pollen developmental pathways.

Rodríguez-Serrano et al. (2012). *Journal of Experimental Botany* 63, 2007-2024.

Solís et al. (2014). *Plant and Cell Physiology*. 55, 16-29.

Funding by Spanish MINECO (BFU2011-23752) and CSIC (PIE 201020E038).

P414

Destabilization of Interaction between Cytokinin Signaling Intermediates AHP1 and ARR4 Affects Root Development in *Arabidopsis*

Vivek Verma, Jayaraman Sivaraman, Prakash Kumar
National University of Singapore, Singapore, Singapore

The eukaryotic variant of the bacterial two-component signaling (TCS) exhibits multi-step phosphorelay (MSP), where phosphoryl group transfer occurs down a His-Asp-His-Asp sequence among signaling proteins. The structural basis of the initial step of TCS has been studied in bacteria, yeast and *Arabidopsis*. We examined the final step of MSP phosphorelay of cytokinin in *Arabidopsis*, the structure-function relationships of which have not been studied so far. Using the crystal structure of ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 1 (AHP1) and a homology model of the response regulator domain of ARABIDOPSIS RESPONSE REGULATOR 4 (Δ ARR4⁽¹⁶⁻¹⁷⁵⁾, type-A response regulator) we generated a structural complex model. Using the model, key amino acid residues for interaction were identified. Selected amino acid residues of ARR4 that were predicted to be involved in the complex formation were mutated. The mutations resulted in either abolition or weaker interaction with AHP1 as demonstrated in yeast two-hybrid interaction studies. Transgenic *Arabidopsis* plants were generated for two mutants of ARR4 that showed weakened interaction with AHP1. Root elongation assay with these transgenic lines showed that weakening of the signal transduction to the negative regulator of cytokinin signaling intermediate, namely, ARR4 results in a stronger cytokinin response. Our study establishes a structure-function relationship for the final step of a eukaryotic MSP signal cascade and explains the hormonal control of root development.

P415

Light-Dependent Alterations in Polyamine Oxidase and Diamine Oxidase Activities of Developing Zucchini Cotyledons Growing in Presence of Plant Growth Regulators

Maya Damyanova, Dessislava Todorova, Zornitsa Katerova, Dzhovani Polizoev, Iskren Sergiev
Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Cotyledons isolated from *Cucurbita pepo* L., cv. Cocozelle var. Tripolis (zucchini) were incubated for 6 days at 28°C in darkness or at 16/8h (light/dark) regime on water supplemented with methyl ester of jasmonic acid (MeJA), cytokinins (BA and 4PU-30) or combinations of them. The analyses of polyamine oxidase (PAO) and diamine oxidase (DAO) were performed in dynamic from 2nd day when cell proliferation processes were started to 6th day when any cell division processes were finished. It was found that during the experimental period both cytokinins applied alone or in combination with MeJA led to a significant suppression of DAO and PAO activities and the effect of the phenylurea type cytokinin was more pronounced – up to 4 times as compared to the relative controls at the end of the experiment. More significant inhibition of DAO and PAO activities was documented when cotyledons were grown in darkness in comparison with those developed under light regime. Less considerable changes were documented when cotyledons were grown on MeJA. Additionally, the results obtained for free proline content showed similar trend of alterations. These data suggest that the catabolic enzymes of polyamines are involved in the regulation of the developmental processes of isolated cotyledons grown under different light regimes.

Acknowledgments: This work was supported by the grant №BG051PO001-3.3.06-0025, financed by the European Social Fund and Operational Programme Human Resources Development (2007–2013) and co-financed by Bulgarian Ministry of Education and Science.

P416

Dynamical Changes in the Antioxidant Enzyme Activities of Developing Cotyledons of *Cucurbita pepo* L. Grown in Presence of Different Plant Growth Regulators

Maya Damyanova, Dessislava Todorova, Zornitsa Katerova, Dzhovani Polizoev, Iskren Sergiev
Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Cotyledons isolated from *Cucurbita pepo* L., cv. Coccozelle var. Tripolis (zucchini) were allowed to develop for 6 days at 28°C on water supplemented with either, methyl ester of jasmonic acid (MeJA), cytokinins (BA and 4PU-30) or combinations of them in darkness or at 16/8h (light/dark) regime. The analyses of catalase (CAT), guaiacol peroxidase (POD), superoxide dismutase (SOD) activities, and hydrogen peroxide (H₂O₂) and total protein contents were performed in dynamic from 2nd day when cell proliferation processes were started until 6th day when any cell division processes were finished. During the experimental period, the CAT and SOD activities and protein content were not changed considerably in control and MeJA-treated cotyledons, while the activity of POD and H₂O₂ content rose significantly. Adding of cytokinins alone or in combination with MeJA into the growth medium provoked an additional increase of POD activity and H₂O₂ content in cotyledons and the effect of phenylurea type cytokinin was more pronounced – up to 6 times as compared to the respective controls. However, the effect of cytokinins on the catalase activity and protein content was the opposite and a significant decrease was observed. Initially, all treatments decreased SOD activity, but during the experimental period it tended to increase and on day 6 the activity in cotyledons grown on 4PU-30 was higher than the control. More obvious alterations in the measured parameters were observed when cotyledons were grown in darkness as compared to those developed under light.

P417

Unravelling Nitric Oxide (NO) Function during Germination and Early Plant Development

Luis Sanz, Isabel Mateos, Pablo Albertos, M. Inmaculada Sanchez-Vicente, Luis Quintero, Tamara Lechón, Noelia Arteaga, Dolores Rodriguez, Oscar Lorenzo
University of Salamanca, Salamanca, Spain

Nitric oxide (NO) is involved in a variety of physiological processes during plant life acting as a key signalling molecule. Our recent research has uncovered a role of NO on primary root growth in *Arabidopsis* (1). However, our current knowledge about the NO signalling pathway is still fragmentary. To identify NO molecular players, we have characterized several mutants encoding transcription factors (TFs) showing ABA- and NO scavenging-insensitive phenotypes during seed germination. Microarray analysis highlighted a putative role of these TFs in the ABA and NO crosstalk. Insights in the posttranslational redox modifications of these TFs will be presented together with their physiological relevance. Additionally, we took advantage of the resources implemented in the frame of the CONSOLIDER-TRANSPLANTA project to identify putative TFs involved in the NO signal transduction pathway. Firstly, we aim to identify direct targets of these TFs by definition of the *cis* consensus promoter element coupled to transcriptional profiling. Furthermore, characterization of TFs able to modify S-nitrosothiol content and enhance or suppress NO-related phenotypes is currently underway. We have obtained a collection of transgenic lines that overexpress 1000 *Arabidopsis* TFs, under the control of the estradiol-inducible promoter, in NO overproducer (*cue1/nox1*) and NO deficient (*atnoa1* and *nia1;nia2*) mutant backgrounds. Finally, these different mutant backgrounds affecting NO homeostasis are being analyzed in terms of crosstalk with key plant hormones.

1. Fernández-Marcos *et al.* (2011) *PNAS* 108:18506-18511.

Acknowledgments: MICINN (BIO2011-26940), TRANSPLANTA-CONSOLIDER (CSD2007-00057), "EcoSeed-311840" ERC.KBBE.2012.1.1-01 and SA239U13 (to O.L.) from Junta de Castilla y León.

P418

Effect of ABA on Certain Genes Expression in *Arabidopsis thaliana*

Nihal Gören-Saglam¹, Vicky Buchanan-Wollaston²

¹Istanbul University, Istanbul, Turkey, ²University of Warwick, Coventry, UK

Senescence is an important developmental procedure which is seen in plants and resulted in death of cell, tissue, organ or the whole plant. Despite of plenty of studies conducted on senescence, its mechanism has not been fully elucidated yet. This may result from the fact that internal and external factors may play a role in the regulation of senescence, solely or together. Of internal factors, especially plant hormones can induce or inhibit senescence. ABA was originally isolated as an abscission-causing factor. It is reported to accelerate senescence. The amount of endogenous ABA shows an increase during senescence. Environmental stress factors such as drought, high salt concentration, high/low temperature accelerate leaf senescence and this resulted of endogenous ABA concentration is increased. During senescence, activity of some of genes is up or downregulated. In this study we analyzed some genes activities after ABA treatment using q-PCR technique in *Arabidopsis thaliana* leaves. These genes are NAC19 (encodes a NAC transcription factor whose expression is induced by drought, high salt, and abscisic acid), NAC4 (Encodes a member of the NAC transcription factor gene family. Its expression is associated with leaf senescence), NAC3 (Encodes an ATAF-like NAC-domain transcription factor), MYB2 (Encodes a MYB transcription factor that possesses an R2R3 MYB DNA binding domain and is known to regulate the expression of salt- and dehydration-responsive genes) and SNAT (Seven-in-absentia family protein). The q-PCR results showed that expression levels of NAC19, NAC4, NAC3 and SNAT were increased significantly after ABA treatment when compared with the control.

P419

REVOLUTA and WRKY53 Connect Early and Late Leaf Development in Arabidopsis

Yakun Xie, Kerstin Huhn, Ronny Brandt, Maren Potschin, Thomas Drechsler, Ulrike Zentgraf, Stephan Wenkel

Center for Plant Molecular Biology, Tuebingen, Germany

As sessile organisms, plants have to continuously adjust growth and development to ever-changing environmental conditions. At the end of the growing season, annual plants induce leaf senescence to reallocate nutrients and energy-rich substances from the leaves to the maturing seeds. Thus, leaf senescence is a mean to increase reproductive success and is therefore tightly coupled to the developmental age of the plant. However, senescence can also be induced in response to suboptimal growth conditions as an exit-strategy, which is accompanied by a severely reduced yield. Here we show that class III homeodomain leucine zipper (HD-ZIPIII) transcription factors, known to be involved in basic pattern formation, have an additional role in controlling the onset of leaf senescence in Arabidopsis. Several potential direct downstream genes of the HD-ZIPIII protein REVOLUTA (REV) have known roles in environment-controlled physiological processes. Here, we report that REV directly and positively regulates the expression of WRKY53, a master regulator of age-induced leaf senescence. HD-ZIPIII proteins are required for the full induction of WRKY53 in response to oxidative stress and mutations in HD-ZIPIII genes strongly delay the onset of senescence. Thus a cross talk between early and late stages of leaf development appears to be essential for reproductive success.

P420

Characterization of E3 Ubiquitin Ligases Involved in Jasmonate Signalling

Sabrina Iñigo, Astrid Nagels Durand, Andrés Ritter, Laurens Pauwels, Alain Goossens
Department of Plant Systems Biology, VIB, Ghent University, Gent, Belgium

As sessile organisms, plants need to adapt to fluctuations in environmental conditions. A complex hormonal network plays a central role in the plant's response to different types of endogenous and exogenous signals, leading to extensive transcriptional, proteomic and metabolomic changes that determine the specifically required response.

Jasmonate and its derivatives (JA(s)) are essential and ubiquitous fatty acid-derived phytohormones. They are involved in plant growth, development and in the response to biotic and abiotic stresses. Upon wounding or pathogen attack plant cells activate several defence mechanisms and produce specific protective specialised metabolites.

It has recently been demonstrated that the Ub-26S proteasome system is connected to almost all aspects of plant development, by acting in the centre of most hormone responses. It was also demonstrated that hormonal crosstalk can occur at the proteolysis level. However, despite the extremely high number of potential E3 ligases encoded by plant genomes, only a few have been specifically characterised and ubiquitination of target proteins has barely been proven.

The aim of our research is to characterize the role and mode of action of E3 Ub ligases involved in JA signalling that were isolated in a transcriptomics and interactomics screens. Their putative targets are being identified after which we study their *in vitro* ubiquitination and confirm protein degradation by proteasome. The phenotypic characterization of plants with loss- or gain-of-function of the E3 ligases and their substrates will enable to establish their function in JA signalling and their importance for plant growth, development and defence in *Arabidopsis thaliana*.

P421

Understanding Genetic Control of Ovule Number in *Arabidopsis thaliana*

Andrea Guazzotti¹, Peter C. McKeown², Antoine Fort², Mara Cucinotta¹, Charles Spillane², Lucia Colombo¹

¹Università degli Studi di Milano, Dipartimento di Bioscienze, via Celoria 26, Milano, Italy, ²Plant and AgriBiosciences Centre (PABC), School of Natural Sciences, National University of Ireland Galway, Galway, Ireland

Seed number is one of the most important crop traits and a target for plant breeding and yield enhancement, needed to meet increasing demand for food, feed, fuel and other plant-derived products. Seed number is inherently linked to ovule number, since ovules, once mature, undergo fertilization and give rise to seeds. A restricted number of master genes controlling ovule number has been identified, some of which have been already characterized (Galbiati et al., 2013). Recently, it has been demonstrated that ovule number in selfed *Arabidopsis thaliana* triploid plants is influenced by parent-of-origin specific genome dosage effects (Duszynska et al., 2013). Triploid plants were generated by crossing tetraploid lines of four different accessions with diploid plants of the same accession, either using the tetraploid as the pollen donor (to produce triploids with an additional paternally-inherited genome); or using the diploid as the pollen donor (to produce triploids with an additional maternally-inherited genome). These triploid lines carried an excess of maternally or paternally derived chromosomes but are genetically identical (isogenic).

Here we describe how to conduct experiments on gene dosage effects on ovule number in *Arabidopsis thaliana* polyploids, in order to find out if the genes related to ovule development are differentially expressed between triploid plants with an excess of maternally or paternally derived chromosomes. This would provide a novel mechanism for parental control of ovule number during plant reproduction.

P422

In Vivo Regulation of SUMO Conjugation

Abraham Mas, Laura Castaño, Maria Lois
Centre for Research in Agricultural Genomics in, Barcelona, Spain

Posttranslational modification with Small Ubiquitin-related Modifier (SUMO) is an essential regulatory mechanism of protein function in eukaryotes. In plants, genetic studies have established a role for SUMOylation in plant development and environmental stress responses. However, the molecular mechanism through which SUMO regulates those biological processes is poorly understood.

SUMO is synthesized as a precursor that is processed by the specific ULP proteases. As a first step in SUMO conjugation, the mature SUMO is activated by the heterodimeric E1 activating enzyme in an ATP-dependent reaction. Next, SUMO is transferred to the E2 conjugating enzyme, which is competent for transferring SUMO to a lysine in the target substrate, although this reaction is facilitated by E3 ligases.

In previous studies, our group has focused in the study of the regulatory role of the heterodimeric E1 (SAE2/SAE1) activating enzyme. We showed that the SUMO E1 contributes to SUMO paralog discrimination, providing a novel mechanism to favor conjugation of the essential AtSUMO1/2 paralogs. In addition, we have established that evolutionary diversification of the E1 small subunit, SAE1, contributes to regulation of SUMO conjugation rate, suggesting that *in vivo* dynamics of SUMO conjugation could be mediated by the E1 activating enzyme.

Recent results pointing to the existence of novel posttranslational modifications that could modulate SUMO activation *in vivo* will be presented.

P423

***In Vitro* Shoot Multiplication in *Vaccinium corymbosum* L. and *Rubus* spp. Cultivars**

Andrea Hricová, Alena Gajošová, Gabriela Libiaková

Institute of Plant Genetics and Biotechnology of the Slovak Academy of Sciences, Nitra, Slovakia

For shoot multiplication, the isolated dormant apical and axillary buds were used. Significantly highest shoot multiplication in *Vaccinium corymbosum* on WPM medium with zeatin 0.5 mg.l^{-1} and IAA 0.2 mg.l^{-1} was achieved in cv. 'Aurora' (Q=3,26). The cultivars 'Chandler', 'Hannah's Choice' and 'Goldtraube' created one homogeneous group with Q= 2.91, 3.05 and 3.08, respectively. Cultivars 'Liberty' and 'Toro' showed the lowest shoot multiplication ability with Q=1.77 and 1.92, respectively. The highest shoot multiplication was observed after 1st subculture (3.16) with lower multiplication in the 2nd (2.40) and 3rd subcultures (2.43).

In *Rubus*, different cultivar ability as well as significant influence of BAP concentrations in MS medium was confirmed on shoot proliferation. There were no significant differences in shoot proliferation between cvs. 'Black Satin' (4.08) and 'Tulameen' (3.56 shoots/explant), similarly like between 'Tayberry' (2.90) and 'Black Jewel' (2.34 shoots/explant), however significant differences were found between both groups of the mentioned cultivars. Regarding BAP concentrations, significant differences were found between 0.5 mg.l^{-1} BAP (with the lowest number of shoots/explant) and 1; 1.5 and 2 mg.l^{-1} BAP (with no significant differences among these concentrations). Higher BAP concentrations showed positive influence on shoot proliferation in tested cultivars. The highest multiplication coefficient was achieved with BAP concentrations $1.5\text{-}2 \text{ mg.l}^{-1}$ in cv. 'Black Satin' (5.86), much like in cv. 'Black Jewel' (4.48 shoots/explant). Our results confirmed that *in vitro* shoot proliferation ability is highly genotype-dependent.

Acknowledgements: The work was supported by VEGA no. 2/0140/14 and by the European Community project no 26220220180: Building Research Centre „AgroBioTech“.

P424

Analysis of a New Wheat Flowering Time Gene QFt.cri-3B.1 on the Growth and Development of Shoot Apex

Ilija Tom Prášil¹, Martina Trávníčková¹, Kateřina Pánková¹, Jana Musilová¹, Zbyněk Milec², Jan Šafář²
¹*Crop Research Institute, Prague, Czech Republic,* ²*Centrum of the Region Haná, Institute of Experimental Botany, Olomouc, Czech Republic*

Two mapping populations of near-isogenic lines derived from chromosome 3B substitution lines of two Czech spring wheat varieties (Sandra and Zlatka) were used to map a new flowering time gene. The 3B chromosome was substituted from landrace Česká přesívka (CP) and the QFt.cri-3B.1 locus was localized on chromosome 3B delimited by markers gwm285-cfa2170. The gene affects flowering time by about four days. Interestingly, under short day conditions it accelerates flowering and under long days the effect is opposite. Experiments aimed at monitoring of spike emergence and the transition of the apical meristem to the reproductive phase were performed to determine the growth phase in which the gene is having an effect. The substitution lines (Sandra CP3B or Zlatka CP3B, carrying QFt.cri 3B.1) revealed a similar development dynamics as their parents up to the stage of floret primordia initiation; then they started to develop differentially in the following aspects: 1) their development was delayed under long days but 2) it was earlier under short days compared to their parents. It seems probable that the new flowering time locus (QFt.cri-3B.1) affects the later stages of apical development, i.e., stages after reaching the terminal spikelet stage. This work has been supported by the European Union Seventh Framework Programme (FP7/2007- 2013) under grant agreement no 289842 (ADAPTAWHEAT).

P425

Symplasmic Communication during Root Epidermal Cell Differentiation

Marek Marzec¹, Aleksandra Muszynska², Michael Melzer², Ewa U. Kurczynska¹

¹Department of Genetics, University of Silesia, Katowice, Poland, ²Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany

It is well known that the process of plant cell differentiation depends on the symplasmic isolation of cells. Before starting the differentiation program, an individual cell or group of cells should restrict their symplasmic communication with neighboring cells.

In the presented work, we tested the symplasmic communication between epidermal cells in the different root zones of parental *Hordeum vulgare* L., plants: cv. 'Karat' with normal root hair development, and two allelic root hairless mutants (rhl1.a and rhl1.b) from the Mutant Collection of Department of Genetics. The results obtained clearly showed that symplasmic communication was limited during root hair differentiation in the parental variety, whereas in both root hairless mutants, epidermal cells were still symplasmically connected in the zone of mature root hairs. Analysis under transmission electron microscope revealed that there are no differences in the ultrastructure of plasmodesmata between mutants and parental variety. However the immunogold labeling of callose showed higher number of callose molecules inside plasmodesmata in the differentiation zone of 'Karat' variety, in contrast to root hairless mutant.

This is the first report about the role of symplasmic isolation in barley epidermal root cell differentiation. Additionally, the presented data show that a disturbance in the restriction of symplasmic communication is present in root hairless mutants.

P426

Meristem Size Regulation in the Arabidopsis Root

Shiqiang Wang¹, Cristina I. Llavata-Peris², Dolf Weijers², Thomas Laux¹

¹BIOSS, Faculty of Biology, Albert-Ludwigs-University Freiburg, Freiburg, Germany, ²Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands

Cell division and differentiation must be coordinated for the correct morphogenesis during development. For the development of the root of *Arabidopsis thaliana*, proximal meristem cells that mainly have cell division activity start to differentiate when they reach the transition zone (TZ). Cytokinin and auxin play critical roles for this developmental switch to ensure the final meristem size in an antagonistic way. The spatiotemporal expression pattern of WUSCHEL-RELATED HOMEODOMAIN (WOX) genes has important functions in plant development. The *wox9* mutant has a short root with reduced meristem size. However, the underlying mechanism is still unknown. Here we address this question by immunoprecipitation of GFP tagged WOX9 and resolved by mass spectrometry. Some potential interacting proteins are found, which gave us a hint that WOX9 may function in the homeostasis of cytokinin level for root meristem size regulation to prevent premature exit of cells from the meristem state.

P427

Influence of Smoke-Water and Smoke-Derived Compounds during the Micropropagation of *Eucomis autumnalis* Subspecies *autumnalis*, a Valuable Medicinal Plant

Nqobile A. Masondo¹, Adeyemi O. Aremu¹, Mack Moyo¹, Stephen O. Amoo¹, Karel Doležal², Jeffrey F. Finnie¹, Johannes Van Staden¹

¹Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, Pietermaritzburg, KwaZulu-Natal, South Africa,

²Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, 783 71, Olomouc, Czech Republic

Eucomis autumnalis (Mill.) Chitt. subspecies *autumnalis* (Hyacinthaceae) is a valuable medicinal plant in African Traditional Medicine. Although the conservation status of this species has not been evaluated, as with most bulbous plants, wild populations are continuously under threat due to over-harvesting and habitat loss via various anthropogenic factors. Thus, in vitro propagation is a viable means of ensuring its conservation. However, mass propagation of medicinal plants should be accompanied with sustained phytochemical production to guarantee their therapeutic efficacy. We evaluated the influence of smoke-water (SW) and karrikinolide (KAR₁), hypothesized to exhibit auxin and cytokinin-like activity, during micropropagation of this species. The phytochemical content of the in vitro regenerants was also quantified. Leaf explants were cultured on Murashige and Skoog medium supplemented with SW (1:500; 1:1000; 1:1500 dilution) or KAR₁ (10⁻⁷; 10⁻⁸; 10⁻⁹ M) and grown for 10 weeks. While shoot proliferation was not enhanced with SW and KAR₁, shoot length (SW 1:1000), root number (KAR₁ 10⁻⁸ M) and root length (SW 1:1500) were significantly higher than in the control. SW (1:1000 and 1:1500) and KAR₁ (10⁻⁷ and 10⁻⁹ M) significantly increased the total phenolics, flavonoids and condensed tannins when compared to the control. The current findings indicate the array of physiological processes influenced by SW and KAR₁ during micropropagation of this species.

P428

Three Classes of Arabinogalactan Proteins are Related with Root Hair Development in *Hordeum vulgare* L.

Marek Marzec¹, Michael Melzer², Iwona Szarejko¹

¹University of Silesia, Katowice, Poland, ²Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Arabinogalactan proteins (AGPs) are a family of glycoproteins that play a crucial role in many biological processes in plants, including cell differentiation and expansion. In the presented work hypothesis about the role of AGPs in barley root hair development was investigated. Obtained results showed that treatment of roots with the active form of Yarif reagent, that binds all classes of AGPs, caused inhibition of root hair tube elongation, or complete lack of root hairs in wild type plants. Moreover, three groups of AGPs, recognized by the LM2, LM14, and MAC207 antibodies, were diversely distributed among root epidermal cells of wild type plants, in a manner that allowed to distinguish trichoblasts from atrichoblasts. Only in trichoblasts and root hair tubes the LM2, LM14, and MAC207 epitopes were present in the cell wall, whereas in atrichoblasts the presence of these AGPs was observed only in the cytoplasm. In root hairless mutant all epidermal cells showed the homogeneous expression pattern and cytoplasmic localization of these epitopes characteristic for atrichoblasts of parent variety. Finally, the specific localization of the epitope recognized by LM2 antibody was observed on the surface of primordia and root hair tubes in all genotypes producing root hairs.

Presented results point out the importance of AGPs in barley root hair development and show that cellular localization of at least three classes of AGPs, recognized by the LM2, LM14, and MAC207 antibodies, is related to differentiation of barley root epidermal cells.

P429

Identification and Functional Analysis of the HvHTD4 Gene Encoding Protein Involved in Strigolactone Signalling in *Hordeum vulgare* L.

Marek Marzec, Piotr Tylec, Damian Gruszka, Iwona Szarejko
University of Silesia, Katowice, Poland

Strigolactones (SLs) are a group of plant hormones described in 2008 as regulators of growth and development, that may inhibit outgrowth of axillary buds and therefore limit shoot branching. They are also known as stimulators of symbiosis with arbuscular mycorrhizal fungi. Moreover, SLs enhance seed germination of root parasitic plants. Recently, the additional roles of SLs were described in plants, for example in the development of root system and root hairs in various species. In presented studies the barley HvHTD4 (High Tillering Dwarf4) gene, encoding α/β hydrolase that is involved in strigolactone signalling, was identified. Bioinformatic analysis revealed that the identified gene is a homolog of the D14, AtD14 and PhDAD2 genes described in rice, Arabidopsis, and petunia, respectively. For functional analysis of the identified gene the TILLING strategy was used to obtain a series of HvHTD4 alleles. Phenotype of plants carrying one of the identified alleles, htd4.d, corresponded to the phenotype of SLs mutants described for other species: plants were semi-dwarf and produced a higher number of tillers, in comparison with the parent variety. Plants with the htd4.d allele were also insensitive to a treatment with GR24, the synthetic analog of SLs, what confirmed the role of HvHTD4 in the SLs signalling pathway. Moreover, the identified mutation in HvHTD4 resulted in higher number of tillers with fertile spikes, hence plants with the htd4.d allele produced increased number of seeds, which makes them very useful as a breeding material for development of improved barley cultivars.

P430

Characteristics of a Root Hair-Less Line of *Arabidopsis thaliana* under Physiological Stresses

Natsuki Tanaka¹, Mariko Kato², Rie Tomioka¹, Rie Kurata³, Yoichiro Fukao³, Takashi Aoyama², Masayoshi Maeshima¹

¹Nagoya University, Nagoya, Japan, ²Kyoto University, Uji, Japan, ³Nara Institute of Science and Technology, Ikoma, Japan

The plasma membrane-associated Ca²⁺-binding protein-2 of *Arabidopsis thaliana* is involved in the growth of root hair tips. Several transgenic lines that overexpress the 23 residue N-terminal domain of this protein under the control of the root hair-specific EXPANSIN A7 promoter lack root hairs completely. The role of root hairs under normal and stress conditions was examined in one of these root hair-less lines (NR23). Compared with the wild type, NR23 showed a 47% reduction in water absorption, decreased drought tolerance, and a lower ability to adapt to heat. Growth of NR23 was suppressed in media deficient in phosphorus, iron, calcium, zinc, copper, or potassium. Also, the content of an individual mineral in NR23 grown in normal medium, or in medium lacking a specific mineral, was relatively low. In wild-type plants, the primary and lateral roots produce numerous root hairs that become elongated under phosphatedeficient conditions; NR23 did not produce root hairs. Although several isoforms of the plasma membrane phosphate transporters including PHT1;1–PHT1;6 were markedly induced after growth in phosphate-deficient medium, the levels induced in NR23 were less than half those observed in the wild type. In phosphate-deficient medium, the amounts of acid phosphatase, malate, and citrate secreted from NR23 roots were 38, 9, and 16% of the levels secreted from wildtype roots. The present results suggest that root hairs play significant roles in the absorption of water and several minerals, secretion of acid phosphatase(s) and organic acids, and in penetration of the primary roots into gels.

P431

Towards Functional Characterization of Retinoblastoma(Rb)-Signaling Pathway in *Physcomitrella patens*

Omar Saleh^{1,2}, Anna Jehle¹, Gertrud Wiedemann^{1,2}, Nelly Horst¹, Eva Decker^{1,2}, Ralf Reski^{1,2}

¹Plant Biotechnology, Faculty of Biology, University of Freiburg, Freiburg, Germany, ²TIP - Trinational Institute for Plant Research, Upper Rhine Region, Upper Rhine Region, Germany

Molecular pathways controlling cell cycle progression and cell division are highly conserved among eukaryotes, including plants. One of these is the Rb-signaling pathway controlling the progression through the G1/S transition checkpoint. The moss *Physcomitrella patens* encodes all Rb-related proteins. Higher plant cells are arrested in the G1, while moss cells are arrested in G2. We aim to study whether the G1/S control of this pathway is conserved in moss or if it is utilized in other contexts in the cell cycle.

Single knockouts (KOs) of *Pprbr;1*, *Pprbr;2*, *Pprbr;3*, *Ppcycd;1*, *Ppcdka;1*, and *Ppe2f4* and *Ppcycd;1/Ppcdka;1* double KOs have been generated. The formation of gametophores was faster in most of these knockouts than in WT. Mild morphological changes occur in these lines and no clear influence on the moss cell cycle progression could be observed, possibly due to functional redundancy. However, some KOs were strongly affected with regard to spore capsule formation indicating a role of Rb pathway proteins in moss generative development. Furthermore, *Pprbr;2/3* doKO and *PpcycD;1/Ppcdka;1/2* triKO plants have been generated. In comparison to WT, the number of G1 phase-arrested cells was drastically lower in *Pprbr;2/3* doKO lines, while it was doubled in *Ppcycd;1/Ppcdka;1/2* triKOs. Microarray analyses of *Ppcycd;1/Ppcdka;1/2* triKOs suggest possible involvements in DNA damage repair among other functions of this pathway in moss.

The results indicate that the cell cycle control of the Rb-signaling pathway is conserved in moss. However, this pathway might also link cell cycle progression to mechanisms involved in DNA recombination and damage repair.

P432

Microspore Reprogramming towards Embryogenesis – Effect of Cold Treatment on Anther and Microspore Culture of Tobacco (*Nicotiana tabacum* L.)

Edith Stabentheiner, Andreas Oberländer, Regina Willfurth
Institute of Plant Science, University of Graz, Graz, Austria

Male microspores can be reprogrammed to shift from the gametophytic to the embryogenic pathway; developmental reprogramming results in haploid (double haploid) embryos instead of mature pollen. This developmental shift is mediated by a stress event, and very often cold stress is this indispensable external trigger. Responsive microspores dedifferentiate; the cells start dividing, form multicellular structures, and an embryo or undifferentiated callus develops.

The influence of cold stress on reprogramming of microspores was examined in tobacco (*Nicotiana tabacum* L.). Young flowers with microspores in the late uninucleate to early binucleate state were exposed to cold stress (4-6°C) for several days to weeks, and embryo and callus formation was followed in anther and microspore cultures. Physiological stress markers were analyzed in the anthers of the cold exposed flowers - total antioxidative capacity, proline, malondialdehyde, and oxidized as well as reduced glutathione as markers for the redox state of the cells. The activities of peroxidase as an overall stress enzyme as well as of invertase, a key enzyme in carbohydrate metabolism, were also investigated.

A cold treatment of 5 to 7 days had a positive influence on embryogenesis in microspore cultures and in anther cultures. Longer cold treatment resulted in a higher mortality rate of the microspores and characteristic changes of the stress markers in the anthers. Callus formation without further development was only observed in microspore cultures but not in anther cultures. Microspore and anther culture are reliable systems and valuably complement one another in studying the reprogramming of microspores towards embryogenesis.

P433

Dormancy Breaking Linked with the Changes of Endogenous ABA, Storage Compounds and Fumarase Activity in Beechnuts

Kateřina Eliášová, Zuzana Vondráková, Bedřich Pešek
Institute of Experimental Botany AS CR, Prague, Czech Republic

The common beech (*Fagus sylvatica* L.) is one of the most important trees in European forestry. Beechnuts belong to the group of non-endospermic seeds with reserves stored predominantly in the cotyledons. They are characterized by deep physiological dormancy, which can be overcome by a moist chilling treatment (stratification). Seed dormancy is a complex physiological process preventing germination during unsuitable climatic conditions. It is controlled genetically and regulated by phytohormones.

We described the changes in embryonic axes as well as in cotyledons of beech embryos during stratification on biochemical and histological level. Our observations were concentrated on the changes of endogenous ABA, activity of fumarase and changes in the distribution and amount of storage compounds - proteins, starch and calcium oxalate.

The release from dormancy interrelated directly with the drop in the endogenous ABA content in both cotyledons and embryonic axes. On the contrary the fumarase activity rose in the course of stratification. Considerable changes in the localization of storage proteins were detected after the first week of stratification. During proceeding dormancy release proteinaceous content of storage vacuoles gradually disappeared, vacuoles diminished or fused forming central vacuoles. Any changes in starch grains and calcium oxalate crystals distribution or amount connected with the dormancy breaking were not detected.

Acknowledgement: This research was supported by the Ministry of Agriculture of the Czech Republic - project No. Q1102A256.

P434

Tomato Flower Abscission Zone - Cellular Mechanisms and Ultrastructure

Aleš Kladnik¹, Marko Chersicola², Marina Dermastia², Magda Tušek Žnidarič², Amnon Lers³
¹University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia, ²National Institute of Biology, Ljubljana, Slovenia, ³Agricultural Research Organization, The Volcani Center, Bet-Dagan, Israel

Abscission in tomato flower pedicel initiates if the developed flower is not fertilized. The unfertilized flowers abscise in a few days after anthesis. The ultrastructure of the abscission zone (AZ) shows a distinct layer of small cells in the dilated part of the pedicel. The abscission can be induced experimentally by removal of the developed flower. The separation process finishes in several hours after induction. The cellular and molecular events are asymmetric at both sides of the AZ. LX RNase is involved in execution of abscission and LX inhibited transgenic plants show delayed abscission. The histology of AZ was examined in wildtype and LX transgenic plants using transmission electron microscopy and light microscopy. LX location was studied using immunolocalization. In the later stages of abscission programmed cell death occurs in the distal part of AZ. The progression of programmed cell death was studied using TUNEL reaction and TEM microscopy. Here we show the different ultrastructural and molecular properties in cells at both sides of the flower AZ.

P435

Arabidopsis Dolichol Kinase, AtDOK1 Involved in Protein Glycosylation in Reproduction

Yuki Nakamura^{1,3}, Chia-En Chen¹, Ying-Chen Lin¹, Kazue Kanehara¹

¹*Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan*, ²*Muroran Institute of Technology, Muroran, Japan*, ³*Japan Science and Technology Agency, PRESTO, Saitama, Japan*

In eukaryotic cells, secretory proteins can be glycosylated at the endoplasmic reticulum (ER) and the Golgi apparatus. Although Arabidopsis possesses many putative N-linked glycoproteins, little is known how N-linked glycosylation takes place. Dolichyl phosphate (Dol-P) is a platform for the pre-assembly of Glc₃Man₉GlcNAc₂ to the recipient proteins in the N-linked glycosylation pathway. In this study, we isolated a putative dolichol kinase (DOK) in Arabidopsis, designated as AtDOK1. AtDOK1 shared overall high amino acid sequence homology with SEC59, a *Saccharomyces cerevisiae* ortholog of DOK. The protein coding sequence of AtDOK1 expressed in the *sec59* complemented defects in temperature-sensitive growth as well as N-linked glycosylation, indicating that AtDOK1 encodes the functional dolichol kinase. Isolation of a T-DNA tagged mutant of AtDOK1, *dok1-1*, demonstrated that heterozygous mutant was defective in both male and female gametophytes. To understand the tissue specificity of AtDOK1 in Arabidopsis, we created transgenic plants that stably express a DOK1-GUS fusion protein on its own promoter. AtDOK1 was specifically expressed in actively growing cells. These evidences suggest indispensable roles of dolichol kinase in Arabidopsis. Possible functions of AtDOK1 will be discussed.

P436

Determinate Growth of the Primary Root of *Pachycereus pringlei* (Cactaceae) and the Role of PLETHORA Transcription Factors

Mayra L. López-Valle¹, Marta Matvienko², Yamel Ugartechea-Chirino³, Joseph G. Dubrovsky¹, Svetlana Shishkova¹, Gustavo Rodríguez Alonso¹

¹Instituto de Biotecnología Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico,

²CLC bio, Cambridge, MA, USA, ³Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico City, Mexico

The primary root of many species of the Cactaceae family exhibits a determinate growth, i.e. the root apical meristem (RAM) becomes exhausted after few cell cycles. As a result, the cells at the root tip become differentiated. This is a unique growth pattern among angiosperms and it has been proposed that the determinate growth of the primary root represents a adaptive advantage to arid habitats. In *Arabidopsis thaliana* the partially redundant PLETHORA (PLT) genes regulate the maintenance of the RAM, and primary and lateral roots of the *plt1 plt2* double mutant exhibit determinate growth. Our research is focused on elucidating the role of PLT genes in the determinate growth of the *P. pringlei* primary root. We previously sequenced the transcriptome of *P. pringlei* primary root tips by mRNA-seq. The reads were de-novo assembled into contigs using the CLC Genomics Workbench. In *P. pringlei* root tips we identified six homologs of *Arabidopsis* PLT genes (PpPLT). Comparison of the virtual expression levels in the primary root in initial phase (when the RAM is still present) and the terminal phase (when the RAM is exhausted) showed that PpPLT genes are down-regulated in the terminal phase. To validate these results, we quantified the expression level of PpPLT by qRT-PCR in the initial and terminal phase and two intermediate growth phases. We also used RACE PCR to amplify the PpPLT cDNA ends. Our data suggest that PpPLT genes are involved in maintenance of the root determinacy developmental program.

P437

Arabidopsis Florigen FT Binds to Diurnally Oscillating Phosphatidylcholine to Promote Flowering

Yuki Nakamura^{1,2}, Fernando Andres², Kazue Kanehara¹, Yu-chi Liu¹, Peter Dörmann³, George Coupland²

¹*Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan*, ²*Max-Planck-Institute for Plant Breeding Research, Cologne, Germany*, ³*Institute of Molecular Physiology and Biotechnology of Plants, University of Bonn, Bonn, Germany*, ⁴*Japan Science and Technology Agency, PRESTO, Saitama, Japan*

Arabidopsis FLOWERING LOCUS T (FT) protein is a component of florigen, a long-range flowering signal transmitted from leaf companion cells to the shoot apex where it initiates flowering. A series of genetic and structural studies suggested that FT functions as a complex along with bZIP transcription factors and 14-3-3 proteins. However, analysis of the crystal structure of FT identified a putative ligand binding pocket predicted to be filled by a phosphate derivative that is not represented in this model. Here, we show that in vitro FT specifically binds the phospholipid phosphatidylcholine (PC). A transgenic approach to increase PC levels in vivo in the shoot apical meristem accelerated flowering whereas reduced PC levels delayed flowering, demonstrating that PC levels are correlated with flowering time. The early flowering was related to FT activity, because expression of two FT-effector genes, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) and APETALA1 (AP1), was increased in these plants. Simultaneous increase of FT and PC in the shoot apical meristem further stimulated flowering, whereas a loss of FT function led to an attenuation of the effect of increased PC. Specific molecular species of PC oscillated diurnally, and night-dominant species were not the preferred ligands of FT. Elevated night-dominant species of PC during the day delayed flowering. We suggest that FT binds to diurnally changing molecular species of PC to promote flowering.

P438

Heterologous Expression of a Hot Pepper Phospholipase A1 *CaPLA1* Improves Biomass and Grain Yield in Rice

Ki Youl Park¹, Hye Jo Min¹, Eun Yu Kim¹, Jiho Seo^{1,2}, Woo Taek Kim¹

¹Yonsei University, Seoul, Republic of Korea, ²Korea Ginseng Corp., Dajeon, Republic of Korea

Phospholipid signaling regulates various cellular processes in higher plants. *CaPLA1*, a hot pepper *sn-1* specific phospholipase A1, was previously reported to be a positive regulator of cell and tissue growth in *Arabidopsis*. To test the possibility that *CaPLA1* can be used to improve biomass in crop plants, the *CaPLA1* gene was constitutively expressed under the control of maize ubiquitin promoter in rice. *CaPLA1*-overexpressing T3 transgenic rice plants (*Ubi:CaPLA1*) showed higher acyl hydrolase activity compared to wild-type plants. The *Ubi:CaPLA1* rice grew faster than wild-type rice in vegetative stage. In reproductive stage, *Ubi:CaPLA1* plants exhibited increased number of tillers and enhanced panicle length and grain yield than those of wild-type rice in paddy field conditions. To address the difference of gene regulation and metabolic components between *Ubi:CaPLA1* and wild-type rice, microarray experiments are performed using 3-weeks-old *Ubi:CaPLA1* leaf RNAs. The results showed that expressions of genes related with the reproductive developmental process and starch metabolism were significantly altered in *CaPLA1* overexpressing rice. These results suggest that *CaPLA1* is functionally relevant in heterologous rice plants and could be used to enhance growth and grain yield in rice (This work was supported by grants from the National Center for GM Crops, Project No. PJ008152 of the Next Generation BioGreen 21 and the National Research Foundation, Project No. 2010-0000782).

P439

Unraveling the Gene Regulatory Networks of Two Sucrose-Responsive Transcription Factors Involved in the Response to Plant Carbon Status and Development

Julia Vogt¹, Jozefus Schippers², Bernd Mueller-Roeber^{1,3}

¹University of Potsdam, Institute of Biochemistry and Biology, Molecular Biology, Karl-Liebknecht-Str. 24-25, Haus 20, 14476 Potsdam, Germany, ²RWTH Aachen University, Institute for Biology I, Molecular Ecology, Worringewg 1, 52074 Aachen, Germany, ³Max-Planck-Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam, Germany

Developmental success of plants depends on the photosynthetic production of energy in the form of sugars. In the light, CO₂ fixation results in the accumulation of sucrose that is required for growth. As carbon availability fluctuates and highly depends on environmental factors it is of interest to understand how plants control the carbon supply allocated to growth.

Sucrose not only functions as a metabolite but also as a signal molecule. Moreover, alterations in sucrose level affect the expression of a large number of genes, including transcription factors (TFs). Currently, only little is known about the gene networks controlled by these sucrose-responsive TFs and how these might integrate the sucrose status into the genetic program controlling growth.

Here, we present work that involves the elucidation of the gene networks controlled by two sucrose-responsive TFs in *Arabidopsis thaliana*. One of them belongs to the NAC family of TFs that are well known for their role in plant development, while the other represents a member of the R2R3-MYB family which is mainly known for their role in stress-responses (changes in environmental conditions). For this, we use a combination of methods including gene expression profiling and phenotyping of transgenic plants, as well as in vitro analyses of the TFs and their targets. Understanding the role of these TFs might shed light on how plants use their carbon status to control plant growth at the molecular level.

P440

Stomatal Development and CO₂ Drawdowns in Leaves of Hypobaric Plants

Jitka Neuwirthova¹, Jiri Santrucek^{1,2}, Martina Drtinova¹

¹Department of Experimental Plant Biology, Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic, ²Institute of Plant Molecular Biology, Centre of the Academy of Sciences of the Czech Republic, Ceske Budejovice, Czech Republic

Reduced total pressure of atmosphere and partial pressures of all comprising gases is experienced by plants grown at high elevation habitats (hypobaric plants, H). The hypobaric plants reduce C_i more than control plants grown at the same C_a. This phenomenon is known for a long time however underlying mechanisms remain unknown. CO₂ supply through stomata or parameters of CO₂ transport through the leaf and/or carboxylation capacity can be responsible. We grew fast growing plant species in hypobaric conditions to test (i) how their stomata development is affected, (ii) if there are any changes in gradient of CO₂ concentration across the leaf intercellular air space (IAS) and (iii) whether they differ in CO₂ drawdown from IAS into chloroplasts compared to plants grown at full atmospheric pressure (baric plants, B).

The C_i values were calculated from stable carbon isotope composition ($\delta^{13}\text{C}$) of leaf biomass and CO₂ gradients inside the leaf were revealed from $\delta^{13}\text{C}$ of epicuticular waxes isolated from opposite leaf sides.

C_i in cotyledons and first leaves of hypobaric plants of *Lepidium sativum* was significantly reduced compared to baric plants. Stomatal density (SD) in H plants did not change but sensitivity of SD to C_i (slope of SD plotted against C_i) increased about 5-times. Preliminary results based on isotopic analyses and leaf anatomy measurements showed no or negligible differences in CO₂ drop across IAS. However the IAS-chloroplast drawdown increased in H plants. We conclude that carboxylation capacity and/or mesophyll conductance is increased in hypobaric plants.

This work was supported by GAJU143/2013/P.

P441

Functional Diversification of Duplicated CYC2 Clade Genes in Regulation of Inflorescence Development in *Gerbera hybrida* (Asteraceae)

Paula Elomaa¹, Inka Juntheikki-Palovaara¹, Sari Tähtiharju¹, Tianying Lan², Suvi Broholm¹, Anneke Rijpkema¹, Raili Ruonala², Victor Albert², Teemu Teeri¹

¹University of Helsinki, Helsinki, Finland, ²University at Buffalo, Buffalo, USA

The complex inflorescences (capitula) in Asteraceae consist of different types of flowers. In *Gerbera hybrida*, the marginal ray flowers lack functional stamens and are bilaterally symmetrical while the central disc flowers with pollen producing stamens and carpels are more radially symmetrical. The CYC2 subclade of the CYC/TB1 like TCP domain transcription factors have independently been recruited to regulate the formation of bilaterally symmetrical flowers in diverse angiosperm plant lineages and to regulate flower type identity in Asteraceae. The six CYC2 subclade genes in gerbera are upregulated in developing ray flower primordia and show largely overlapping gene expression domains. At the level of single flowers, their expression domains show a spatial shift from the dorsal pattern typical for so far studied species with bilaterally symmetrical flowers, suggesting that the expression change may have evolved after the split of Asteraceae. Functional analysis indicates redundancy for GhCYC2, GhCYC3 and GhCYC4 in regulating ray flower identity, but they also regulate ray flower petal growth by affecting cell proliferation until the final size and shape of the petals is reached. Moreover, our data shows functional diversification for the GhCYC5 gene. Ectopic activation of the GhCYC5 function increases flower density of the inflorescences suggesting that GhCYC5 may promote flower initiation rate during expansion of the capitulum. Our data thus indicates that modification of the ancestral network of TCP factors has, through gene duplications, led to establishment of new expression domains and to functional diversification.

P442

Transcriptional Network of Phenylpropanoid Pathway in Arabidopsis Peroxidase 52 Mutants

Francisco Fernández-Pérez¹, Federico Pomar², M^a Ángeles Pedreño¹, Esther Novo-Uzal¹
¹Universidad de Murcia, Murcia, Spain, ²Universidade da Coruña, A Coruña, Spain

Lignins result from the oxidative polymerization of three hydroxycinnamyl (p-coumaryl, coniferyl and sinapyl) alcohols in a reaction mediated by peroxidases, yielding H, G and S units, respectively. The AtPrx52 from Arabidopsis is a basic peroxidase involved in syringyl lignin formation. We have previously shown that the suppression of AtPrx52 causes a decrease in lignin content accompanied by a reduced S/G ratio. Here, we take advantage of two different mutant lines to evaluate the effect of AtPrx52 suppression in the expression of genes involved in the phenylpropanoid pathway, mainly in the route of lignin biosynthesis, as well as other genes implicated in cell wall formation. Results obtained by qPCR showed a decrease in *atprx52* of PAL, 4CL, CCR, CAD and F5H expression, all involved in lignin formation. Moreover, *CesA7*, *CesA8* and *FRA8* expression was also reduced in the stems of *atprx52* plants, which suggests a decrease in cellulose and xylan deposition in secondary cell walls. The gene expression of two transcriptional factors, MYB58 and MYB103, implicated in lignin and S monomers formation, respectively, was also reduced in *atprx52* plants. On the other hand, expression of both SMT8 and CHS, involved in sinapate esters formation from sinapyl alcohol and flavonoid biosynthesis, respectively, was up-regulated in *atprx52* plants. Our results suggest the suppression of the last step of syringyl lignins formation affects the whole phenylpropanoid biosynthesis and agrees with the decrease in S lignins in interfascicular fibres previously reported. Acknowledgments: FFP and ENU hold FPU and JdC grants, respectively, from MICINN (Spain).

P443

Cellulose Synthase Genes in the Flax Genome

Dmitry Galinousky¹, Tsimafei Padvitski¹, Natalya Anisimova¹, Nikolay Pydiura², Galina Bayer², Yaroslav Pirko², Alla Yemets², Yaroslav Blume², Lyubov Khotyleva¹, Alexander Kilchevsky¹
¹*Institute of Genetics and Cytology of NAS of Belarus, Minsk, Belarus,* ²*Institute of Food Biotechnology and Genomics NAS of Ukraine, Kyiv, Ukraine*

The genome of flax (*Linum usitatissimum* L., cultivar Bethune, CDC) was assembled in 2012 and provided by Mike Deyholos for Phytozome v9.1. The genome sequence is available now for searching of genes of interest. The aim of our work was to find the cellulose synthase genes (CesA) in the flax genome.

We have used characterized CesA genes from *Arabidopsis thaliana* for similarity search in flax genome. Twenty two sequences of CesA gene candidates were found in the flax genome. Together with orthologous genes *A. thaliana* genome those sequences were subjected to phylogenetic analysis. The cellulose synthase like (Csl) gene (AF232907) was used as an outgroup. As a result 11 sequences (Lus10001619, Lus10022982, Lus10013851, Lus10010024, Lus10009248, Lus10026609, Lus10030455, Lus10026610, Lus10030453, Lus10012119, Lus10002134) were depicted in a group with the outgroup, therefore denoted sequences were referred to Csl genes. The other 11 sequences were found to be orthologs of CesA-genes from *Arabidopsis*.

Thus we have identified four CesA genes in the flax genome: CesA1 (Lus10018902), CesA3 (Lus10007538 and Lus10039607), CesA8 (Lus10007296 and Lus10029245,) and CesA6 (Lus10022449, Lus10003526, Lus10002939, Lus10002940, Lus10006161 and Lus10041063). Previously we have demonstrated an expression of CesA1, CesA7, CesA9, CesA4 genes in fiber flax (cultivar Blakit, Belarus). The partial disagreement between experimental and bioinformatical data we can explain by different origin of flax cultivars, used as the subject.

P444

Caulogenesis of Two Varieties of Flax (*Linum usitatissimum* L.) Cultured In Vitro

Aneta Adamczuk, Irena Siegien, Iwona Ciereszko

Department of Plant Physiology, Institute of Biology, University of Bialystok, Bialystok, Poland

Flax plants have been the focus of a great deal of both applied and basic research in a plant cell and biotechnology studies in recent years. In vitro regeneration response in terms of the number of produced shoots is an important factor determining the success of plant micropropagation.

The caulogenic ability of hypocotyl explants of oil and fibre flax in different media, supplemented with plant growth regulators, was investigated in this study.

Hypocotyl explants of 7-day-old seedlings of oil flax (*Linum usitatissimum* L., cv Szafir) and fibre flax (cv Selena) were cultured on Murashige and Skoog (MS), Gamborg (B5) and MS+B5 (macronutrients + micronutrients and vitamins) medium. The caulogenic effect of various growth regulators: benzyloaminopurine (BA), kinetin, tidiazuron and 2,4-dichlorophenoxyacetic acid (2,4-D) was also tested. Cultures were incubated for 30 days in a growth chamber under controlled conditions. CO₂ exchange was measured with the use of an infrared CO₂/H₂O analyzer (LI-6262, USA). Chlorophylls and carotenoids content was determined in 5-, 15- and 30- day-old cultures and in 7-day-old seedlings. The measured intensities of net photosynthesis and dark respiration, as well as chlorophylls and carotenoids content were different, depending on a cultivar of flax and the stage of growth. The regeneration of shoots from explants of flax cv Szafir was effective on all tested media, whereas from cv Selena - only on B5, MS+B5 medium. When the concentration of BA was at least twenty times higher than 2,4-D, the regeneration of shoots was the most effective, especially from cv Szafir explants.

P445

Winter Beet - A New Way of Cultivating Sugar Beets

Katia Schuetze, David Wurbs, Sarah Burgdorf, Elena Ernst, Karin Fiedler, Iris Grocholl, Mandy Haberzettl, Stefan Huellen, Margarita Mantai, René Neumann, Jasmin Schmidt, Viktoria Sendler, Daniela Thormann, Marion Wirth, Josef Kraus
KWS SAAT AG, Einbeck, Germany

Sugar beet is the second most important crop for sugar production after sugar cane. Traditionally sugar beet plants are sown in spring and harvested in late autumn. It is estimated that an extension of this vegetation period can lead to a yield increase of up to 30%. Therefore KWS is developing "winter beets" that can be sown in autumn and harvested in autumn the following year. These plants present an additional winter crop choice for farmers and furthermore the increased yield allows an extended harvest period which is very attractive for the sugar factories.

To realize this concept it is necessary to increase the winter hardiness and to suppress bolting and flowering in spring. Additionally a switch system allowing seed production is required. We have identified several candidate genes conferring bolting and flowering resistance. A promising candidate is BvVil1, a plant homeodomain (PHD) finger-containing protein and a key component of the sugar beet vernalization pathway. In order to increase the frost tolerance of sugar beet we are working on both breeding and transgenic approaches.

P446

Disruption of PME Activity Alters Hormone Homeostasis and Triggers Compensatory Mechanisms Controlling Adventitious Rooting

Stéphanie Guéin^{1,2}, Gaëlle Mongelard², Hervé Demailly², Ondrej Nowak³, Kieran Lee⁴, Sophie Bouton¹, Petra Amakorova³, Miroslav Strnad³, Paul Knox⁴, Gregory Mouille⁵, Jerome Pelloux¹, Laurent Gutierrez²

¹EA3900-BIOPI Université Picardie Jules Verne, 33 Rue St Leu, Amiens, France, ²CRRBM Université Picardie Jules Verne, 33 Rue St Leu, Amiens, France, ³Laboratory of Growth Regulators & Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University, Olomouc, Czech Republic, ⁴School of Molecular and Cellular Biology, Faculty of Biological Sciences, Irene Manton Building 6.94b, University of Leeds, Leeds, UK, ⁵IJPB, UMR1318 INRA-AgroParisTech, Bâtiment 2, INRA Centre de Versailles-Grignon, Route de St Cyr (RD 10), Versailles, France

We recently showed that the decreased PME activity in *Arabidopsis pme3* mutant, in which the PECTIN METHYLESTERASE 3 gene was knocked-out, led to increase the degree of methylesterification (DM) of pectins as well as the adventitious rooting in hypocotyl. In order to gain insights into the relationship between DM of pectins and adventitious rooting, we analyzed the *pme36* knock-out line. In wild-type, PME36 is expressed during seed maturation and in seedling hypocotyl up to 72-hours after germination. As expected, *pme36* displayed a decrease in PME activity during seed maturation and a resulting increase in the DM of pectins in the mature seed. Unexpectedly, 48-hours after germination, PME activity in dark-grown hypocotyl of *pme36* was higher than in the wild-type, leading to a decrease in the DM of pectins and in the adventitious rooting in the mutant. While confirming the positive correlation between DM of pectins and adventitious rooting, these results highlight the existence of a mechanism overcompensating the absence of PME36 in *pme36* hypocotyl. We found that this compensatory mechanism involved transcriptional regulations, other PME genes being overexpressed in *pme36* hypocotyl. In addition, looking at hormone contents and assessing the expression of genes involved in the hormone signaling pathways shown to control adventitious rooting, we found a strong alteration of hormone homeostasis in *pme36* hypocotyl. Altogether these results suggest that adventitious rooting is controlled by a regulatory network involving crosstalk between hormone signaling and PME activity, which is modulated through a compensatory mechanism triggered by variations in DM of pectins.

P447

The Time Course of Acquisition of Competence and In Vitro Organ Induction in Tomato (*Solanum lycopersicum* L. cv Micro-Tom)

Marcela Notini, Lázaro Peres
ESALQ/USP, Piracicaba, São Paulo, Brazil

Organogenesis can be divided into two phases: acquisition of competence and the induction of a new organ. *Solanum lycopersicum* L. cv Micro-Tom (MT) is an alternative model plant for regeneration studies, since it has, like other species of commercial interest, differences in this pathway, when compared to *Arabidopsis*. This study aimed to determine the time necessary for acquisition of competence and induction of roots, callus and shoots in MT. To determine the time required for root, callus and shoot induction, in vitro MT cotyledon explants were subjected to gradual transference from the shoot-, callus- and root-inducing medium (SIM, CIM and RIM) to the basal medium (BM), varying the number of days that the explants remained in each medium. To determine the time spent for acquisition of competence during shoot formation, the RIM was gradually replaced by SIM within the established days to shoot induction. Similarly, SIM replaced RIM and CIM to define the time for acquisition of competence during root and callus formation, respectively. It was determined that shoot and callus induction occurs in the first 8 days of incubation, and that root induction occurs within 3 days. The acquisition of competence occurs in 2 days for both shoot and root formation and could not be set for callus. Taken together, our results suggest that the acquisition of competence is a non media-dependent initial developmental pathway common for different organs, which contrasts to the induction phase, since it takes different times and media compositions for each organ formed.

P448

Transcriptome Analysis (RNA-Seq) Associated with the Phase of Acquisition of Organogenic Competence in Tomato (*Solanum lycopersicum* L. cv Micro-Tom)

Mariana Azevedo¹, Erika Jesus³, Antonio Figueira¹, Marie-Anne Van Sluys³, Lázaro Peres^{1,2}
¹CENA-USP, Piracicaba, SP, Brazil, ²ESALQ-USP, Piracicaba, SP, Brazil, ³IB-USP, São Paulo, SP, Brazil

Among the factors related to the higher in vitro regeneration capacity of *Solanum peruvianum* L, it is the presence of the dominant allele Rg1. The RG1 gene is required for shoot formation from roots and others explants and was mapped on chromosome 3 between the BETA-CAROTENE HYDROXYLASE (CrtR-b) and PHYTOENE SYNTHASE (PSY1) genes. The Rg1 allele was introgressed into the background MT, which has small size and fast reproductive cycle. Among the steps to in vitro organogenesis, the acquisition of competence and induction phases are the most important for new organ formation. Through the sequential transfer of explants from SIM to BM and RIM to SIM, we found that MT-Rg1 reduces the time required for the induction of shoots in one day (6 days on SIM for MT), due to the reduction of the time required for competence acquisition in one day (2 days for MT). We performed RNA-Seq analysis (SOLiD platform) to identify genes differentially expressed in MT and MT-Rg1 during the phase of acquisition of competence. It was observed more down-regulated than up-regulated genes. Due to the large number of differentially expressed genes, some parameters were used for selection of genes that would have their expression validated by qRT-PCR in different in vitro regeneration tests. 14 genes (GRAS10, PP7, LACASE1A, GLYCOSYLTRANSFERASE, PECTIN-METHYLESTERASE, GLUCURONOSYLTRANSFERASE, FARNESYL-SYNTHASE, ADENYLYL-SULFATE-REDUCTASE, PHOSPHADENOSINE-REDUCTASE, DIACYLGLYCEROL-KINASE, ABC-TRANSPORTER, CYP-71-B20, YABBY-LIKE, ALPHA-AMYLASE) among 361 genes differentially expressed were validated in qRT-PCR. Transgenic plants are being constructed for functional confirmation of the candidate genes as controlling organogenic competence.

P449

***Solanum pennellii* Introgression Lines Controlling Root Formation Capacity**

Maísa Pinto, Lázaro Peres
Esalq-USP, Piracicaba, São Paulo, Brazil

Despite the importance of roots for plants' nutrient and water acquisition, little is known about the genetic basis of the capacity to form these organs. We characterized the in vitro root-regeneration capacity of a set of introgression lines (ILs), each containing a small chromosome segment of *Solanum pennellii* LA716 introgressed and mapped into the tomato cultivar M82 (*Solanum lycopersicum* L.). Among the lines evaluated, ILs 5-4, 5-5, 7-4, 9-1, 11-3 and 11-4 presented the highest ability to form roots in vitro, and the ILs 2-5, 2-6, 3-4, 8-1, 8-2 and 12-2 the lowest. The alleles conditioning high root formation are probably located in the bins 5H (IL 5-4 and IL 5-5), 7A or 7D (IL 7-4) and 11F (IL 11-3 and IL 11-4). The alleles conditioning low root formation are probably located in the bins 2K (IL 2-5 and 2-6), 3G (IL 3-4) and 8D (IL 8-1 and IL 8-2). According to our previous work, the bins 5H, 7A, 7D e 11F do not seem to increase the ability to form shoots in vitro. Taken together, these results suggest that the chromosomal regions discovered here are involved in the specific phase of root induction. The expression of the main genes present in the bins was analysed using public data (RPKM) and candidate genes controlling root formation were proposed. The confirmation of the impact of such genes on root formation using transgenic plants will provide for their exploration as an agricultural trait.

P450

Scleromorphic Features and Vacuolar Phenolic Inclusions in *Leucospermum* (Proteaceae). Anatomical and Ultrastructural Studies in Control and *In Vitro* Plants

Carmen Alfayate¹, Emma Suárez², Candelaria Vera-Batista³

¹Dpto. Bioquímica, Microbiología, Biología Celular y Genética. Facultad de Biología. Universidad de La Laguna, La Laguna, Tenerife, Canary Islands, Spain, ²Dpto. Biología Vegetal. Facultad de Farmacia. Universidad de La Laguna, La Laguna, Tenerife, Canary Islands, Spain, ³Dpto. Ingeniería, Producción y Economía Agrarias. Escuela Técnica Superior de Ingeniería Agraria. Universidad de La Laguna, La Laguna, Tenerife, Canary Islands, Spain

Introduction. Proteaceae comprises about 80 genera with about 1700 species, distributed over a wide range of temperature and habitats in the Southern Hemisphere continents. Proteas have numerous scleromorphic anatomical structures in their leaves that protect the mesophyll from excess solar radiation. Among the defense and protection mechanisms are also secondary metabolites and phenols with antioxidant properties. The present study aims to describe the anatomical organization and the ultrastructure of the leaves in control and in vitro plants belonging to the *Leucospermum* genus.

Methods. Leaves from plants grown in field and in vitro from *L. patersonii*, L. 'Flame Spike', L. 'Succession I' and L. 'Tango' were collected and fixed for examination under light, transmission and scanning electron microscopes.

Results. Cuticle of leaves from control plants was markedly thicker than cuticle from in vitro plants leaves. Trichomes were simple and had a striate cuticle in 'Flame Spike' and 'Tango' varieties. The unilayered epidermis contained phenolic deposits as electron-dense drops in *L. patersonii* leaves. Ultrastructural observations in 'Flame Spike' and 'Tango' control leaves presented a palisade parenchyma with cells containing phenolic deposits that occupied the entire vacuolar volume, and spongy parenchyma cells with phenolic deposits as scattered droplets. Mesophyll from in vitro plants showed a poorly organized parenchyma, poorly developed vascular bundles with scarce sclerenchyma. Phenolic deposits were less abundant in tissues of micropropagated plants.

Conclusions. Anatomical and ultrastructural observation revealed alterations in leaves of micropropagated plants. These modifications included thin cuticle formation, narrow vascular bundles and low levels of phenolic compounds.

P451

Anatomy, Ultrastructure and Histochemical Study from Aerial Parts of *Ceropegia fusca* Bolle (Asclepiadaceae)

Carmen Alfayate, Lucía Rodríguez

Dpto. Bioquímica, Microbiología, Biología Celular y Genética. Facultad de Biología. Universidad de La Laguna (ULL), La Laguna, Tenerife, Canary Islands, Spain

Background: *Ceropegia fusca* Bolle (Asclepiadaceae) is an endemic chamaephyte from dry rocky areas in the south of the islands of Tenerife and Gran Canaria (Canary Islands), belonging to the association *Ceropegio fuscae* - *Euphorbietum balsamiferae* ('*tabaibal dulce*'; Rivas-Martínez et al. 1993). Its stems produce a thick liquid latex, used in traditional medicine in wound-healing and as a disinfectant, besides being an antidote for the toxic effects of *Euphorbia canariensis* L. ('*cardón*'). The plant extract has been chemically analyzed and presented compounds C-21 steroidal glycosides (García 2011) exhibited significant cytostatic activity, at inhibitory concentrations (IC₅₀) for 72 hours, against HL-60, A-431 and SK-MEL-1 cell lines (García et al. 2011).

The present study aims to describe the anatomical organization, ultrastructure and histochemical composition from the aerial parts of *C. fusca*.

Methods: Stems and leaves were collected in Tenerife and fixed for examination under light, transmission and scanning electron microscopes. Samples of fresh stems were subjected to histochemical tests.

Results: The most significant anatomical features of the stems and leaves is the presence a thick cuticle, stomata in crypts and large continuous branched lactiferous tubes, distributed in the mesophyll, cortex and medula of the parenchyma. The cuticle showed positive reaction in the lipidic tests and the lactiferous tubes presented walls and latex rich in callose and lipids

Conclusions: These structures provide a protection against solar radiation and latex compounds could help against predators e.g. herbivores.

P452

Fruit Ripening and Quality of Tomato Mutants Affecting Growth Habit and Flowering

Jonata Freschi¹, Ivan Sestari², Mariana Azevedo³, Lázaro Peres^{1,3}

¹ESALQ-USP, Piracicaba, SP, Brazil, ²UFSC, Curitibanos, SC, Brazil, ³CENA-USP, Piracicaba, SP, Brazil

The aim of this work was to determine the impact of mutations affecting the vegetative/reproductive transition and the organization of reproductive structures on tomato ripening. The classical mutants Sp, j2, sft, uf and rin were used, all them introgressed into Micro-Tom (MT) cultivar. The rin mutant is a loss of function in a MADS-Box transcription factor necessary for fruit ripening, which was used as a positive control. Fruits were marked at anthesis and collected in mature green stage before to be stored at 20 °C. Ethylene production and carbonic gas were measured every 3 days by gas chromatography until the ripening of the fruits. In parallel, a Minolta colorimeter was used to quantify visual parameters of epicarp color. Once the fruits become ripe, quality parameters such as brix, total pigments and ascorbic acid content were determined. sft fruits had the shortest ripening period but delayed the climacteric and produces higher levels of ethylene during this period when compared to MT. On the other hand, uf and j2 delayed the climacteric and ripening, besides they produced less ethylene during the climacteric. Our results suggest that sft has a role in the ethylene biosynthesis pathway, increasing this hormone synthesis, while uf and j2 may act in the opposite way.

P453

Perianth Modifications in Basal Angiosperm Magnolia - The Role of ABC Genes in Shaping Perianth Phenotype

Magdalena Wroblewska, Alicja Dolzblasz, Beata Zagorska-Marek
University of wroclaw, Wroclaw, dolnoslaskie, Poland

The origin and development of a flower, a characteristic structure of angiosperms, became one of the most intensively studied problems in modern evolutionary and developmental plant biology. As a result, the ABC model and its variations, describing the correlation between specific genes function and floral diversity, was proposed. Perianth of a flower displays extensive diversity in size, color and morphology. It can be differentiated (sepals and petals) or alternatively, undifferentiated (tepals only) like generally accepted for Magnolia genus. Nevertheless, there are exceptions, like in *Magnolia acuminata*, *M.liliiflora* and *M.stellata*, where the organs of first whorl are the smallest, green and sepal-like. Our anatomical and morphological studies together with the expression level of MADS-box genes in different floral organs of Magnolia strongly suggest the identity of the first whorl as a true calyx. The sepal identity of these elements was confirmed by lack of B-genes expression (AP3/PI) and the presence of A class gene transcript (AGL6). Interestingly, in *M.stellata* there is another interesting phenomenon - the petalody, in which additional perianth elements are arranged in spiral phyllotaxy. These spirally arranged elements have transcripts of both AGL6 (A class gene) and AG (C class gene). A gradual decrease in the expression of AGL6 accompanied by an increase of AG in additional perianth elements was detected, but no morphological gradual transition was evident. Our results prove the accuracy of "sliding boundary" hypothesis for perianth evolutionary change and propose a variation of a "fading borders" model for petalody formation in Magnolia genus.

P454

Variability of Flower Development in Concern to the Changes of the Pollination Type

Svetlana Zimnitskaya¹, Elizaveta Kamotina¹, Elizaveta Bolotnik²

¹Ural Federal University, Ekaterinburg, Russia, ²Ural Medical University, Ekaterinburg, Russia

Cytological and morphological studies of the development peculiarities of white clover flowers from wild populations of the Urals were done. Light, confocal and electron microscopies were used for the study. Previously it was shown that effective fertilization in some legumes including white clover could be the result of both cross- and self-pollination. We have found that the restructuring of the generative system and changes in flower incompatibility occurs during development and maturation of the flower. Flower transition from xenogamy to autogamy depends on environmental factors (temperature, humidity, lack of pollinators), as well as internal ones. Among them flower age and genetic predisposition to formation of a complex system of pollination. Seven paths of flower development have been described. All of them have shown similarity in the early stages and primacy of xenogamy. However, more or less attributes of changes in incompatibility accumulate during development. Flowers of different types differ in relative growth rate of perianth and generative elements. The state of the stigma surface cells and anther fibrous layer, physiological condition of pollen grains and ovules, changes the status of the stigma cuticle, the thickening of the cell walls, stigma surface area are also important. The utmost paths of development are flowers in which there is no any changes in the system of pollination and those that effective self-pollination occurs. Intermediate forms demonstrate the predisposition of the pollination system changes but retain xenogamy.

P455

A Floral Induction System in *Arabidopsis thaliana*

Frank Wellmer¹, Diarmuid O'Maoileidigh¹, Bennett Thomson¹, Andrea Raganelli²

¹Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland, ²Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria

The emergence of flowers in *Arabidopsis thaliana* follows a spiral phyllotaxy, such that no two flowers are at the exact same stage of development on any one inflorescence. Consequently, it is impossible to collect stage-specific tissue without the use of time-consuming techniques such as laser capture microdissection. A Floral Induction System (FIS), which allows the synchronization of flowers from an inflorescence-like meristem, has been developed to circumvent this difficulty.

The original floral induction system was based on hormone-dependent activation of an ectopically expressed APETALA1 protein that is fused to the rat glucocorticoid receptor. To enhance the usefulness of this system, we have focused on 1) mimicking the endogenous *AP1* expression pattern and 2) expanding the repertoire of chemicals we can use to activate AP1. These modifications would allow us 1) to imitate *Arabidopsis* flower development more accurately than the previous FIS and 2) increase the number of chemically inducible transgenes we can introduce into the FIS background. To this end, we can examine the effects of perturbing gene function in an inducible and stage-specific manner. This would ultimately allow us to gain a detailed insight into the complex gene regulatory networks that govern flower development.

P456

Developmental Regulation of Cell Division by the RBR Complex with E2FB in Leaf Pavement Cells and Meristemoids

Tünde Leviczky¹, Binish Mohammed², Aladár Pettkó-Szandtner¹, Beatrix Horváth^{2,3}, Safina Khan², Anita Kovács¹, Ben Scheres³, László Bögre², Zoltán Magyar¹

¹*Institute of Plant Biology, BRC, HAS, Szeged, Hungary,* ²*School of Biological Sciences, Royal Holloway University of London, Egham, UK,* ³*Department of Molecular Genetics Utrecht University, Utrecht, The Netherlands*

Leaf growth relies on the number of cells produced before pavement cells exit proliferation and the number of daughter cells produced through asymmetric stem cell divisions of meristemoids. E2FB is an activator of cell cycle genes in actively proliferating pavement cells in young developing leaves. In accordance, pavement cells of *e2fb* mutant prematurely exit cell proliferation while elevated E2FB level delays this exit. Overexpression of a dominant negative E2FB form, E2FB^{ΔTA-RBR}, which is unable to activate cell cycle genes, also results in early exit from proliferation. During the cell cycle exit, RBR represses E2FB activity. We found that E2FB elevates RBR both transcriptionally and on protein level but also elevates CYCD3;1 transcript level leading to RBR inactivation. This connectivity could provide the underlying mechanism for switch of cell proliferation to cell cycle exit and differentiation. Therefore elevated E2FB level can alter this switch in cells started to differentiate and they reenter proliferation. In leaf meristemoids, change in E2F function has totally different outcomes. On one hand, *e2fb* mutation results in extra symmetric divisions of the meristemoids to produce stomata clusters. On the other hand overexpression of the mutant E2FB^{ΔTA-RBR} construct expands the transit amplifying cells produced by meristemoid stem cells. RBR together with E2FB regulates these processes as a repressor complex. Thus E2FB has dual functions regulated by RBR. (1) RBR represses E2FB activity, dependent on its phosphorylation, regulating cell cycle exit in pavement cells, (2) RBR forms a repressor complex with E2FB to regulate stem cell divisions in meristemoids.

P457

Norway Spruce Vegetative Buds during Ecodormancy and Endodormancy in Natural Conditions

Aleksandra Staszak, Tomasz A. Pawlowski, Marzenna Guzicka
Institute of Dendrology, Kórnik, Poland

Woody plants from temperate zone developed mechanisms that enable them to adapt to the seasonally changing climatic conditions. This study concerns the key issues associated with development and adaptive processes in changing environmental conditions. *Picea abies* was the object of the study. Embryonic shoots isolated from vegetative buds were collected from the middle part of tree crown between September and May, in two successive years. Only one selected clone was a source of the material due to the fact that differences in phenology between different clones exist. Proteomic analyses were a focus of the study. Two-dimensional electrophoresis (2D SDS-PAGE) was applied. Proteome maps with an average of 294 Comassie blue stained spots were established. A set of 19 spots showed significant changes between endodormancy and ecodormancy. 68 spots differed significantly between the stages of endodormancy and resumption of mitotic activity, and 75 between ecodormancy and resumption of mitotic activity. The changes are probably a result of frost stress during ecodormancy. Additionally, differences in proteome maps between terminal and lateral buds are presented. Using LM, TEM and CLCM microscopy, structural changes were observed between bud endodormancy and ecodormancy. Changes in cells from embryonic shoot took place primarily at the level of their ultrastructure. A pattern in these changes was described. Changes in symplastic isolation pattern and starch distribution pattern were of crucial importance for spruce buds dormancy. We will present connection between proteomic and microscopic analysis. The research has been financially supported by the National Science Centre, Poland, project no NN303806940.

P458

Transcriptional Regulation by Components of the Ubiquitin System in *Arabidopsis*

Katarzyna Hanczaryk¹, Frank Wellmer¹, Emmanuelle Graciet^{1,2}

¹Trinity College Dublin, Dublin, Ireland, ²National University of Ireland Maynooth, Maynooth, Co. Kildare, Ireland

In eukaryotes, the ubiquitin system is essential for the regulation of protein stability and it has been shown to control about every physiological process in the cell. The conjugation of the small protein ubiquitin to substrate proteins requires the activity of different types of enzymes. In particular, so-called E3 ubiquitin ligases recognize specific degradation signals in a substrate protein and catalyze the conjugation of ubiquitin to this substrate. After addition of several ubiquitin molecules, the protein substrate can be targeted for degradation by the 26S proteasome. In eukaryotes, including plants, the degradation of transcriptional regulators by the ubiquitin system has been shown to be essential for the regulation of transcription. Different mechanisms for a role of the ubiquitin system in the regulation of transcription have been suggested, some of which involve the indirect interaction of E3 ubiquitin ligases with the promoter elements of some transcription factors' target genes. We used the model plant *Arabidopsis thaliana* to study the possible roles of a core component of SCF-type E3 ubiquitin ligases, the protein ARABIDOPSIS SKP1 HOMOLOGUE 1 (ASK1), in the regulation of transcription. Our results suggest that the regulation of various physiological processes depends on the binding of ASK1, and likely associated SCF complexes, to the promoter region of target genes.

P459

Computational Identification of Putative Barley Transglutaminases

Agnieszka Zmienko¹, Anna Samelak-Czajka^{2,3}, Marek Figlerowicz^{1,2}, Jolanta Legocka³, Ewa Sobieszczuk-Nowicka³

¹*Institute of Bioorganic Chemistry, Polish Academy of Sciences, ul. Noskowskiego 12/14, 61-704 Poznan, Poland,* ²*Institute of Computing Science, Poznan University of Technology, ul. Piotrowo 2, 60-965 Poznan, Poland,* ³*Department of Plant Physiology, Faculty of Biology, Poznań Adam Mickiewicz University, ul. Umultowska 89, 61-614 Poznan, Poland*

To date, the cDNA sequences of two apparently non-orthologous genes whose products possess TGase activity have been described in plants. The first gene codes for the Arabidopsis AtPng1p protein. The other report concerns the highly similar Zea mays TGases (TGZ15, TGZ21). A computational search for barley proteins with a high homology to AtPng1p or TGZ15/TGZ21 proteins was performed.

One predicted barley protein (HvPng1-like) has been identified as exhibiting a high degree of similarity to the AtPng1p reference protein sequence. HvPng1-like has a high level of similarity to multiple proteins from both monocots and dicots, all of which possess a conserved core domain and whose Cys-His-Asp residues form the catalytic triad, a characteristic of TGases.

When the maize TGZ21 protein was used for the identification of barley homologs, four predicted proteins with partial homology were identified. We refer to this putative protein as HvTGZ21-like. HvTGZ21-like shows a very high degree of similarity to TGZ21 at the N-terminus, but the C-termini of the two proteins are drastically different. A more detailed investigation revealed that although numerous monocot cDNA sequences are highly similar to the TGZ15/21 cDNA sequences, these two maize cDNAs lack a conserved G in position 747. The unique deletion in the cDNA sequences resulted in a frameshift in their amino acid sequences with respect to the other Poaceae. That differs TGZ15 and TGZ21 from HvPng1-like or AtPng1p.

Acknowledgement: This work was partly supported by NSC research grant no N N303 418236.

P460

Expression Analysis of the Hvpng1-Like Gene (Barley Transglutaminase) in Dark-Induced Senescence

Ewa Sobieszczuk-Nowicka¹, Agnieszka Zmienko², Anna Samelak-Czajka^{1,3}, Marek Figlerowicz^{2,3}, Jolanta Legocka¹

¹Department of Plant Physiology, Faculty of Biology, Poznan Adam Mickiewicz University, ul. Umultowska 89, 61-614 Poznan, Poland, ²Institute of Bioorganic Chemistry, Polish Academy of Sciences, ul. Noskowskiego 12/14, 61-704 Poznan, Poland, ³Institute of Computing Science, Poznan University of Technology, ul. Piotrowo 2, 60-965 Poznan, Poland

Transglutaminases (TGases) are enzymes that catalyze the post-translational modification of proteins. Plant TGases are involved in the growth and differentiation processes. They are related to fertilization, greening, pollen germination, abiotic and biotic stresses.

We have already presented experimental evidence that the barley TGases are activated during the dark-induced leaf senescence, which corresponds to the enhanced local TGase accumulation and activity. The activity of transglutaminases through senescence can be controlled not only by the post-translational regulation but also by transcription. The most studied plant gene coding for a protein with TGase activity is Arabidopsis AtPng1p. It has been shown that in conditions of undisturbed growth, the AtPng1p gene undergoes constitutive expression at a low level in all plant organs during various stages of development and under various light conditions. We observed the same expression pattern for the HvPng1-like gene, which was computationally identified on the basis of its high sequence homology to AtPng1p. We also analyzed expression changes of the HvPng1-like gene during the dark-induced senescence of barley leaves. The level of transcript increased at the onset of the senescing process (day 3, when the mechanisms associated with the degradation of cell structures begin to be activated), remained elevated up to day 10, and then dropped on day 12.

Our real-time RT-PCR experiments showed that expression of HvPng1-like increased as early as senescence was induced and was associated with its progress.

Acknowledgement: This work was partly supported by NSC research grant no N N303 418236.

P461

Effect of Yield of Seed Product When Onion Seed Production House Shade Treatment of Flowering Period (*Allium cepa*. L)

Eul-Tai Lee, Cheol-Woo Kim, Eom-Ji Hwang, Young-Seok Kwon, Sang-Gyeong Bae, In-Hu Choi
Bioenergy Crop Research Center, National Institute of Crop Science, RDA,, Muan, Jeonnam, Republic of Korea

In Korea onion often copes with heavy rainfall and long rainy days during flowering season. In order to increase fertility rate of onion by avoiding rains unheating vinyl house cultivation has been developed. However, after flowering stage in mid-June, high temperature in the vinyl house affects onion fertility. This problem decreases the seed quality and yield of onion. The objective of this study was conducted to investigate the effect of light shading materials (methods) on seed production and yield. Early maturing (sinseonhwang) mid maturing (sunpower) onion varieties was used. The flowering of Sinseonhwang and Sunpower began from May 19 and May 24, respectively. The light shading was treated in May 28. As controls, 35% shading net and non-shading was used. Fertility rate of onion was the highest in White shade (63.9%) and the lowest in control (60.7%). Thousand grain weight was higher in green shade (+0.24g) and white shade (+0.08g) than control (4.15g). Seed yield was the highest in white shade (34.98kg/100m²) whereas the lowest in (35%) shading net (29.71kg/100m²). Seed yield was high in white shade treatment while the germination rate was the lowest (77.9%).

P462

Isolation and Characterization of the Quintuple Spa-q Cop1 Null Mutant to Analyze the Role of the Repressor of Photomorphogenesis COP1/SPA Early in Arabidopsis Development

Natalia Maria Ordonez Herrera, Ute Hoecker
University of Cologne, Koeln, Germany

Light is a critical environmental factor that controls several developmental processes through the plant's life cycle. Developmental modifications are coordinated by the action of the photoreceptors and downstream components such as the COP1/SPA complex. COP1 is an E3 ubiquitin ligase that acts in concert with the four members of the SPA family (SPA1-SPA4) to promote the degradation of positive regulators of the light signaling pathway. Both null mutants *cop1-5* and the *spa-q* quadruple mutant lacking all four SPA functions undergo normal embryogenesis and develop into photomorphogenic seedlings. However, the *cop1-5* mutant seedling arrests development at the cotyledon stage whereas *spa-q* survives and completes its life cycle. The latter indicates that COP1 has some residual activity even in the absence of SPA proteins. To investigate whether SPA proteins have any activity in the absence of COP1, we isolated a quintuple *cop1-5 spa-q* mutant and compared its phenotype with that of the *spa-q* mutant. Results from this analysis will be presented.

P463

New Mendelian Locus from Tomato Wild Relative Controlling Leaf Shape and Internode Length

Frederico Jesus, Marcela Notini, Lázaro Peres
ESALQ/USP, Piracicaba, São Paulo, Brazil

Natural genetic variation is a powerful tool to exploit genes or alleles harbored in the wild relatives by crossing them with the domesticated species. Using this approach, we introgressed a new Mendelian locus coming from *Solanum galapagense* into *Solanum lycopersicum* cv. Micro-Tom (MT). We named it Galapagos dwarf (Gdw), a semi-dominant trait that shortens internodes and increases leaf dissection. Gdw plants were always smaller than MT, due to their short internodes, same as Gdw/+ plants. Other noticeable feature of the Gdw locus is its dissected leaves. We assessed leaflet emission on the first seven leaves, and we included the Petroselinum (Pts) isogenic in our analysis. Plants harboring the Gdw locus formed more primary leaflets than MT and Pts. The striking effect of the Gdw locus on leaf dissection was observed in the number of secondary leaflets emitted. Secondary leaflet formation was observed only in plants harboring one or two copies of the *S. galapagense* locus Gdw. Intercalary leaflets were observed only on the seventh leaf on MT. Pts emitted intercalary leaflets on the sixth and the seventh leaves, producing 2 leaflets on each. Once again, the presence of the Gdw locus contributed to increase leaf dissection, since plants harboring it produced a higher number of intercalary leaflets than MT or Pts. Next, we would like to assess the expression of some genes in the beginning of leaf development, checking if genes like *TKn2*, *TKn1*, *Ga20ox*, *Ga2ox* are differentially expressed in the meristem and in the earlier leaf primordium.

P464

The Arabidopsis Tonoplast Contains Only a Very Small Proportion of the N-Glycoproteome, Unlike the Mammalian Lysosomal Membrane

Ilaria Fojadelli¹, Andrea Caprera³, Alessandra Rocchetti¹, Alessandra Stella¹, Barbara Weder², Enrico Martinoia², Alessandro Vitale¹, Emanuela Pedrazzini¹

¹*Istituto di Biologia e Biotecnologia Agraria, CNR, Milano, Italy*, ²*Institute of Plant Biology, University of Zurich, Zurich, Switzerland*, ³*Parco Tecnologico Padano, Milano, Italy*

N-glycosylation is one of the most important modifications of proteins synthesized by the secretory pathway. About one third of secretory proteins are N-glycosylated and their glycans are very often modified when proteins travel through the Golgi complex. It has been recently shown that the N-glycoproteomes of different eukaryotes mostly evolved after the phylogenetic divergences between plants, fungi, nematodes, insects, and vertebrates (*Zelinska et al., 2012 Mol. Cell 46, 542-548*). We have now analyzed *in silico* the percentage of N-glycosylated proteins in the tonoplast or plasma membrane (PM) of Arabidopsis and have verified our predictions experimentally. The results show that the tonoplast is virtually devoid of proteins with Golgi-modified glycans and contains only a small proportion, if any, of the N-glycoproteome, which is instead enriched in the PM. Lysosomes are considered the animal equivalents of plant vacuoles, because of shared abundance of hydrolytic enzymes and position along the secretory pathway. However, our results show that a much higher percentage of N-glycoproteins is present on the lysosomal membrane than in the tonoplast and that the very few tonoplast N-glycoproteins are much less extensively glycosylated. Thus, lysosomes and vacuoles had a divergent evolution in the composition of their membranes. This questions the hypothesis that the extensive N-glycosylation of lysosomal membrane proteins has evolved as a protection against the luminal hydrolases that are abundant in this compartment. Supported by the Italian Ministry of Education, Universities and Research (PRIN2010CSJX4F) and the EU Training Network VaTEP (MRTN-CT-2006-035833).

P465

Characterisation of Endophytic Fungi within Three *Sphagnum* Species from Clara Bog, Co. Offaly, Ireland, Followed by Bioactive Isolation and Identification: Implications for Enclosed Environment Systems in Space

Michelle McKeon-Bennett^{1,2}, Patrick Murray², Trevor Hodkinson¹

¹Department of Botany, School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland, ²CELLS Research Group, Department of Applied Science, Limerick Institute of Technology, Limerick, Ireland

This work represents the first report of the mutualistic relationships between endophytic fungi and three species of native *Sphagnum* moss found on the Special Area of Conservation & National Heritage Area of Clara Bog, Co. Offaly.

The application of the ion-exchange ability of *Sphagnum* moss to water remediation and recourse recovery within an Enclosed Environment has been investigated by the author at NASA's Space Life Science Laboratory, Kennedy Space Centre, Florida. While this research indicated that *Sphagnum* could be utilized in this manner, it resulted in yet more questions, specifically in relation to microbial interactions and growth mechanisms within the *Sphagnum* test beds. Micro-static ability of three different *Sphagnum* moss species has been observed but the molecular mechanisms of this activity have not been defined.

It is postulated that endophytic fungi growing mutualistically with the plant may produce secondary metabolic compounds responsible for the micro-static effects.

DNA for fungi will be isolated from 100ug samples of *S. cuspidatum*, *S. magellanicum* and *S. fallax* used to identify the fungal endophytes using standard barcoding primers. Genetic fingerprinting will also be utilised to type the endophytes, followed by culturing of the fungi with a view to isolating and characterisation of the micro-static compounds via LCMSMS.

P466

Identification of a Recently Active *Prunus*-Specific Miniature Inverted Repeat Transposable Element with Considerable Evolutionary Implications

Attila Hegedus¹, Ossama Kodad², Eva Stefanovits-Banyai³, Júlia Halász¹

¹Corvinus University of Budapest, Department of Genetics and Plant Breeding, Budapest, Hungary,

²École Nationale d'Agriculture de Meknès, Meknès, Morocco, ³Corvinus University of Budapest, Department of Applied Chemistry, Budapest, Hungary

Miniature inverted repeat transposable elements (MITEs) are known to contribute to the evolution of plants but only limited information is available for MITEs in *Prunus* genome. We have identified a MITE with structural features (its 349-bp size, 82-bp terminal inverted repeats and 9-bp target site duplications) that are consistent with this MITE being a putative member of the Mutator-like transposase superfamily. The element was provisionally labelled as *FaSt* (*Falling Stone*). *FaSt* showed a preferential accumulation in the short AT-rich segments of the euchromatin region of the peach genome. DNA sequencing and pollination experiments have been performed to confirm that nested insertion of *FaSt* into the *S-haplotype-specific F-box* gene of apricot resulted in the breakdown of self-incompatibility. The structure and emergence of the nested insertion suggest within-locus integration in response to abiotic stress conditions. A bioinformatics based survey of the known *Rosaceae* and other genomes and a newly designed PCR-assay verified the *Prunoideae*-specific occurrence of *FaSt* elements. Phylogenetic analysis suggested a recent activity of this MITE in the *Prunus* genome. In addition, *FaSt* was also declared in a *Myb* gene suggesting further phenotypic consequences. This is the first study to report on a presumably active MITE in the *Prunus* genus that might have exhibited direct and indirect genome shaping forces. The breakdown of the self-incompatibility barrier *per se* was of crucial importance during the evolution of *Prunus* species.

This study was financed by the OTKA K84290, PD 78124, János Bolyai Scholarship and the M/L n°3287 and TÉT-10-1-2011-0505 Moroccan-Hungarian Programme grants.

P467

Ginkgo's Limited Physiological Plasticity at Low [O₂]:[CO₂] May Have Contributed to Its Near Extinction across the Triassic-Jurassic Boundary (200 Million Years Ago)

Charilaos Yiotis¹, Christiana Evans-Fitz.Gerald¹, Tracy Lawson², Jennifer C. McElwain¹

¹School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland,

²School of Biological Sciences, University of Essex, Colchester, Essex, UK

Contrary to the traditional view of plant evolution as a strict series of reproductive and vegetative innovations, the importance of co-evolution between plants and Earth's atmosphere has been largely overlooked. The present study aimed to investigate the inherent physiological plasticity of basal, extant species following two months acclimation in a low [O₂]:[CO₂] atmosphere. Mature plants from 2 angiosperm (*Drumys winteri* and *Chloranthus spicatus*), 2 pteridophyte (*Osmunda claytoniana* and *Cyathea australis*) and one gymnosperm (*Ginkgo biloba*) species were grown for two months in 2 walk-in CONVIRON BDW40 chambers running at 16% [O₂]-1900 μmolmol⁻¹ [CO₂], while 2 more chambers running at 21% [O₂]-400 μmolmol⁻¹ [CO₂] served as control. After the conclusion of the acclimation period, gas exchange and chlorophyll fluorescence measurements were performed on leaves that had reached maturity prior to the beginning of the experiment. The low [O₂]:[CO₂] acclimation induced a significant reduction of $V_{c_{max}}$ and J_{max} in all species, yet this down-regulation of photosynthesis had little effect on the operating, light saturated photosynthetic rate (A_{max}), which was slightly increased in the angiosperm and pteridophyte species. However, F_v/F_m was found to be severely decreased in *Ginkgo biloba* and moderately decreased in the 2 angiosperms when grown in a low [O₂]:[CO₂] atmosphere. We argue that the observed photo-damage is a result of the reduced J_{max} coupled with an apparent inability to divert excess electrons to sinks other than the C₃ and C₂ cycles, and that this may help explain why Ginkgoales nearly went extinct as CO₂ increased across the Triassic-Jurassic boundary.

P468

The E-MoVe! Project: Everything in Biology Makes Sense in the Light of (Plant) Evolution

Antonella Grano, Mario De Tullio
University of Bari, Bari, Italy

Dobzhansky's well-known aphorism that Nothing in biology makes sense, excepting in the light of Evolution (Am. J. Biol. Teach., 1973, 35:125-129) is still a guiding light. Plants are ideal organisms for teaching the basics of evolution, but surprisingly this opportunity has not been fully exploited. The E-MoVe! (Evolution of the Plant World) project is funded by the Italian Ministry of Education, and aims at establishing and implementing a centre for science education in the specific field of Plant Evolution, based in the Botanical Garden Museum of the University of Bari (Italy). Our permanent exhibition is a tool for designing, validating and disseminating teaching activities on plant evolution. The project also aims at establishing a network of collaborations with National and International institutions. We are presenting here three interactive games for teaching plant evolution: 1. The time machine (basic), in which the player travels in time to see plants from the past ages. 2. Plant evolution and biodiversity (intermediate), with the player picking the picture and the scientific name of a given plant. 3. Gene expression and flower morphology (advanced), based on the ABC model of specification of floral organs. The player, by regulating the expression of genes involved in the determination of flower shape, interacts with the development of the Arabidopsis flower.

P469

Resin Glands in the Androecia of Two Weakly Related *Clusia* Species: Structure and Secretion

Silvia Machado¹, Bárbara Haiad²

¹UNESP, IBB, Botucatu, SP, Brazil, ²UFRJ, Museu Nacional, Rio de Janeiro, RJ, Brazil

Production of resin as floral resource is unusual and known in representatives of few angiosperm genera. In Neotropical genus *Clusia*, half of the species presents resiniferous flowers; this character emerged independently three times, being a relatively old phenomenon. We analyzed the morphology and functioning of resin glands in the androecia of *Clusia fluminensis* and *C. lanceolata*, placed in two of the three different clades in which floral resin production arose. Both taxa are southeastern Brazilian dioecious shrubs, having resiniferous flowers with strongly distinct androecia and pollinated by bees that use resin in nest construction and defense. We aimed to characterize the morphology of resin glands and verify if the cellular aspects of resin secretion process differ in such weakly related species. Resin is produced by subepidermal secretory cavities. Epithelial cells, in the secretory stage, present cell machinery typical of resin production being the modified plastids the more conspicuous organelles. Medium lamellae dissolution occurs along the radial walls of the epithelial cells and the intercellular spaces are occupied by secretion that migrates toward the lumen. The resin is released via rupture points on the distal surface of stamens and staminodes. In both species, resin synthesis, compartmentalization and releasing of secretion occur in the young flower buds, pre-anthesis buds and flowers at anthesis, respectively. The cell machinery related to resin production and the chemical identity are quite similar, suggesting that the acquisition of the character, in an evolutionary point of view, could be related to biogeographical conditions and presence of specific pollinators.

P471

The Influence of Elevated CO₂ and Subambient O₂ on Stomatal Function in Different Evolutionary Plant Groups

Christiana Evans-Fitz.Gerald¹, Charilaos Yiotis¹, Tracy Lawson², Jennifer C. McElwain¹

¹University College Dublin, Dublin, Ireland, ²University of Essex, Colchester, Essex, UK

Both CO₂ and O₂ have influenced the evolution of life on Earth: atmospheric oxygen is essential for aerobic respiration in eukaryotic cells, while CO₂ is used by plants as a primary substrate for photosynthesis. Plant stomatal function is also influenced by both gases: increased [CO₂]_{atm} leads to a decrease in stomatal conductance (g_s) through physiological and/or morphological adaptation; and O₂ supplies some of the energy needed for stomatal movement. Three major plant grades have been dominant over the past 450 million years: pteridophytes during most of the Palaeozoic, gymnosperms during the early Mesozoic and angiosperms during the Late Mesozoic to Cenozoic. Changes in stomatal function in these plant groups may have been a key factor influencing the evolutionary pattern observed in the fossil record. Representatives from each plant group were grown in a high CO₂ (1900 μmol mol⁻¹), low O₂ (16%) atmosphere in order to investigate the effect of these two gases on stomatal opening in response to light. The results suggest that under a high CO₂/low O₂ atmosphere, stomatal opening in response to an environmental trigger (light) is species-specific and not determined by plant functional group. It is possible that the low O₂ atmosphere (16%) impacted the energetics of stomatal movement in these species, however the high CO₂ concentration (1900 ppm) may also have contributed to the observed reduced conductances. Future work measuring stomatal opening responses of new leaves of these species under the same atmospheric conditions will allow further conclusions to be made.

P472

Analysis of Genetic Relationships among Pulse Genotypes Based on AFLP Markers

Lizica Szilagyi, Cistinel Relu Zala

University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania

Pulses such as dry beans and lentils have undoubtful importance, both for their nutritional value as well as the positive impact on the environment and agronomic benefits. This study was undertaken to evaluate genetic diversity and relationships among twenty common bean cultivars and among nine lentil genotypes using amplified fragment length polymorphism (AFLP) markers. Two groups of seven AFLP primer combinations were used to amplify a total of 72 polymorphic fragments (61.26%) in common bean and 69 polymorphic fragments (49.60%) in lentil. The level of similarity was higher than 65% for lentil, suggesting that genetic diversity between lentil genotypes analyzed is relatively low. Genetic similarity in bean genotypes vary greatly (38% - 86%), depending on the pairs of genotypes, on the subgroups and groups. The dendrograms generated with UPGMA (Unweighted Pair Group Method with Arithmetic mean) cluster analysis of the Jaccard's similarity coefficient matrices revealed two major clusters for each crop (A and B). The common bean genotypes were clustered according to the gene pools of origin, cluster A included cultivars whose genomic background is mainly from Middle American gene pool, and cluster B included cultivars with parents from the Andean gene pool. Lentil genotypes were grouped according to their macro- and microsperma type and their geographical origin. In the future bean and lentil breeding programs, artificial crosses among genotypes from clusters A and B with less similarity will allow a larger segregation and the combination of different favorable alleles.

P473

Competitive Capability of *Robinia pseudoacacia* with Native Species in an Old-Growth Broadleaf Forest

Mirko Umberto Granata², Loretta Gratani¹, Francesco Sartori², Laura Varone¹, Rosangela Catoni¹
¹Department of Environmental Biology, Sapienza University of Rome, Rome, Italy, ²Department of Earth and Environmental Sciences, University of Pavia, Pavia, Italy

Air temperature and CO₂ concentration increasing over recent decades have determined novel environmental conditions which might act as a potent agent of natural selection among species, resulting in a competition between invasive over co-occurring native species. An enhanced phenotypic plasticity may play an important role in successful colonization of new habitats by invasive species. The objective of the present research was to analyze the competitive capability of *Robinia pseudoacacia* with the co-occurring species in a broadleaf deciduous forest (Natural Reserve Siro Negri, Italy) by the analysis of plasticity. The results show that the mean plasticity index (mean of morphological, anatomical, physiological traits) is the highest for *Q. robur* (0.39), followed by *R. pseudoacacia* (0.38), *A. campestre* (0.34), *C. avellana* (0.33), and *P. alba* (0.29). Moreover, the highest *R. pseudoacacia* morphological plasticity (0.56) contributes to its adaptability. Considerations can be made for the conservative management of the forest carried out since the establishment of the Reserve, which has probably limited the presence of *R. pseudoacacia* until 1980 when it became established. Thus, it is important to maintain this type of management in the future since creating gaps could allow a greater seed regeneration of *R. pseudoacacia* over *Q. robur* because of the higher growth rate. Consequently, land use is a fundamental determinant in shaping vegetation composition with important implications for forest management. In particular, forests with old-growth characteristics, like the one investigated, are important reference sites for more natural management approaches involving in a broad range of ecosystem functions and services.

P474

Some Eco-Physiological Properties of Invasive Plant Japanese Knotweed

Matilda Djukic, Danijela Djunisijevic Bojovic, Mihailo Grbic, Dragana Skocajic, Marija Markovic
Faculty of Forestry, Belgrade, Serbia

Reynoutria japonica Houtt. was ranked in a group of 100 the most invasive organisms (IUCN International Union for Conservation of Nature). The damages of invasive plants are the great challenge in environment and biodiversity protection and it is very important to study their ecophysiological properties, reproduction, spreading and taxonomy.

Japanese knotweed is very common, with a great spreading potential, luxury growth on the sunny, open and wet habitats .It can be found near the roads, railways, river banks and on degraded urban areas and very difficult to eradicate.

In this paper it was investigated leaves stomata at plants from various habitats, age and vitality as well as the rhizome allelopathic influence.

It was found that number of stomata was larger on the lower than on the upper side of leaves in all populations and ecological conditions. Stomata index was significantly different between chlorotic and vital leaves but there were no difference between young, undeveloped and totally developed leaves. Stomata index was significantly higher at leaves from more sunny in compare with plants from shade sites. Rhizome extract had an inhibitory effect on seed germination and seedling growth at *Ailanthus glandulosa* and *Ulmus pumila* but acted as a stimulating at *Platanus acerifolia*.

Stomata analysis and allelopathic properties can help in better understanding of physiological processes of invasive plants. This can help to found the best measures how to control and destroy them.

Key words: *Reynoutria japonica* Houtt., invasive plant, stomata, allelopathy

P475

EU-COST Action on "Sustainable management of *Ambrosia artemisiifolia* in Europe" (COST FA1203-SMARTER): Opportunities and Challenges

Heinz Müller-Schärer, Suzanne Lommen
University of Fribourg, Fribourg, Switzerland

The EU -COST Action FA1203 on "Sustainable control of *Ambrosia artemisiifolia* L. (Asteraceae) in Europe (SMARTER)" was successfully launched in February 2013 and will last for four years. Thirty-three countries have already signed the Memorandum of Understanding and over 180 researchers with specialists in weed research, invasive alien species management, ecology, aerobiology, and economics are registered participants of SMARTER. COST Actions interlink nationally funded research projects and enable and finance conferences, working groups, training schools and research exchanges. SMARTER aims to initiate and develop long-term and sustainable control methods, to integrate these into existing mechanical and chemical control measures, and to quantify the success of these measures both for agriculture and health. The focus is on biological control methods with insects and fungi (especially using alien species from the area of origin of *Ambrosia*) and vegetation management to achieve a competitive plant cover. For this, we develop and parameterize models, starting from the population dynamics of *Ambrosia*, on the impact of control measures on the frequency and distribution of *Ambrosia* and finally on pollen counts and allergy occurrences, each with both ecological and economic components. The necessary data are derived from the many experiments that we carry out in well-coordinated studies across Europe. SMARTER will allow the various stakeholders to select optimal habitat- and region-specific combinations of control methods.

P476

***Ailanthus altissima* - Gain from the Pain?**

Jürgen Lernbeiss, René Rehorska, Julia Jamnig, Gerald Pichler, Hartwig Pfeifhofer, Maria Müller
Institute of Plant Sciences, Graz, Austria

Ailanthus altissima and *Fallopia japonica*, so called invasive plant species, became notorious in middle Europe due to their rapid growth and regenerative capabilities. They are also considered to be a major threat to native plant species.

The long term agenda of our research is to "gain from the pain" by utilizing these invasive plant species, by extracting their valuable bioactive compounds. Our actual research is focused on quassinoids, triterpenoids, which occur in *Ailanthus altissima*. Quassinoids show a large range of bioactive effects like growth inhibition and phytotoxicity. Nevertheless, the phytotoxic mechanisms must be understood, before usage of quassinoids or even crude extracts from *Ailanthus altissima* become reasonable.

To investigate the mechanisms of phytotoxicity, we tested crude extracts of *Ailanthus altissima* roots as well as leaves in modified cress tests and treated a heterotrophic plant cell suspension culture (tobacco BY2) with the same extracts.

The statistical analyses of the cress tests show drastic negative effects on the germination and growth of cress radicles and hypocotyls even at very low application rates of applied extracts. Further we found that cell division and biomass gain of the tested plant suspension cultures were distinctly inhibited at similar low doses of the extract, although the cells stayed vital.

These results also lead us to the conclusion, that some plant suspension cultures may be viable models for investigating allelopathic mechanisms, too.

P477

Allelochemicals from *Sesbania virgata* (Cav) Pers. Seed Affect Carbohydrate Metabolism and Growth of *Leucaena leucocephala* (Lam) de Wit Seedlings

Daiane Salete Broch Mignoni, Marcia Regina Braga
Botanical Institute, São Paulo, Brazil

Allelochemicals can contribute to the success of invasive species. *Sesbania virgata* (sesbania) seeds exudate the phytotoxin (+)-catechin, which has been related with its invasive behavior. *Leucaena leucocephala* (leucena) has also allelopathic potential, mainly attributed to the presence the allelochemical mimosine. This work evaluated the effects of leucena seed exudates on germination of rice and tomato and the effects of sesbania seed on germination, early growth and mobilization of carbohydrate reserves of leucena seed and seedlings. Rice and tomato seeds were germinated with leucena exudates (1 mg mL^{-1}). A co-germination experiment was performed with increasing number of sesbania seed (1 to 5) together with one leucena seed. Germination of both experiments was evaluated daily, and on the fifth day, the length of the root and shoot seedlings were measured. Furthermore, soluble carbohydrates were also quantified. Leucena exudates collected at 72 hours, decreased germination of rice seeds (15%), besides reducing in 48% and 26% root and shoot length, respectively. In tomato, germination was less affected by the exudates; however, there was a 90% reduction of the root length. In co-germination, sesbania delayed leucena germination in 65%, and drastically reduced root length (97%). An increase on levels of soluble sugars and oligosaccharides raffinose series was observed in leucena seeds indicating that reserve mobilization was also affected by sesbania. Despite the allelopathic potential of leucena as an exotic, invasive and successful species, sesbania drastically affects its growth and metabolism (FAPESP, CNPq).

P478

Antioxidant Activity of Leaf Extracts of Invasive Balsams

Irma Sliumpaite, Lina Zybartaitė, Ramunas Vilcinskas, Daiva Ambrasienė, Algimantas Paulauskas, Eugenija Kupcinskiene
Vytautas Magnus University, Department of Biology, Kaunas, Lithuania

Penetration of alien plant species is among the biggest threats to biodiversity. Strong antioxidant activity might influence competition capacity of invasive species. The aim of present study was to evaluate leaf extracts of *Impatiens parviflora* and *Impatiens glandulifera* for their content of total phenolic compounds, radical scavenging activity using DPPH and ABTS assays. Twenty populations of each *I. parviflora* and *I. glandulifera* were collected in Lithuania. Powdered leaves were extracted with ethanol. Radical scavenging activity of leaf extracts against stable DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) was determined spectrophotometrically. For DPPH assay the free radical scavenging capacity was expressed as Trolox (tetramethylchromane-2-carboxylic acid) equivalent (TE) antioxidant capacity. ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation decolouration was measured spectrophotometrically. The content of total phenolic compounds in plant extracts was determined by Folin-Ciocalteu method. For *I. parviflora* populations TE ranged in the interval of 0.03 and 7.43 mg/g dried extract, meanwhile extracts of *I. glandulifera* ranged in the interval 5.22-11.32 mg/g. Another antioxidant activity screening method ABTS radical cation decolourisation assay showed that *I. glandulifera* extracts possessed higher radical scavenging activity than *I. parviflora*. The total phenolic content of the extract of *I. parviflora* leaves in terms of gallic acid equivalent (GAE) ranged in the interval of 8.77 to 30.39 GAE mg/g dried extract. Total phenolic content of the extracts of *I. glandulifera* leaves ranged in the interval of 42.33 to 88.16 GAE mg/g. *I. glandulifera* had stronger antioxidant effect and accumulated more phenolic compounds compared to *I. parviflora*.

P479

Diversity of *Impatiens parviflora* Populations According to Inter Simple Sequence Repeat Loci

Rasa Janulioniene, Lina Zybartaite, Ramunas Vilcinskas, Algimantas Paulauskas, Eugenija Kupcinskiene

Vytautas Magnus University, Department of Biology, Kaunas, Lithuania

Studies of genetic diversity of plant species started from isozyme analyses, followed by DNA markers which do not require preliminary sequence information: random amplified polymorphic DNA (RAPDs) and inter simple sequence repeats (ISSRs). Till now ISSR markers are widely used for angiosperm analyses solving out both theoretical and practical problems like genotyping of medicinal and agricultural plants, explaining phenomenon of endemism and invasion. Climatic shifts within recent decades created favourable conditions for invasive species flourishing in more Northern parts of Europe. Presently *Impatiens parviflora* is one of the most widely spread invasive plants of Baltic States. Compared to the other alien species of Europe genetic studies of *Impatiens parviflora* have not received adequate attention. Our study was aimed at evaluation of genetic variability of *I. parviflora* populations growing in Lithuania. Throughout the country 21 population (15 individuals in each) was selected and analysed according to 4 ISSR markers. Percentage of polymorphic DNA loci (% P), Nei's gene diversity, Shannon's information index per population ranged respectively 10.2–25.9, 0.030–0.078, 0.046–0.121. Genetic distances among populations were in the interval 0.083–0.405. Molecular diversity between populations (76 %) was much higher than interpopulation variability. These results will be discussed comparing similar scope data obtained by RAPD markers. Analyses of *I. parviflora* at ISSR loci show that in Northern Europe invasion process is very complex and gets reflection in genetic structure.

P480

Characterization of the *RIBA* Gene Family in *Arabidopsis thaliana*

Hanna-Maija Hiltunen, Boris Hedtke, Bernhard Grimm
Institute of Biology Humboldt University, Berlin, Germany

Riboflavin serves as a precursor for flavoenzymes (FMN and FAD) and is essential for all living organisms. The bifunctional RIBA enzymes comprised of GTP cyclohydrolase II (GCHII) and 3,4-dihydroxy-2-butanone-4-phosphate synthase (DHBPS) perform the two initial steps of riboflavin biosynthesis in plants. The occurrence of at least three members in the *RIBA* gene family is highly conserved among angiosperms. In *Arabidopsis* all three isoforms were shown to be plastid localized, expressed in all tissues and at all developmental stages. The *riba1* mutant is embryo lethal indicating that lack of *AtRIBA1* is not substituted by the simultaneous expression of *AtRIBA2* and *AtRIBA3*. Partial *RIBA1* gene silencing causes a strong bleaching phenotype due to reduced flavin levels. In addition transgenic plants with different *RIBA* gene constructs were used for physiological characterization of responses to modified flavoenzyme contents. We further examined reasons why *RIBA2* and *RIBA3* fail to compensate for a lack of *RIBA1* expression. A sequence alignment revealed amino acid exchanges in conserved peptide domains, which were shown to be responsible for the loss of *in vitro* and *in vivo* activity of the GCHII or the DHBPS domain in *AtRIBA2* and *AtRIBA3*, respectively (Hiltunen et al., 2012). The current hypothesis is that the combined overproduction of the monofunctional *AtRIBA2* and *AtRIBA3* in *riba1* enables to compensate deficiency of *AtRIBA1*. As it is not excluded that steric hindrance of monofunctional isoforms compromises riboflavin metabolism, different potentially bifunctional fusion RIBA proteins were designed for the complementation of *riba1* and the results are presented.

P481

Methanol as an Energy Substrate for *Chlamydomonas reinhardtii*

Serhii Stepanov

M.G. Kholodny Institute of Botany, Kyiv, Ukraine

Methanol in low concentrations stimulates growth of autotrophic batch culture *Chlamydomonas reinhardtii*. Since methanol is a carbon-containing but nitrogen-free compound, its assimilation may influence biosynthesis of components, the accumulation of which depends on the C/N ratio and in the first place, amino acids and proteins. The aim of the present work is to elucidate as the methanol application affects the content of free amino acids and soluble proteins in *C. reinhardtii* cells grown in the presence of 50 mM methanol. To elucidate how the methanol application affects the processes of energy transformation, the rate of photosynthesis, respiration and content of reduced nicotinamide coenzyme NAD(P)H were determined. The intracellular content of NAD(P)H was increased after addition of methanol. Without illumination, NAD(P)H content increased only slightly. It was shown that intracellular concentration of free amino acids increased by 31% as a result of 50 mM methanol addition, and their composition has changed: the concentrations of glutamate, glutamine, alanine, serine, threonine, and tyrosine rose, and that of methionine decreased. Content of soluble protein also increased by 30% eliminating the decisive contribution of proteolysis in amino acids content. Thus methanol has a positive effect on the nitrogen assimilation as evidenced by increase in the concentration of soluble proteins and free amino acids. This effect may be connected with the increase of respiration and NAD(P)H content induced by methanol addition. The results suggest that methanol can be not only the carbon source but also takes part in energy metabolism in *C. reinhardtii* cells.

P482

Proteome and Phosphoproteome Changes in Response to 24-Epibrassinolide in Wheat Seedlings

Kristina Ivanova, Ruslan Yuldashev, Azamat Avalbaev, Farida Shakirova
Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences, Ufa, Russia

Brassinosteroids are plant hormones that regulate wide range of growth and developmental processes through numerous regulatory systems. In our previous study we have found a pronounced growth-stimulating and protective effects of 24-epibrassinolide (EBR), active representative of brassinosteroids, on wheat plants that have indicated its active influence on cell metabolism which is based on protein synthesis. One of the essential regulatory mechanisms of cellular activity is post-translational modifications of proteins, and special attention is given to protein phosphorylation. Proteins might be phosphorylated on various amino acid residues, in particular, on tyrosine residues. Tyrosine phosphorylation has a significant role in the recognition and subsequent development of cell response to extracellular signals, particularly, phytohormones. In this regard, the aim of this study was proteomic and phosphoproteomic analysis of response to 24-epibrassinolide in wheat seedlings. We have revealed that EBR caused the promotion of protein metabolism, and this was evidenced by an intensification of protein signals in a wide range of molecular weights and isoelectric points. Meanwhile, EBR caused significant changes in the level of protein tyrosine phosphorylation. Using MALDI-TOF MS there were identified about 30 proteins and phosphotyrosine polypeptides responsive to EBR treatment, some of which are involved in key physiological processes such as photosynthesis, plant growth and development. The obtained data suggests an important role of brassinosteroids in the activation of protein metabolism which is the basis for promotion of plant growth and development.

This work is supported by Grant № 14-04-00731_a of the Russian Foundation for Basic Research.

P483

Molecular Induction of Flower Abscission in *Vitis vinifera* L. as a Response to C- Shortage and to Exogenous Gibberellic Acid

Sara Domingos^{1,2}, Vânia Cardoso¹, Hugo Nóbrega², António Eduardo Leitão¹, José Cochicho Ramalho¹, Cristina Moniz Oliveira², Luis Filipe Goulao¹

¹BioTrop – Instituto de Investigação Científica Tropical (IICT), Lisbon, Portugal, ²Instituto Superior de Agronomia - Universidade de Lisboa (ISA/UL), Lisbon, Portugal

Abscission enables shedding of vegetative and reproductive organs and is triggered by developmental, hormonal and environmental cues. Due to its economic importance and role as a woody plant model, grapevine stand as an attractive species to characterize the molecular mechanisms that regulate flower abscission. Here, we provide insights on the regulation of abscission in response to two different stimuli: shading imposition and gibberellic acid (GA3) spraying. The experiment was conducted during 2013 in Portugal using 'Thompson Seedless' under field conditions. Flower drop, leaf gas exchanges and vegetative development were assessed and inflorescences were collected at fixed time points for global metabolOMICs, targeted biochemical and gene expression analyses. Both treatments were successful in increasing flower abscission (drops of 99% for shade and 83% for GA3) comparing to the control (63%). Leaf area, chlorophyll content and gas exchange parameters were reduced in the shade, whereas no differences were found in the GA3 treatment. Shade led to a C/N imbalance due to C-shortage, increased amino acids and markers of oxidative stress, and less polyamines, sugars and other energy molecules. GA3 application resulted in a lower magnitude changes and emphasis was on a decrease of several N-compounds and increase of proline and lysolipids levels. MAPK(n) and ethylene/auxin receptor-related gene expression was conducted by qrt-PCR and the results show different, often contrasting, patterns according to the treatment. The overall results suggest that both shade and GA3 application promote changes on physiologic, metabolomic and transcriptomic patterns, disclosing that triggering of flower abscission proceeds following different pathways.

P484

Genetic Analysis of Pathways for Nucleotide Sugars Needed for Plant Cell Walls

Claudia Geserick, Stefanie Endres, Rebecca Reboul, Anja Geretschläger, Robert Behmüller,
Raimund Tenhaken
University of Salzburg, Salzburg, Austria

Cell wall polymers are synthesized by glycosyltransferases using nucleotide sugars as glycosyl donors. We are studying de novo and salvage pathways for major nucleotide sugars of plant cell walls by characterizing knockout mutants and overexpressor lines in different pathways. Changes in cell wall polymers are tightly connected with plant development. Mutants with altered levels of inositol show enhanced resistance to plant nematodes. Surprisingly, recycling of sugars into nucleotide sugars is essential for plant growth and reproduction.

P485

Different Pathways of Mannitol Metabolism in Land Plants and Brown Algae

Patricia Bonin^{1,2}, Agnès Groisillier^{1,2}, Zhanru Shao³, Sylvie Rousvoal^{1,2}, Gurvan Michel^{1,2}, Sophie Goulitquer⁴, Anaïs Guibert^{1,2}, Stefan Krahulec⁵, Simon Dittami^{1,2}, Bernd Nidetzky⁵, Delin Duan³, Catherine Boyen^{1,2}, Thierry Tonon^{1,2}

¹Sorbonne Universités, UPMC Univ Paris 06, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, Roscoff, France, ²CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, Roscoff, France, ³Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China, ⁴Plate-forme MetaboMER, CNRS and UPMC, FR2424, Station Biologique de Roscoff, Roscoff, France, ⁵Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Graz, Austria

Brown algae represent photosynthetic organisms distantly related to land plants and animals. They are mostly found in the intertidal zone, a harsh and frequently changing environment. Among their metabolic features, mannitol is used for carbon storage, as well as osmoprotectant and antioxidative.

Mannitol is produced by land plants from fructose-6-phosphate (F6P) via mannose-6-phosphate and mannitol-1-phosphate (M1P) in source tissues, and then translocated to sink tissues where it is recycled to F6P for use as a carbon and energy source (Stoop *et al.*, 1996). In brown algae, mannitol synthesis involves the direct reduction of F6P into M1P, by a mannitol-1-phosphate dehydrogenase (M1PDH), and the hydrolysis of the phosphoric ester of M1P by a mannitol-1-phosphatase (M1Pase) (Iwamoto and Shiraiwa, 2005). This latter step also occurs in some plants (Grant and Rees, 1981; Rumpho *et al.*, 1983). However, little is known on these pathways at the molecular level in both types of organisms.

Taking benefit of the annotation of the model brown alga *Ectocarpus siliculosus* genome (Cock *et al.*, 2010; Michel *et al.*, 2010), we started deciphering its mannitol metabolism, in particular M1PDH and M1Pase steps, by combining analysis of endogenous activities, kinetic parameters of recombinant enzymes, and gene expression under different physiological conditions (Rousvoal *et al.*, 2011; Groisillier *et al.*, 2014). Such results will serve to implement a kinetic modeling approach, to better understand the functioning and the importance of the mannitol metabolism in brown algal physiology. Knowledge gained in algae should also benefit research on such metabolic process in land plants.

P486

Identification and Expression Analysis of Structural Genes Involved in Flavonoids and Anthocyanin Biosynthesis Expression Along the Maturation of *Rubus* sp. Var. Lochness

Daniel Garcia-Seco¹, Yang Zhang², Javier Gutierrez-Mañero¹, Cathie Martin², Beatriz Ramos-Solano¹
¹San Pablo CEU University, Madrid, Spain, ²John Innes Center, Norwich, UK

Blackberry (***Rubus*** sp.) is especially rich in plant polyphenols (Kaume et al., 2012). One subclass of plant polyphenols are flavonoids, a large group that includes anthocyanins, known as being strong natural antioxidants, together with carotenoids, ascorbate and tocopherols (Moyer et al., 2001); however, benefits of flavonoids for health are beyond a simple antioxidant benefit (Martin et al., 2013). Knowledge of its biosynthesis pathway and its development along the fruit ripening stage is essential to control and improve the blackberry's quality. In this research, the main structural genes that control the flavonoids and anthocyanin pathway in *Rubus* sp. Var. Lochness were identified by the 3'RACE technique.

Chalcone Synthase (CHS), Flavanone-3-hydroxylase (F3H), Flavonoid-3',5'-hydroxylase (F3'5'H), Dihydroflavonol 4-reductase (DFR), Anthocyanidin synthase (ANS), Flavonol synthase (FLS), Flavonoid 3'-hydroxylase (F3'H), Actin (ACT), were sequenced for the first time in *Rubus* sp. Var. Lochness. The expressions of these genes were studied by qRT-PCR. Glutathione S-transferase (GST) and Anthocyanidin reductase (ANR) expressions were also studied but not sequenced as they were previously sequenced.

In the earlier ripening stage of the fruit (the green state), the expression of all genes analyzed were very low. However, during the intermediate state, in the red state, the genes more upstream in the pathway, CHS, F3H, F3'5'H, DFR and ANS, had the highest expression pattern. On the other hand, the last gene in the route, ANR, showed the highest expression pattern in the most mature state of the fruit (the black state).

P487

Using Fundamental Knowledge Relating to Pyridine Alkaloid Synthesis by Members of the Nicotianoideae, Family Solanaceae, for Improved Molecular Farming Outcomes

John Hamill¹, Huai-Yian Ling⁴, Giorgio DeGuzman², Suzanne Ryan², Kathleen Deboer⁵, Assunta Pelosi², Alan Neale², Diane Webster², Amanda Walmsley³

¹Deakin University, Geelong, Victoria, Australia, ²Monash University, Melbourne, Victoria, Australia,

³Bionic Institute, Melbourne, Victoria, Australia, ⁴CSL Ltd, Melbourne, Victoria, Australia, ⁵CSIRO, Perth, West Australia, Australia

Recent studies reveal that production of nicotine-type alkaloids is an effective, wound-stress inducible chemical defensive strategy employed by species of the Solanaceous subfamily Nicotianoideae which includes the genus *Nicotiana* and the (mainly Australian) tribe Anthocercideae. Parallel studies into the molecular genetics of pyridine alkaloid synthesis have increased understanding relating to the evolution of mechanisms which facilitate synthesis of these alkaloids *in planta*. Gene-based technology has also enabled genetically engineered plants and hairy root systems to be developed which are free of toxic alkaloids but which synthesise high levels of useful therapeutic proteins such as plant-made vaccines. Direct administration of transgenic plant extracts by injection, or inclusion in the diet of animals, can elicit effective immune responses to potentially pathogenic viruses and microorganisms. As these Nicotianoideae species are highly amenable to genetic modification, can produce large amounts of biomass rapidly when grown as plants in field conditions or as hairy root systems *in vitro* and given that they are not part of the human food chain, they have potential for widespread use as vehicles to produce low cost and effective vaccines for veterinary disease prevention programs.

P488

Changes in the Expression of Genes Involved in Fructan Metabolism and Alteration in Redox Pairs during Wheat (*Triticum durum* L.) Kernels Maturation

Sara Cimini, Vittoria Locato, Maria Grazia D'Egidio, Laura De Gara
Food Science and Human Nutrition Unit, Campus Bio-Medico University, Rome, Italy

Wheat grains have recently received attention as source of bio-active molecules having positive effects on human health. These molecules include soluble carbohydrates, such as fructans, interesting molecules from a nutritional point of view by acting as prebiotics; as well as antioxidant molecules, such as polyphenols. The present work is part of a wider study in which we have shown that immature wheat kernels have a very high content in fructans (20-30% of dry matter) and increased levels of ascorbate and glutathione (1). We have also reported that during wheat kernel maturation fructans undergo changes in their polymerization levels and branching profile (2). In order to have further information on fructan metabolism in wheat analyses of gene expression of the enzymes responsible for fructan biosynthesis and degradation have been carried out in *Triticum durum* kernels starting from 7 days after anthesis up to the end of maturation processes. The levels of molecules conferring antioxidant properties (polyphenols, ascorbate and glutathione) as well as the content of starch, fructans, water and dry matter have also been monitored during the same period. The alterations in the levels of the analyzed molecules and in the gene expression of the enzymes responsive for fructans metabolism are discussed in relation to the physiological process of wheat kernel maturation as well as in the variation of nutritional value of the maturing kernel.

1) De Gara et al., J. Exp. Bot. 54: 249-258, 2003.

2) Verspreet et al. Plant Cell Physiol. 54: 2047-2057, 2013.

P489

Identification and Transcriptional Profiling of Candidate Flavonoid Biosynthesis Genes in Two Apricot Genotypes Differing in Polyphenolic Contents

Eva Stefanovits-Banyai¹, Peter Pfeiffer², Nora Papp¹, Laszlo Abranko^{1,3}, Attila Hegedus²
¹Corvinus University of Budapest, Department of Applied Chemistry, Budapest, Hungary, ²Corvinus University of Budapest, Department of Genetics and Plant Breeding, Budapest, Hungary, ³Research Centre for Natural Sciences, Institute of Organic Chemistry, Budapest, Hungary

Fruits are rich sources of antioxidants including flavonoid polyphenolic compounds. Commonalities and differences are well-known in the polyphenolic contents of various genotypes of the same fruit species, which suggest alterations in the biosynthesis pathway of fruit tissues. We examined the flavonoid metabolism in two different apricot (*Prunus armeniaca* L.) genotypes characterized by markedly different total antioxidant capacities and polyphenolic contents using HPLC-ESI-(Q)TOF MS and molecular genetics. The hybrid 'Preventa' with outstanding polyphenolic content was compared with the traditional Hungarian cultivar 'Gönci magyarkajszli'. Degenerate primers were designed based on gene sequences from closely related species and a homology based approach was used to identify flavonoid genes in *P. armeniaca*. We were successful in determining partial cDNA sequences of some candidate genes encoding the key enzymes in the phenylpropanoid and flavonoid biosynthesis pathways including phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, chalcone synthase, chalcone isomerase, flavanone 3-hydroxylase, flavonoid 3'-hydroxylase, flavonol synthase, dihydroflavonol 4-reductase, anthocyanidin synthase, anthocyanidin reductase, leucoanthocyanidin reductase and UDP glucose:flavonoid 3-O-glucosyltransferase. We determined transcript levels of the genes encoding the identified enzymes using quantitative real-time PCR (qRT-PCR) analyses. By comparing gene expression profiles of the tested apricot genotypes with their polyphenolic composition, the enzymes responsible for metabolomic differences were declared. The outstanding antioxidant capacity of 'Preventa' is, at least partly, due to the high catechin contents in fruit flesh, which is a consequence of the elevated expression of several genes involved in the flavanol biosynthesis.

This study was financed by the OTKA K84290, OTKA PD 100506 and János Bolyai Research Scholarship grants.

P490

A Metabolomics Approach to Study the Effects of Pre- and Postharvest Factors on Braeburn Browning Disorder

Darwish Hatoum, Maarten Hertog, Geeraerd Annemie, Nicolai Bart
KU Leuven, Leuven, Belgium

Braeburn (*Malus domestica* Borkh.) is a popular apple cultivar worldwide. During storage, Braeburn is susceptible to the development of an internal browning disorder (Braeburn Browning Disorder; BBD). The incidence and intensity of BBD vary with batch and growing season. The objective of this study is twofold: 1) to study the effects of various pre- and postharvest conditions on the metabolic profile of Braeburn apple; and 2) to identify a biomarker that can predict the occurrence of BBD.

Braeburn was grown in an orchard of the experimental tree fruit research station (RSF-pcfruit) in Sint-Truiden (Belgium) under 8 different treatment combinations of different levels of calcium and potassium fertilisation with different levels of triazole fungicides. Fruit were harvested at two dates (early and late), with cortex samples being collected at harvest and after 2 weeks, 4 weeks, 2 months, 4 months and 6 months of controlled atmosphere storage at 1°C and under 8 different combinations of 2 levels of O₂ (optimal versus low), CO₂ (optimal versus high) and 1-MCP (treatment versus no treatment). Metabolomics analyses were performed using a GC-MS based protocol optimized for apple. In this study, about 30 metabolites were identified and quantified from the polar extracts of the Braeburn apple cortical tissue.

By comparing the at-harvest metabolic profile of the samples, the effects of the different preharvest treatments is determined. Further, by analysing the metabolic profile of the samples from the different storage conditions, a differentiation is made between the fruit ripening related changes and the BBD specific changes.

P491

***In Vivo* Monoubiquitination at Lys624 and Phosphorylation at Ser7 of Anaplerotic CP21 Phosphoenolpyruvate Carboxylase in Germinating Sorghum Seeds Suggest a Novel Pattern of Posttranslational Modifications for a Metabolic Enzyme**

Isabel Ruiz-Ballesta¹, Ana-Belén Feria¹, Guillermo Baena¹, Hong Ni², Yi-Min She², William C. Plaxton³, Cristina Echevarría¹

¹Universidad de Sevilla, Sevilla, Andalucía, Spain, ²Shanghai Center for Plant Stress Biology, Shanghai, China, ³Queen's University, Kingston, Ontario, Canada

Phosphoenolpyruvate carboxylase (PEPC; EC 4.1.1.31) is an important enzyme that plays a pivotal role in numerous physiological processes in plants. Two immunoreactive PEPC polypeptides of approximately 110- and 107-kDa (p110 and p107) have frequently been observed on immunoblots of cereal seed extracts¹. Recent PEPC studies have provided examples of regulatory monoubiquitination of a metabolic enzyme². In the present work, a monoubiquitinated PEPC heterotetramer of p110:p107 subunits was purified from germinated sorghum seeds. Mass spectrometry revealed that: i) both subunits originated from the same *CP21 PEPC* gene, ii) p110 is a monoubiquitinated version of p107, and iii) p110's monoubiquitination site is Lys624³. The purified PEPC was not phosphorylated, as indicated by immunoblotting with phospho-site specific antibodies. By contrast, immunoaffinity chromatography of clarified extracts coupled with immunoblotting indicated that *in vivo* phosphorylation of the subunits occurred at PEPC's conserved Ser7 phosphorylation site. In addition, *in vivo* radiolabeling demonstrated ³²Pi incorporation into immunoprecipitated p110 and p107 subunits. This was corroborated by kinetic studies which detected a significant increase in PEPC's *I*C₅₀ for L-malate from dry to 48 h germinating seeds. Results of the current study suggest a novel pattern of post-translational modification of plant PEPC, as it appears that the same p110 subunit can be simultaneously phosphorylated and monoubiquitinated *in vivo*.

1. Feria *et al.*, (2008). *Plant Physiol* 148: 761-774.
2. Uhrig *et al.*, (2008). *J Biol Chem* 283: 29650-29657.
3. Ruiz Ballesta *et al.*, (2014). *J. Exp. Bot* 65: 443-451.

Acknowledgments: Funding by project AGL2012-35708 from 'MEC' and grant from 'NSERC'.

P492

Growth and Proteomic Analysis of Tomato Fruits in Wild Type and *flacca* Mutant

Radmila Stikic¹, Zorica Jovanovic¹, Milena Marjanovic¹, Mireille Faurobert², Nadia Bertin³, Biljana Vucelic-Radovic¹

¹ Faculty of Agriculture, University of Belgrade, Belgrade, Serbia, ² National Institute for Agricultural Research – Unit UGAFL, Avignon, France, ³ National Institute for Agricultural Research – Unit PSH, Avignon, France

To better understand the metabolic regulation of tomato fruit growth and the role of plant hormones ABA, the proteomic analyses in the phase of intensive growth of fruits of wild type (WT) and *flacca* mutant (ABA-deficient) was done. The phase of fruit development was determined by following fruit growth rate in the experiment conducted in controlled conditions. These results showed that lower fruit growth rate in *flacca* fruits compared to the fruits of WT plants, resulted in significantly smaller of both final fruit size of *flacca* and ABA content in a pericarp. For proteomics analyses, pericarps of investigated fruits were collected in the phase of intensive cell expansion. Phenol method was used for protein extraction, while proteins were separated by two-dimensional gel electrophoresis (2-DE) and analyzed by LC-MS/MS mass spectrometry. Identification was done using the SGN tomato unigene database. Proteins were classified according to their function in several categories: related to carbon and amino acid metabolism, protein translation, processing and degradation, energy metabolism, cell wall related, oxidative stress, stress defense, and other metabolic process. In total 50 proteins showed variation between two genotypes. Most proteins related to carbon, amino acid metabolism and protein translation, processing and degradation showed increased expression in WT compared to *flacca*. These results indicate that faster metabolic flux in WT compared to *flacca*, might be responsible for higher fruit growth rate and final bigger fruit size in WT than in *flacca*.

Acknowledgment: research supported by EU FP7 project AREA and national project TR31005.

P493

Regulation of Oil and Protein Accumulation in Developing Lupin Seeds

Slawomir Borek¹, Stanislaw Pukacka², Stanislaw Stawinski³, Katarzyna Nuc⁴

¹Department of Plant Physiology, Adam Mickiewicz University, Poznan, Poland, ²Institute of Dendrology, Polish Academy of Sciences, Kornik, Poland, ³Plant Breeding Station Smolice Division in Przebedowo, Murowana Goslina, Poland, ⁴Department of Biochemistry and Biotechnology, Poznan University of Life Sciences, Poznan, Poland

The research was conducted on seeds of yellow lupin (*Lupinus luteus* L.), white lupin (*Lupinus albus* L.) and Andean lupin (*Lupinus mutabilis* Sweet). The lipid content in mature lupin seeds is 6%, 7-14% and 20%, respectively. However, the main storage compound in lupin seeds is protein, which content may vary from 38% (white lupin) up to 45-50% (yellow and Andean lupin). Experiments were conducted: *i*) on developing cotyledons (isolated from developing seeds) grown *in vitro* for 96 h on liquid mineral medium supplemented with sucrose (60 mM), asparagine (35 mM) or nitrate (35 mM), *ii*) developing pods grown in quasi *in vitro* conditions on above described media, and *iii*) under field conditions with application of the nitrate fertilizer (40 or 80 kg N per ha; application: before sowing, at nodulation stage and at pods filling stage).

Experiments conducted on isolated cotyledons grown *in vitro* shown that asparagine significantly enhanced the negative relation in accumulation of storage lipid and protein i.e. decreased oil content and enhanced the protein level. Contrary to asparagine, nitrate caused the positive relation i.e. significantly and simultaneously increased the storage lipid and protein accumulation. Similar effect of asparagine and nitrate was observed also in the developing pods. However, the positive effect of nitrate was clearly less visible. Unfortunately, no effect of nitrate was observed under field conditions. Application of nitrate fertilizer did not cause changes in seed lipid and protein content.

This work was supported by the National Science Centre (Grant NN310003540).

P494

Does Pexophagy (Autophagic Degradation of Peroxisomes) Occurs in Cells of Lupin Sugar-Starved Embryo Axes?

Slawomir Borek¹, Ewelina Paluch¹, Stanislaw Pukacka²

¹Department of Plant Physiology, Adam Mickiewicz University, Poznan, Poland, ²Institute of Dendrology, Polish Academy of Sciences, Kornik, Poland

Experiments were conducted on embryo axes of yellow lupin (*Lupinus luteus* L.), white lupin (*Lupinus albus* L.), and Andean lupin (*Lupinus mutabilis* Sweet). Embryo axes were isolated from imbibed seeds and cultured *in vitro* for 96 h on liquid mineral medium in six variants: medium with 60 mM sucrose (+S) or without sucrose (–S) and on media additionally supplemented with asparagine (+S+Asn and –S+Asn) or sodium nitrate (+S+NO₃ and –S+NO₃).

The lipid level was significantly higher in sugar-starved embryo axes than in sugar-fed axes of three lupin species. Such result is opposite to many literature data and is difficult to interpret. However, sugar starvation (–S) irrespective of Asn and NO₃ nutrition, caused significant increase in cell vacuolization and decrease in phosphatidylcholine level. These results pointed on advanced autophagy. In cells of sugar-fed axes autophagy did not occur. In cells of embryo axes fed with asparagine (–S+Asn) the decomposition of autophagic bodies was remarkably slower than in cells of axes non-fed with asparagine (–S). This allowed to more or less precise identification of cell components which were degraded during autophagy. Inside vacuoles of –S+Asn axes some autophagic bodies were very similar to structures located in cytosol which may be recognized as peroxisomes. Other autophagic bodies contained structures which may be also identified as peroxisomes. Ultrastructure of cells of axes fed with nitrate (–S+NO₃ and +S+NO₃) was very similar to –S and +S, respectively.

This work was supported by the National Science Centre (Grant NN310003540).

P495

Cell Wall-Bound Molecular Forms of β -Glucosidase of Pea: Activity, Physical and Chemical Properties, Impact of Hypoxic Stress

Antonina Ershova, Aynur Fatullaeva
Voronezh State Pedagogical University, Voronezh, Russia

β -Glucosidase (EC 3.2.1.21) catalyzes hydrolysis of β -glycosidic bond in β -D-aryl- and alkylglucopiranosides and plays an important role in exchange processes of cell. In pea seedlings together with cytoplasmic β -glucosidase an ion-bound and adsorbed on cell wall forms were discovered. Activity and properties of cell wall-bound forms of β -glucosidase in pea plants under oxygen deficit and high concentrations of CO_2 (24h) were studied. Electrophoretically homogeneous enzymatic agents of adsorbed form with specific activity of 303.1 U/mg of protein and ion-bound form - 451.7 U/mg of protein were obtained. Under aeration both molecular forms of β -glucosidase separated specific for this plant isosuccinimide- β -glycoside (K_m 0.32 and 0.93mM) and p-NPG (K_m 0.45 and 1.03mM) with highest speed. Less affinity was shown towards β -gentiobiose, salicin and was no separation of galactopyranosides with α - and β -bonds. Under hypoxia and CO_2 -media an activity of both forms and rate of the separation of the substrates were changing that affected kinetic parameters of K_m and V_{max} . Hydrogen peroxide tolerance as one of most stable ROS types of both molecular forms of β -glucosidase was increasing. Shift of pH optimum for adsorbed form from 4.8 to 4.6 and for ion-bound one from 5.0 to 4.8 was noted. Obtained results indicate significant changes of physical and chemical properties of both molecular forms of β -glucosidase in plants under hypoxia and CO_2 -media. It can be assumed that activity increase of cell wall-bound forms of β -glucosidase of pea plants under such conditions can enhance free glucose content needed for respiratory exchange under oxygen deficit.

P496

Antioxidant Features and PAL Gene Expression of Blueberry Cultivated on an Andisol at Different N Addition

Erwin Yañez Mansilla, Paula Cartes, Marjorie Reyes Diaz, Alejandra Rivera Fonseca, Miren Alberdi
Universidad de La Frontera, Temuco, Chile

There are variable evidence about the N effect on phenolic accumulation and antioxidant capacity in plants. In the present study we tested the effect of increasing N doses on the antioxidant features and phenylalanine ammonia-lyase (PAL) gene expression in highbush blueberry on Andisol. Plants were grown at greenhouse conditions and the following N treatments were applied: 0, 20, 40 and 80 kg N ha⁻¹. The results showed that N concentration was raised in leaves and roots at increasing N doses. Lipid peroxidation was increasing at 40 kg N ha⁻¹ and was exacerbated at 80 kg N ha⁻¹ treatment accompanied by detrimental effect on CO₂ assimilation and relative growth. Total phenols and antioxidant capacity was reduced at leaf N concentration above 16 g kg⁻¹ DW. This fact agreed with the significant negative correlation between N concentration and total phenols or antioxidant capacity. Plants without N additions show an increasing phenolic acids and flavonols levels. In fact, a positive correlation in leaves between total phenols was found for caffeic acid, rutin or mirecetin at 16 g N kg⁻¹ DW. Relative gene expression was high at 0 kg ha⁻¹ and we observed correlation between antioxidant capacity and PAL gene expression. Thus, our study found that N concentration in leaves until 16 g kg⁻¹ DW does not reduce antioxidant capacity and maintain the nutritional quality of blueberry leaves.

ACKNOWLEDGMENTS: FONDECYT project 1110726. PhD CONICYT Scholarship and the Office of Research Universidad de La Frontera, Chile.

P497

Effect of Growth and Phytochemical Synthesis in Rice Seedling Based on Light Quality

Sun-Hyung Lim¹, Jae Kwang Kim², Jong-Yeol Lee¹, Young-Mi Kim¹, Sun-Hwa Ha³

¹Department of Agricultural Biotechnology, National Academy of Agricultural Science, RDA, Suwon, Republic of Korea, ²Department of Life Science, Incheon National University, Incheon, Republic of Korea, ³Department of Genetic Engineering and Crop Biotech Institute, Kyung Hee University, Yongin, Republic of Korea

Light is the primary energy source and plays the pivotal roles in plant growth and development. In addition, light has been known to affect the phytochemicals accumulation in plant, as key signaling element. Several studies have been conducted to investigate the effect of light quality on plant morphological process and phytochemical synthesis process, however, the influence of individual light quality on specific plant morphogenesis and phytochemical biosynthesis still remains unraveled. Understanding the effects of light quality on plant morphogenesis and phytochemical production may provide useful information to adjust or enhance the biomass and phytochemical synthesis in plant.

To investigate these effects on plant growth and phytochemical synthesis, rice seedlings were grown under the different light sources; blue (B), red (R), white (W) and dark (D). Seedling growth was significantly different according to the light sources: shorter plants with wider leaf blades were observed in B, long coleoptile length in D and narrow leaf blades in R compared to W. Furthermore, the intracellular content of secondary metabolites such as terpenoids and phenolic compounds was highest in B. Through mRNA profiling, the expression of genes being related in plant growth and phytochemical synthesis was displayed to be changed under B light. Currently, we are exploring the novel target genes in association with a two-for-one in the regulation of two different phytochemical synthesis along with the seedling phenotype changed by B light.

P498

Evolution of Carbohydrates in Tissue Culture Medium and Leaves of *Guzmania* 'Hilda' Plantlets

Ruth Lembrechts, Johan Ceusters, Veerle Verdoodt, Maurice De Proft
KU Leuven, Leuven, Belgium

Tissue culture is a commonly used technique for the propagation of bromeliads. As sucrose being a carbohydrate source and important for root development, it is mostly added to the culture medium. In this research *Guzmania* 'Hilda' plantlets were grown *in vitro* for 8 weeks on a rooting medium with variable initial sucrose concentrations (0, 22.5, 25, 40 g/L). It was showed that already after 4 weeks almost all sucrose is gone from the media (except for the highest sucrose concentration 40 g/L), but in the meanwhile glucose and fructose (not present at the start) was formed. During the next 4 weeks also in the medium with 40 g/L all the sucrose was gone and more and more hexoses were measured. Knowing the plants had nothing of the transport sugar sucrose left in the media after 4 weeks, there should be other mechanisms that explain the growth of the plants for the last 4 weeks under *in vitro* conditions. It was seen that after 4 and 8 weeks the plants had a sucrose concentration dependent amount of starch in their leaves, so one possibility is that they use this stored carbohydrates to support growth at times when no sucrose was available in the medium. Actually in this research it was shown that the amount of starch in the leaves was lower after 8 than after 4 weeks of growth, suggesting the plants used the starch source.

P499

Beyond Camalexin: The Biosynthesis of Inducible Indolic Compounds in Cruciferous Plants

Stefanie Mucha¹, Alexandra Chapman¹, Dirk Walther², Christoph Böttcher³, Erich Glawischnig¹
¹TU München, Heisenberg research group at the Lehrstuhl für Genetik, Freising, Germany, ²MPI
Molecular Plant Physiology, Bioinformatics, Golm, Germany, ³IPB, Induced Pathogen Defense, Halle,
Germany

Pathogens induce the biosynthesis of tryptophan-derived indole glucosinolates and indolic phytoalexins in Brassicaceae. More than 50 distinct compounds are known, each of them typically restricted to a few species. In *Arabidopsis thaliana*, in addition to the characteristic phytoalexin camalexin, derivatives of indol-3-carbaldehyde (ICHO) and indole-3-carboxylic acid (ICOOH) accumulate after pathogen attack. We have comprehensively analyzed the composition of these metabolites and show that they accumulate in total amounts similar to camalexin. They are synthesised from indole-3-acetonitrile (IAN), which is converted to ICOOH by CYP71B6 and Aldehyde Oxidase 1 (AAO1).

To study an alternative model for indolic phytoalexin biosynthesis we introduced *Thellungiella salsuginea* (= *Eutrema salsugineum*). The major phytoalexins in this species are 1-methoxybrassinin and wasalexin, which are probably synthesised via indole glucosinolates. To identify biosynthetic genes we are following a comparative transcriptomics approach. In addition we have introduced genes of camalexin biosynthesis with the aim to engineer phytoalexin profiles.

P500

Function of the Tandem Genes CYP71A12 and CYP71A13: Establishment of a Double Mutant

Teresa Müller¹, Alexandra Chapman¹, Cornelia Götz¹, Robert Morbitzer², Thomas Lahaye², Erich Glawischnig¹

¹TU München, Heisenberg research group at the Lehrstuhl für Genetik, Freising, Germany,

²Universität Tübingen, ZMBP, Allgemeine Genetik, Tübingen, Germany

Camalexin is the major phytoalexin of *Arabidopsis thaliana*. Its biosynthetic pathway via tryptophan is transcriptionally induced upon pathogen attack and specific abiotic stresses. One important step in this pathway is the conversion of indole-3-acetaldoxime (IAOx) to indole-3-acetonitrile (IAN). It is catalysed by the closely related cytochrome P450 monooxygenases CYP71A12 and CYP71A13, which are localized in tandem. We have characterized their kinetic parameters and their specific product spectra and demonstrated by targeted CoIP that CYP71A13 forms homodimers and interacts with CYP71B15 (PAD3), the last enzyme of the pathway. *Cyp71a12 cyp71a13* double knockout lines were created by using TALE-nucleases. After induction, these plants produce only traces of camalexin in contrast to the corresponding single knockouts.

P501

Bioactive Compounds and Antioxidant Activity of Blueberry Cultivars Produced under Organic Farming in Portugal

Berta Gonçalves, Ana Paula Silva, Alfredo Aires, Sofia Correia, Sónia Ferreira, Eunice Bacelar, Ana Barros

CITAB, Universidade de Trás-os-Montes e Alto Douro, UTAD, Vila Real, Portugal

Blueberries (*Vaccinium corymbosum* L.) are recognized resources of natural antioxidants, such as phenolic compounds and vitamins. However, in Portugal the cultivation of blueberries is still relatively new and there are no studies on the antioxidants in these fruits produced under organic farming. Therefore, in the present study, the phenolic composition, vitamin C and total antioxidant capacity of four blueberry cultivars (Camellia, Duke, Legacy and Palmetto) organic produced in Avanca region, north of Portugal, during 2013 were investigated. Phenolic compounds and vitamin C were determined by HPLC-UV/DAD and antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method. There were significant differences in phenolic composition and vitamin C among cultivars. Camellia cultivar showed the highest concentration of vitamin C, but lower concentration of phenols. On the other hand, Legacy cultivar presented higher content of chlorogenic acid, caffeic acid and delphinidin-3-O-galactoside, Palmetto cultivar had higher content of delphinidin-3-O-glucoside, cyanidin-3-O-galactoside and cyanidin-3-O-glucoside and Duke cultivar higher delphinidin-3-O-arabinoside and cyanidin-3-O-arabinoside contents. Palmetto cultivar had higher content of total phenolic compounds follow by Duke, Legacy and finally Camellia cultivar. This work confirms the richness in phenolics of organic blueberries with potential benefits in human health.

Acknowledgements: This work was supported by Project INNOFOOD - INNOVATION in the FOOD sector through the valorization of food and agro-food by-products - NORTE-07-0124-FEDER-0000029, financed by the North Portugal Regional Operational Programme (ON.2 - O Novo Norte) under the National Strategic Reference Framework (QREN), through FEDER, as well as by PIDDAC through FCT/MEC.

P502

Phenotypic Analysis of Starch Structure in Barley TILLING Mutants

Francesca Sparla¹, Claudia Pirone¹, Giuseppe Falini², Ermelinda Botticella³, Francesco Sestili³,
Valentina Talamè⁴, Riccardo Bovina⁴, Silvio Salvi⁴, Roberto Tuberosa⁴, Paolo Trost¹

¹Department of Pharmacy and Biotechnology, Bologna, Italy, ²Department of Chemistry "G. Ciamician", Bologna, Italy, ³Department of Agriculture, Forestry, Nature and Energy, Viterbo, Italy, ⁴Department of Agricultural Sciences, Bologna, Italy

Starch is the predominant carbohydrate of the barley grain (62 to 77% of dry weight) where it is packed into granules of different size (A-granules with a diameter of 10-40 µm, and B-granules with a diameter smaller than 10 µm), containing amylose and amylopectin in 1:3 ratio.

The targeting-induced local lesions in genomes (TILLING) approach was previously used to identify 29 new allelic variants in genes related to starch metabolism in barley (cv. Morex). Here, 12 lines carrying missense or non-sense mutations in genes known to be expressed in the endosperm during grain filling, BMY1 (beta-amylase 1), GBSSI (Granule-Bound Starch Synthase I), LDA1 (Limit Dextrinase 1), SSI (Starch Synthase I) and SSIIa (Starch Synthase IIa), were analyzed for starch structure and crystalline phases.

As compared to wild type grains, five mutations were found associated to anomalous starch phenotypes: a BMY1 mutant showed higher starch content; a GBSSI mutant had lower starch content and near-waxy phenotype; a LDA1 mutant had higher starch content with more abundant B-type granules; a SSI mutant resulted in higher A:B granule ratio; a nonsense mutation in SSIIa was associated to shrunken seeds with higher amylose:amylopectin ratio and different type of crystalline phases.

P503

Study on the Cross-Talk between the MVA and MEP Pathways in Polyisoprenoid Biosynthesis

Agata Lipko¹, Michel Rohmer², Magdalena Kania³, Witold Danikiewicz³, Ewa Swiezewska¹

¹*Institute of Biochemistry and Biophysics, Warsaw, Poland*, ²*Université de Strasbourg, CNRS, Institut Le Bel, Strasbourg, France*, ³*Institute of Organic Chemistry, Warsaw, Poland*

Isoprenoids are produced by all living organism but they are exceptionally abundant and diverse in plants. Polyisoprenoid alcohols, linear polymers of five-carbon units are divided into two groups depending on their structure : α -unsaturated polyprenols and α -saturated dolichols. Plant polyprenols are found in photosynthetic tissues, wood, seed and flowers while dolichols are present mainly in roots. Both groups are identified in cells as mixtures of prenologues. Despite extensive studies, biological role of polyisoprenoid alcohols has not yet been fully elucidated. Studies on biosynthetic origin of dolichols in plant hairy root culture have suggested the involvement of both, the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways in their synthesis.

To further elucidate the biosynthetic origin of plant polyisoprenoid alcohols in planta experiments have been designed. After feeding of hydroponically grown *Arabidopsis thaliana* plants with deuterium labelled specific precursors of both the MVA or MEP pathways ([3-(²H₃)methyl] mevalonolactone and [5,5-²H₂]-1-deoxy-D-xylulose, respectively), incorporation of deuterium to particular classes of isoprenoids was investigated. Dolichols and polyprenols as well as the main end-products of the MVA pathway (phytosterols) and MEP pathway (chlorophylls and carotenoids) were isolated from the leaves and roots of treated plants and analyzed by GC-MS and LC-MS. Obtained results confirmed that both pathways contribute to biosynthesis of polyisoprenoid alcohols in roots and leaves of *A.thaliana*. These results provided also some insight into the phenomenon of the cross-talk of the MVA and MEP pathways during the biosynthesis of other isoprenoids.

P504

Lipid Remodeling after Heat Stress

Stephanie Müller, Martin Müller, Agnes Fekete
University of Wuerzburg, Wuerzburg, Germany

As sessile organisms plants have evolved the potential to minimize the detrimental effects of high temperatures by triggering the heat shock response directly after heat exposition. The aim of our work is to understand the metabolic reprogramming after heat stress by using ultra performance liquid chromatography coupled to high resolution mass spectrometry. Utilizing metabolite fingerprinting, we identified triacylglycerols (TG) as lipids which accumulate eight- to tenfold at 37° C compared to 22° C. TG accumulation was determined to be dependent on the duration of the treatment and also on the temperature. It could also be shown that the polyunsaturated TGs accumulated extra chloroplastically by rapid remodeling of structured lipids. TG accumulation is not under the control of the master heat shock factors since quadruple mutant deficient in HSF1's displayed normal heat-induced TG accumulation. TG synthesis upon heat seems to be regulated post transcriptionally because a very fast decrease was observed when the plants were transferred back to optimal growth condition after heat treatment. It could also be shown that the TG accumulation is not specific for *Arabidopsis thaliana* but seems to be a general heat response in plants.

P505

Assessing Isoform Specificity of Arabidopsis 14-3-3 Proteins by SPOT Peptide Array

Sabina Visconti¹, Lorenzo Camoni¹, Roberta Pallucca¹, Giovanni Cesareni¹, Simona Panni², Patrizia Aducci¹

¹Department of Biology, University of Rome "Tor Vergata", Rome, Italy, ²Department DiBEST, University of Calabria, Rende, Italy

14-3-3 proteins are a family of dimeric proteins that accomplish a wide range of regulatory roles in eukaryotes through phosphorylation-dependent interaction with a wide range of client proteins. In plants, these proteins regulate primary metabolism, ion transport, cellular trafficking, gene transcription and hormone signaling.

In *Arabidopsis thaliana* thirteen 14-3-3 isoforms are expressed. Based on a phylogenetic analysis, Arabidopsis 14-3-3s can be divided into two major groups, named ϵ and non- ϵ . The relatively large number of 14-3-3 isoforms, as well as the abundance of 14-3-3 target proteins, has raised the issue of functional specificity. It is not clear whether 14-3-3s can accomplish specific functions by binding their targets in an isoform-specific manner.

In order to clarify this issue, we analyzed the interaction of seven Arabidopsis 14-3-3 isoforms, representative of the ϵ and non- ϵ group with known Arabidopsis targets.

To this purpose, we have used the SPOT-synthesis technology to array a number of phosphopeptides matching known or predicted 14-3-3 binding sites present in a number of clients participating to carbon and nitrogen metabolism, or involved in signal transduction pathways. This approach demonstrated isoform specificity in the recognition of several target peptides, suggesting a distinct role of ϵ and non- ϵ group members in the regulation of cellular processes.

P506

Nitrogen Remobilization in *Arabidopsis* and *Brassica napus*

Michaël Moison¹, Mathilde Orsel², Anne Marmagne¹, Fabienne Soulay¹, Marianne Azzopardi¹, Jean-Christophe Avice³, Jérémy Just⁴, Boulos Chaloub⁴, Michèle Reisdorf-Cren¹, Céline Masclaux-Daubresse¹

¹INRA-AgroParisTech, Institut Jean-Pierre Bourgin (IJPB) UMR1318, Saclay Plant Science, 78000 Versailles, France, ²INRA, Institut de Recherche en Horticulture et Semences (IRHS), 42 rue Georges Morel, BP 60057, 49071 Beaucouze Cedex, France, ³INRA, UMR INRA-UCBN 950, Ecophysiologie végétale, Agronomie et Nutrition N, C, S, Normandie Université, Université de Caen Basse-Normandie, 14000 Caen, France, ⁴INRA-CNRS, Unité de Recherche en Génomique Végétale, 2 rue Gaston Crémieux, CP 5708, 91057 Evry Cedex, France

Rapeseed, despite a high nitrogen (N) uptake efficiency, is characterized by a low global nitrogen use efficiency due to a high N loss during shedding of the senescing leaves, main source organ for N remobilization to support grain filling. Glutamine and asparagine are essential for N translocation in phloem saps and enzymes involved in their biosynthesis are of special interest (cytosolic glutamine synthetase encoded by GLN1 genes and asparagine synthetase encoded by ASN genes, respectively). Some of these genes are known to be induced in leaves during senescence and to reassimilate the ammonium released from protein degradation and amino acid catabolism. GS1 isoforms were known to be expressed in different tissues (as for ASN isoforms) and to display different kinetic properties. Furthermore, co-localisations exist between QTL for GS activity and for yield traits in several species showing that GS activity is important for yield in plants. Studies were performed on both *Arabidopsis* and *Brassica napus* by a combination of molecular, physiological and genetic approaches in order to understand the role of GLN1 and ASN genes in N metabolism (Orsel, Moison et al., 2014). ¹⁵N labelling experiments were designed to investigate nitrogen remobilization efficiency in *gln1* and *asn* *Arabidopsis* mutants.

This study helps deciphering the role of each GLN1 and ASN isoform in plant adaptation to low and high nitrate conditions and find solutions to improve yield and nitrogen remobilization efficiency in the crop species like *Brassica napus*.

Orsel, Moison et al. (2014) Journal of Experimental Botany, doi: 10.1093/jxb/eru041

P507

Functional Analysis of AtNPF3.1

Laure C. David, Patrick Berquin, Françoise Daniel-Vedele, Sylvie Ferrario-Méry
Institut Jean-Pierre Bourgin, Unité Mixte de Recherche 1318 INRA-Agro-ParisTech, SPS, Versailles, France

The plant NRT1/PTR FAMILY (NPF) encodes membrane proteins involved in nitrate, peptide and hormone transport. Phylogenetic analysis divides the NPF family in eight clades (Léran et al., 2014). The smallest subfamily contains the cucumber CsNPF3.2, the first plant nitrite transporter that has been described, which is localized at the chloroplast (Sugiura et al., 2007).

To decipher the function of the ortholog AtNPF3.1 in *Arabidopsis thaliana*, we analyzed its expression pattern using transgenic lines GUS expressing under the control of the AtNPF3.1 promoter. Mutants and over-expressors lines have been studied under a wide range of abiotic stresses. We also showed that in contrast to cucumber, AtNPF3.1 protein is located in the plasma membrane. The AtNPF3.1 protein has been characterized as a nitrate/nitrite transporter in vitro (Pike et al., 2014). We then discuss, given these different features, its potential role within the plant.

Léran, S., Varala, K., Boyer, J.-C., Chiurazzi, M., Crawford, N., Daniel-Vedele, F., David, L., Dickstein, R., Fernandez, E., Forde, B., et al. (2014). A unified nomenclature of NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER family members in plants. *Trends Plant Sci.* 19, 5-9.

Pike, S., Gao, F., Kim, M.J., Kim, S.H., Schachtman, D.P., and Gassmann, W. (2014). Members of the NPF3 Transporter Subfamily Encode Pathogen-Inducible Nitrate/Nitrite Transporters in Grapevine and *Arabidopsis*. *Plant Cell Physiol.* 55, 162-170.

Sugiura, M., Georgescu, M.N., and Takahashi, M. (2007). A Nitrite Transporter Associated with Nitrite Uptake by Higher Plant Chloroplasts. *Plant Cell Physiol.* 48, 1022-1035.

P508

Exploiting the Genetic Variation Apparent in Wild Tomato Species to Define Pathway Structure and Metabolic Regulation of Phenylpropanoid Metabolism

Takayuki Tohge¹, Regina Wendenburg¹, Pierre Frasse², Ilse Balbo¹, Marc Lohse¹, Anthony Bolger¹, Patrick Giavalisco¹, Lothar Willmitzer¹, Bjoern Usadel¹, Zoran Nikoloski¹, Mondher Bouzayen², Alisdair R. Fernie¹

¹MPIMP, Potsdam/Golm, Germany, ²Université de Toulouse, Toulouse, France

The increase in quality and productivity on the crop cultivations was offset by the narrowing of crops genetic base which has led to greater susceptibility to environmental stress. However complete genome information for several plant species facilitates another approach for crop breeding design. The approach of metabolomics-assisted breeding is a systematic strategy for introducing high quality traits on the basis on information of natural variance and diversity of metabolite accumulation. In order to perform this strategy, we have to know not only metabolites displaying natural variation but also their underlying biosynthetic pathway and to find the key genes for the productivity of the target metabolites. Tomato represents a useful crop model given its genome is available and its diploid nature renders genetics relatively facile. An LC-MS and GC-MS based global metabolite profiling and microarray analysis were combined to allow comparisons between the relative metabolic levels of leaves and fruits of *S. lycopersicum* and seven wild species tomato that can be crossed with it. Several secondary metabolites such as flavonols and chlorogenic acid accumulated to significantly different levels in the various *Solanum* species and the different tissue types. Based on the result of metabolite profiling, we performed pathway prediction for finding novel genes involved in tomato flavonoid biosynthesis by integration analysis with microarray analysis and publically available RNAseq data. We will discuss similarities of differences between the levels of variance observed between the different metabolite classes for the purpose of metabolomics-assisted breeding.

P509

Diadinoxanthin De-Epoxidation in Thylakoid Membranes of *Pheodactylum tricorutum* Acclimated to Low and Moderate Temperatures

Monika Bojko, Monika Olchawa-Pajor, Malgorzata Jemiola-Rzeminska, Dariusz Latowski, Kazimierz Strzalka

Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

Xanthophyll cycles play important role in photoprotection mechanisms of photoautotrophs such as algae or plants. The photoprotection is connected with enzymatic removal of epoxy groups of xanthophyll pigments under high light conditions. One of common known xanthophyll cycles is a diadinoxanthin cycle in which epoxy group is removed from diadinoxanthin and diatoxanthin is created. This conversion takes place e.g. in diatoms with involvement of the enzyme diadinoxanthin de-epoxidase. In one of the diatoms, *Phaeodactylum tricorutum* (CCAP 1055/1 strain which whole genome was sequenced) three forms of de-epoxidases were identified.

The de-epoxidation reaction of diadinoxanthin cycle in thylakoid membranes isolated from *Ph. tricorutum* CCAP/1055/1 strain adapted to different growth temperatures has been studied.

The diadinoxanthin de-epoxidation was measured in thylakoid membrane isolated from *Ph. tricorutum* adapted to 12 and 20°C growth temperature. The reaction was initiated by the addition of sodium ascorbate and carried out at 12 and 20°C. The level of xanthophyll pigments (diadinoxanthin as substrate and diatoxanthin as products) was analyzed HPLC chromatography.

The analysis of the kinetics of diadinoxanthin de-epoxidation measured in isolated thylakoids shows that adaptation and growth temperatures of *Ph. tricorutum* had effects on reaction velocity. In chloroplast isolated from diatoms adapted to 12°C the rate of reaction was 20 to 30% higher than that in chloroplast isolated from *Ph. tricorutum* adapted to 20°C. However, the significant effect of *Ph. tricorutum* growth temperature on $Dtx/(Dtx+Ddx)$ [%] ratio in isolated thylakoid membranes was not observed.

This work was supported by project No. 2011/01/M/NZ1/01170

P510

Isolation and Functional Analysis of Cytochrome P450 SIP450-03 Gene from Tomato

Ji Sun Park, Ah Young Kim, Se Hee Park, Hyun Min Kim, Sang Hoon Ma, Seo Young Park, Mi Jin Jeon, Young Hee Joung*
Chonnam National University, Gwangju, Republic of Korea

Cytochrome P450 superfamily is a group of enzymes that catalyze the oxidation of organic substance. In plants, cytochrome P450 enzymes have diverse function such as synthesis or catabolism of hormone or signaling molecules and synthesis of structural macromolecules or pigment and defense compounds. There are 457 cytochrome P450 gene (including pseudogenes) in the tomato genome. The SIP450-03 gene was isolated from tomato (*Solanum lycopersicum* cv. Micro-Tom) using by RT-PCR. The SIP450-03 encoded sterol demethylase which related to sitosterol and brassinosteroids biosynthesis. The SIP450-03 cDNA was cloned into plant expression vector and controlled with CaMV35S promoter. The SIP450-03 over-expressed construct was transformed into tobacco (*Nicotiana tabacum* cv. Xanthi NC) and tomato. The transformation rate of SIP450-03 gene was very low in shoot induction media without ABA, while transformation rate was increased in ABA contained media. This result suggest that SIP450-03 enzyme is involved in active sterols synthesis in the transgenic plants and it might affect ABA sensitivity. To characterize function, the SIP450-03 gene was cloned into pCW vector for E.coli expression. The SIP450-03 enzyme was purified from E.coli and analyzed their function.

Key words: cytochrome P450, Sterol demethylase, transgenic tomato

*Corresponding author: Tel 82-62-530-5202, e-mail: yhjyoung@jnu.ac.kr

P511

Identification and Characterization of the Hot Pepper (*Capsicum annuum*) NADPH-Cytochrome P450 Reductase Genes

Hyun min Kim, Ga-Young Lee, Ah Young Kim, Se Hee Park, Sang Hoon Ma, Seo Young Park, Ji Sun Park, Mi Jin Jeon, Chul-Ho Yun, Young Hee Joung*
Chonnam National University, Gwang-ju, Republic of Korea

Plant NADPH-P450 reductase (CPR) is membrane protein play important function which transfer the electron to diverse plant P450s. We isolated two CPR genes (CaCPR1, CaCPR2) from cDNAs of hot pepper (*Capsicum annuum* L. cv. Bukang). The expression levels of CaCPRs mRNA in various hot pepper tissues were determined by quantitative PCR analysis. All transcripts of CaCPR genes were detected in leaves, flower and fruit tissues. The expression level of CaCPR1 was higher than CaCPR2 from all tested tissues and it was gradually increased during fruit ripening. To investigate the enzymatic properties, the CaCPR genes was heterologously expressed and purified in *Escherichia coli*. The enzymatic properties of CaCPR1 were confirmed by measurement of characteristic absorption spectrum and catalytic activities, which were assessed using protein and chemical substrates including P450, cytochrome c, cytochrome b₅, MTT, and CTC. In particular, CaCPR1 could support abscisic acid 8'-hydroxylation of purified plant CYP707A70 (ABA 8'-hydroxylase) better than rat CPR and FDX/FDR. These results reveal that the CaCPR1 is a major CPR in most of the tissues in hot pepper and that the overall enzymatic properties of CaCPR1 are quite similar to those of other typical CPR enzymes from other sources including mammalian tissues.

Keywords: Plant NADPH-cytochrome P450 reductase, heterologous expression, reduction

*Corresponding author: Young Hee Joung (Tel 82-62-530-5202, e-mail: yhjoung@jnu.ac.kr)

P512

Isolation and Functional Analysis of the Cytochrome P450-19 (SIP450-19) Gene from Tomato

Seo Young Park, Ah Young Kim, Se Hee Park, Sang Hoon Ma, Hyun Min Kim, Ji Sun Park, Mi Jin Jeon, Young Hee Joung*

Chonnam National University, Gwangju, Republic of Korea

Cytochrome P450 enzyme is large and diverse protein family that catalyze the oxidation of organic substrate. Plants have thousands of cytochrome P450 which are catalyzed various biochemical reactions, such as hormones, defense related compounds and secondary metabolites synthesis. In case of tomato, there are 457 cytochrome P450 genes include pseudogenes. The SIP450-19 gene was isolated from tomato (*Solanum lycopersicum* cv. Micro-Tom) cDNA. The SIP450-19 plays a role of ferulate 5-hydroxylase (F5H) that is involved in phenylpropanoid pathway and related to lignin synthesis. The SIP450-19 cDNA was cloned into plant expression vector and controlled with 35S promoter. The over-expressed construct was introduced into tobacco (*Nicotiana tabacum* cv. Xanthi-nc) and tomato (*Solanum lycopersicum* cv. Micro-Tom) using *Agrobacterium*-mediated transformation. The SIP450-19 over-expressed transgenic tobaccos showed higher level of lignin contents compared to wild type tobacco. To investigate function of the SIP450-19, the SIP450-19 was cloned into pCW vector and expressed in *E. coli*. The *E. coli* expressed SIP450-19 was purified and enzymetic properties were confirmed at in vitro.

Keywords: cytochrome P450, Ferulate 5-hydroxylase, transgenic tobacco

*Corresponding author: Young Hee Joung (Tel 82-62-530-5202, e-mail: yhjoung@jnu.ac.kr)

P513

Phytyl Epoxide Affects Accumulation of Phytosterols and Polyisoprenoids in the Hairy Roots of *Arabidopsis thaliana*

Karolina Skorupinska-Tudek¹, Tomasz Rowicki², Ewa Swiezewska¹

¹*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland,* ²*Warsaw University of Technology, Warsaw, Poland*

Dolichols and phytosterols are common constituents of plant tissues. Despite the knowledge on the subsequent steps of their biosynthetic pathways the mechanisms responsible for their regulation remain unknown. Interestingly, modulatory effect of epoxidated isoprenoids on cholesterol and ubiquinone biosynthetic pathways has earlier been reported in mammalian cells.

In order to study the influence of isoprenoid epoxides on phytosterols and dolichol accumulation in vitro hairy roots of *Arabidopsis thaliana* were cultivated in the Murashige and Skoog liquid medium supplemented with different concentrations (20µM up to 2mM) of phytyl epoxide.

Results of the estimations have shown both the qualitative and quantitative changes in the profiles of accumulated lipids.

The HPLC/UV studies revealed that in the presence of 20µM and 200µM phytyl epoxide the additional family of longer dolichols was accumulated (Dol-19 to Dol-23 with Dol-20 dominating) in contrast to control condition when only one-family mixture of dolichols with Dol-15 dominating was found.

Dolichol accumulation was highly induced in the presence of phytyl epoxide and reached 790% of the control for 2mM phytyl epoxide.

The GC-FID analysis showed that hairy roots of *A. thaliana* accumulated phytosterols i.e. campesterol, stigmasterol, β-sitosterol and traces of brassicasterol. The highest sterol accumulation (318% of the control) was noted for 20µM phytyl epoxide treatment whereas 1mM and 2mM phytyl epoxide resulted in the decrease of the sterol content (25% and 4% of the control, respectively).

These results indicate that phytyl epoxide might influence the metabolic pathways leading to the formation of phytosterols and dolichols in plants.

P514

The Role of Cell Wall Invertase in Inhibition of Photoassimilate Translocation and Re-Arrangement of Carbon Metabolism under Nitrate Nutrition

Svetlana Batasheva, Golsoyar Bakirova, Guzel Akhtyamova, Larisa Khamidullina, Vladimir Chikov
Kazan Institute of Biochemistry and Biophysics, Kazan, Russia

We investigated the influence of nitrate fertilization on photosynthesis, carbon metabolism and photoassimilate translocation in tomato plants, in which the expression of cell wall invertases Lin6 and Lin8 in source leaves was suppressed by RNA-interference (Lin8RNAi-plants). In 15 h after the fertilization of tomato plants with KNO₃ solution an attached leaflet was exposed to ¹⁴CO₂ for 2 minutes, and in 6h after the exposure the allocation of ¹⁴C-assimilates along the plant was investigated. Control wild type and transformed plants almost did not differ in intensity of ¹⁴CO₂ assimilation, partitioning of ¹⁴C among photosynthetic products and allocation of ¹⁴C along the plant, but fertilization of plants with KNO₃ revealed great differences between them. In wild type plants nitrates inhibited ¹⁴C-assimilate export from the ¹⁴C-leaflet. In Lin8RNAi-plants there was no prominent inhibition of ¹⁴C-assimilate export from the ¹⁴C-leaflet, but nitrates relatively increased ¹⁴C-assimilate translocation upwards and accumulation of ¹⁴C in all leaves. Under nitrate nutrition in both plant types there was an increase in ¹⁴C distribution to amino and organic acids, but Lin8RNAi-plants did not respond to nitrates with a drastic decrease of ¹⁴C incorporation into sugars and the sucrose/hexoses ratio as wild type plants did. Thus, the activity of cell wall invertase is necessary for enhanced sucrose hydrolysis in the apoplast and inhibition of sucrose loading to phloem in leaves under nitrate nutrition. However, the initial target of nitrate is probably assimilate translocation along the phloem rather than cell wall invertase activity.

The work was partially supported by RFFI grant 14-04-31606.

P515

The Role of Plasma Membrane Proton Pump AHA2 in the Regulation of Growth and Development of Roots in *Arabidopsis thaliana* Induced by Nitrogen Signal

Ewa Młodzinska

Institute of Experimental Biology, Wrocław, 50-328, Poland

The aim of presented study is the molecular and physiological characterization of the one isoform of the plasma membrane proton pumps (AHA2), and its role in nitrogen-dependent growth and development of roots of *Arabidopsis thaliana*. It is known that roots have developmental responses towards availability of different mineral nutrients, which include processes such as initiation of lateral roots primordia, their elongation and increasing of the root biomass. However, the signal transduction mechanism, which enables roots to sense changes in different mineral environment and match their growth and development patterns to actual conditions in soil, is still unknown. Most recent comments focused on nitrogen and its role in the modification of the root architecture of the *Arabidopsis thaliana*. As yet, not all elements of the signal transduction pathway leading to the perception of the nitrate stimulus are known, leading to anatomical changes of root, which help plant roots to optimize exploration of the soil. The results of this study suggest that the plasma membrane proton pump AHA2 is a component of the nitrate signaling cascade and participates in the regulation of root growth and development in response to variable nitrogen source. Our data indicate that primary and lateral root length were considerably decreased in *aha2* lines than in WT. Moreover, using a genetically encoded pH biosensor, the differences in the apoplastic pH of root apical zone in seedlings exposed to different nitrogen source have been revealed.

P516

Engineering of Rosmarinic Acid and Icetexanes Diterpenoids Biosynthetic Pathways in *Salvia* Species

Laura Bassolino, Emanuela Giacomelli, Annalisa Giovannini, Antonio Mercuri, Barbara Ruffoni
CRA-FSO, Sanremo, Italy

Plant exudates, derived from the secretory structures of *Salvia* species, are a valuable source of biologically active metabolites such as polyphenols and diterpenoids that have been widely adopted in traditional medicine due to their diverse phytochemicals properties. Rosmarinic acid (RA) is a polyphenolic acid well-known for its antioxidant and anti-inflammatory properties. Icetexanes, belonging to diterpenoids family, act as inhibitors of microbial biofilms and cancer chemopreventive. This study aims to increase the relative concentration of these compounds *in planta* through a molecular farming approach. We set up *in vivo* and *in vitro* propagation protocols for *S. dolomitica* and *S. corrugata* to enhance biomass yield. Two genes, the *Hydroxyphenylpyruvate reductase* (SoHPPR) and the *Kaurene synthase like 1* (SmKSL1), encoding for putative enzymes responsible for the RA and diterpenoids synthesis, have been cloned into the pK7FWG2 binary vector and overexpressed in different *Salvia spp.* as well as in *N. tabacum*, through *A. tumefaciens* mediated transformation. *N. tabacum* has been included due to its easy *in vitro* management and high transformation performances. Further, transformed *Salvia* plants will be assessed qualitatively and quantitatively for their RA and diterpenoids content. It will be also interesting to test whether the regenerated *N. tabacum* plants overexpressing the SoHPPR gene are able to synthesize rosmarinic acid.

P517

Metabolism of Plant Antioxidants under Stress Conditions

Galina Tchoupakhina, Lubov Skrypnik, Pavel Maslennikov, Raisa Poltavskaya, Pavel Feduraev
Immanuel Kant Baltic Federal University, Kaliningrad, Russia

Redox status is one important biophysical parameter characterizing the functional state of plant cell. This status sensitively responds to environmental change. There is a balance between oxidation and reduction processes under normal physiological conditions. However under the influence of unfavourable factors intensification of reactive oxygen species (ROS) accumulation is observed. By now it is exactly known that ROS play key roles in the regulation of the most processes in plant life, but high ROS level causes cells damage. So the ROS content in plant cell is regulated by enzymatic and non-enzymatic antioxidants. Therefore, antioxidant metabolism in plants closely depends on environmental factors.

A study on the antioxidant metabolism in plants affected by various abiotic factors was conducted at the Laboratory of Natural Antioxidants (Immanuel Kant Baltic Federal University, Kaliningrad, Russia). Particular attention is paid to the study of ascorbic acid system, bioflavonoids, glutathione. In laboratory experiments the effect of monochromatic and polychromatic different intensity light, low positive temperatures, heavy metals, deficiency of macro-, and micronutrients on the biosynthesis of ascorbic acid, anthocyanin, catechin, glutathione, carotenoids was observed. Furthermore, according to the results of work a bioindication express method was designed, in which the level of anthocyanins in plants can be considered as a test for contamination by various pollutants.

Long-term field studies have revealed regularities of antioxidant accumulation in Baltic region's plants. In addition, in this research plants with high antioxidants were identified. These plants can be used as raw material for the biotechnological industries.

P518

Regulation of Wheat Seedlings Proteome and Phosphoproteome by 24-Epibrassinolide and 6-Benzylaminopurine: A Comparative Analysis

Ruslan Yuldashev¹, Azamat Avalbaev¹, Kristina Ivanova¹, Regina Gilmanova², Natalia Petrova², Evgenia Fedina², Fatima Karimova², Farida Shakirova¹

¹*Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences, Ufa, Russia,* ²*Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Centre, Kazan, Russia*

Earlier we have received priority data about 24-epibrassinolide-induced fast and stable 2-fold accumulation of cytokinins (CK) in wheat seedlings. These results indicate involvement of 24-epibrassinolide (EBR) in the regulation of CK metabolism in wheat plants and suggest the important role of endogenous CK in the manifestation of physiological action of EBR on seedlings.

It is well-known that both groups of hormones have a pronounced growth-promoting effect based on their ability to regulate plant genome, transcriptome and, finally, proteome. The aim of this study was comparative proteomic analysis of response to EBR and 6-benzylaminopurine (BAP) in wheat seedlings. There was investigated the action of EBR and BAP on synthesis of proteins and their level of tyrosine phosphorylation. Then the most responsive to these hormones proteins were identified by MALDI-TOF MS.

We have revealed that EBR and BAP treatment in general caused similar proteome changes in wheat seedlings. Both hormones also equally promoted the level of protein tyrosine phosphorylation. Using MALDI-TOF MS there were identified about 20 proteins and phosphotyrosine polypeptides equally responsive to EBR and BAP treatment, some of which are involved in key physiological processes such as photosynthesis, plant growth and development.

Thus the obtained data supports an important role of endogenous cytokinins like a hormonal intermediates in the manifestation of physiological action of EBR on wheat seedlings.

This work is supported by Grant № 14-04-00731_a of the Russian Foundation for Basic Research.

P519

Nitrate Reductase, Glutamine Synthetase and Glutamate Dehydrogenase Activity in Bromeliad *Alcantarea imperialis* (Carrière) Harms Cultivated *In Vitro* in Different Nitrogen Sources

Flavia Maria Kazue Kurita, Vívian Tamaki
Instituto de Botânica, SMA, São Paulo, SP, Brazil

Alcantarea imperialis is a bromeliad used in landscaping and is endemic of the Serra dos Orgãos/RJ/BR, so conservation measures are necessary, and *in vitro* culture can be a tool. An important aspect of this technique is the mineral supply of the culture medium. Nitrogen (N) is the main component of amino acids, nucleic acids, chlorophyll and coenzymes. This can be found in two forms, nitrate (NO_3^-) and ammonium (NH_4^+). There are no studies for this plant related to activity of nitrogen enzymes and different nitrogen source. This study aimed to evaluate the activity of nitrate reductase (NR), glutamine synthetase (GS) and glutamate dehydrogenase (GDH) in plants of *A. imperialis* cultured *in vitro* with different nitrogen sources. Plantlets were deposited in *in vitro* treatments with Murashige and Skoog medium, with different nitrogen sources (NO_3^- and/or NH_4^+) with different concentrations (5, 15, 30 and 60 mM). After six months, the analysis of nitrogen metabolism enzymes (NR, GS and GDH) and biometric parameters were made. The results showed that when combining the two nitrogen sources at a concentration of 30 mM, the activities of NR and GS are higher, corroborating with biometrics results. GDH activities were higher in 60 mM NO_3^- , showing that the source can influence the response of the plant. In conclusion the activity of NR and GS in leaves of *A. imperialis* cultured *in vitro*, is greater with 30 mM of $\text{NO}_3^-/\text{NH}_4^+$ after six months.

P520

A Lower Root Surface pH as a Possible Adaptive Response to Sulfur Deficiency in Brassica

Martin Reich, Marten Staal, J. Theo M. Elzenga, Luit J. De Kok
University of Groningen, Groningen, The Netherlands

Active acidification of the rhizosphere by plant roots is well documented during e.g. phosphate (2) and iron deficiency (3) and is considered as an adaptive response to increase the solubility of these immobile nutrients. But also in the case of the very mobile nitrate, deficiency leads to an instant acidification of the rhizosphere (4) probably because the alkalizing effect of both nitrate uptake and assimilation present under optimal supply diminishes under deficiency and proton extrusion becomes dominant.

Sulfate is taken up by plant roots cells via sulfate/proton symporters and the efficiency of the uptake is strongly dependent on the external pH (5). By using the non-invasive Microelectrode Ion Flux Estimation method (MIFE) it was observed, that sulfate-deficient seedlings of Chinese cabbage not only have a higher sulfate/proton symport activity but also consistently exhibited a lower root surface pH compared with seedlings grown under sufficient sulfur supply. This is indicating an adaptive response of plant roots to sulfate deficiency in means of increasing the proton motive force over the root plasma membrane and thereby making sulfate/proton symport more efficient.

- (1) Hawkesford, M.J. and De Kok, L.J. (2006) *Plant Cell Environ.* 29: 382.
- (2) Yan, F. et al. (2002) *Plant Physiol.* 129: 50.
- (3) de Vos, C. R. et al. (1986) *Plant Physiol.* 81: 842-846.
- (4) Moorby, H. et al. (1985) *Plant Soil.* 84: 403-415.
- (5) Hawkesford, M. J. et al. (1993) *Planta.* 190: 297-304.

P521

Molecular Characterization of Genes Involving in the Methylerythritol 4-phosphate Pathway and the Prenyltransferase Steps in Rice

Min Kyoung You¹, Sun-Hyung Lim², Sun-Hwa Ha¹

¹Graduate School of Biotechnology and Crop Biotech Institute, Yongin, Republic of Korea, ²National Academy of Agricultural Science, Suwon, Republic of Korea

Among two pathways of the cytosolic mevalonate (MVA) pathway and the plastidial methylerythritol 4-phosphate (MEP) pathway for isoprenoid synthesis, the MEP pathway is composed of eight essential enzymatic steps by DXS, DXR, IspD, IspE, IspF, IspG, IspH and IPI to synthesize isoprenoids, which play a role as building blocks for the biosynthesis of diverse isoprenoid compounds from pyruvate and D-glyceraldehyde 3-phosphate. The synthesized isoprenoids are condensed by prenyltransferase, GPP-, FPP- and GGPP synthases, to produce the key branchpoint intermediates of cytosolic and plastid isoprenoids, monoterpene (geranyl diphosphate, GPP), diterpene (geranylgeranyl diphosphate, GGPP), sesquiterpene (farnesyl diphosphate, FPP), respectively. Since there has been only a few reports about essential component genes related to those pathways in rice, 10 genes of MEP pathway and 8 genes of prenyltransferase were identified based on GenBank database and the expression patterns on various developmental stages and rice organs, and the subcellular localization of those genes were characterized. Also, the useful transit peptides for plastid targeting were developed and several essential components for expression efficiency were analyzed and selected using flow cytometric analyses of protoplasts expressing fluorescent proteins. These useful informations were taken together to be applied for developing the efficient and unique strategies of metabolic engineering in rice. Additionally, the methodology of a flow cytometric analyses of protoplasts was improved as a useful tool system for comparative analysis in this study.

P522

Housekeeping Gene Selection for Expression Level Normalization in the Medicinal Plant *Catharanthus roseus*

Teresa Martínez-Cortés¹, Inês Carqueijeiro^{1,2}, Fernanda Niño^{1,3}, Sara Bettencourt^{1,2}, Jacob Poiller⁴, Alain Goossens⁴, Mariana Sottomayor^{1,2}

¹IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Rua do Campo Alegre, 823, 4150-180, Porto, Portugal, ²Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007, Porto, Portugal, ³ICBAS – Instituto de Ciências Biológicas Abel Salazar, Universidade do Porto, Rua do Campo Alegre, 823, 4150-180, Porto, Portugal, ⁴Department of Plant Systems Biology, Flanders Institute for Biotechnology (VIB) and Department of Plant Biotechnology and Genetics, Ghent University, Ghent, Belgium

The leaves of *Catharanthus roseus* accumulate in very low levels the terpenoid indole alkaloids (TIAs) vinblastine and vincristine, widely used in anticancer treatments, what has made of this plant an object of intensive study for decades. The TIA pathway is highly complex, involving more than 30 biosynthetic steps and several subcellular compartments and cell types, with intense research under way to uncover the full pathway, its regulation and compartmentation mechanisms. Previously, in a differential transcriptomic analysis by cDNA-AFLP, we observed that the housekeeping gene commonly used for *C. roseus*, the 40S ribosomal protein S9 (RPS9), was not equally expressed in all the cell types tested. Therefore, the cDNA-AFLP profiles were screened for the presence of better housekeeping genes, and 21 tags with similar expression levels throughout the different organs/cell types were initially selected. This selection was narrowed to three genes by screening of the respective expression levels at the Medicinal Plants Genomic Resource: the 40S ribosomal protein S28 (RSP28), the 60S ribosomal protein L24 (RSPL24) and the ADP-ribosylation factor 2 (ADP2). Quantitative RT-PCR further confirmed that the three genes were all very similarly expressed across several *C. roseus* organs and cell types, even for the ones showing a very different expression of RSP9. Overall, these results indicate that *C. roseus* RSP28, RSPL24 and ADP2 may be used as robust reference genes, providing an important tool to be used in future work involving expression level normalization, e.g. RNA-seq and qPCR, namely for the characterization of the valuable *C. roseus* TIA pathway.

P523

Identification and Analysis of Plant Thiamine/TPP Transporters

Bandar Aljuaid, Christine Raines
Essex University, Colchester, UK

Thiamine pyrophosphate (TPP or ThDP) serves as a cofactor in universal metabolic pathways including glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle, and is essential for the proper functioning of all organisms. Recently, several steps of plant thiamine biosynthetic pathway have been characterized, and a mechanism of feedback regulation of thiamine biosynthesis via riboswitch has been unraveled. In plant, thiamine is made in the chloroplasts and then transferred to the cytosol to form the active form of thiamine, thiamine pyrophosphate (TPP). The mitochondria and chloroplasts must import thiamine pyrophosphate (TPP) from the cytosol because both organelles contain TPP-dependent enzymes. In Arabidopsis, two members of the mitochondrial carrier family (MCF), AtTpc1 and AtTpc2, export TPP to the mitochondria, but chloroplast TPP carrier is still unknown. This project is going to identify thiamine chloroplast transporter(s) and investigate mitochondrial thiamine transporters. As a result, expression of these mitochondrial TPP transporter genes are similar in Arabidopsis leaves when they are treated with TPP. In addition, there are chloroplast transporters that have homology with mitochondrial TPP transporters and it could be a chloroplast thiamine transporter.

P524

Participation of the *Medicago truncatula* Seed-Specific Glutamine Synthetase (MtGS2b) in Storage Protein Synthesis

Liliana Silva^{1,2}, Ana Seabra¹, Helena Carvalho¹

¹Institute for Molecular and Cell Biology, Porto, Portugal, ²Department of Biology, Faculty of Sciences, University of Porto, Porto, Portugal

Grain legumes represent an important source of plant proteins for feed and food. Protein accumulation in legume seeds is strictly related to N remobilization, a process wherein glutamine synthetase (GS) plays a crucial role. We have previously shown that the model legume *M. truncatula* contains a second gene encoding a plastid located GS (MtGS2b), which is specifically expressed in the seeds (Seabra et al, 2010 BMC Plant Biol, 10:183). To investigate the physiological function of this gene, we characterized a Tnt1 retrotransposon mtgs2b insertion line (NF16182) obtained from the *M. truncatula* mutant collection (Tadege et al, 2008, Plant J 24:335-347).

Molecular and genetic analysis revealed that the retrotransposon is inserted in exon 12 and that the Tnt1 homozygous mutant plants are knocked-out for MtGS2b protein expression. A careful phenotypic analysis was performed during seed formation, but no major morphological or developmental changes between the mtgs2b knockouts and the wild type plants were detected. Also, seed germination does not seem to be affected by the absence of the seed specific GS2 isoenzyme. However, an analysis of the seed proteome revealed the absence of a protein of approximately 50 kDa, identified by MALDI-TOF as a legume seed storage protein legumin, in the mtgs2b mutant. These results suggest a specific involvement of MtGS2b in the synthesis of this legume seed storage protein. Further work is underway to understand the involvement of MtGS2b in the N-metabolic pathways underlying protein storage production in *M. truncatula* seeds.

P525

Diurnal Rhythm Activity of Glutamine Synthetase and Glutamate Dehydrogenase in Bromeliad *Alcantarea imperialis* (Carrière) Harms Cultivated *In Vitro* at Different Concentrations of Nitrate

Flávia Maria Kazue Kurita, Vívian Tamaki
Instituto de Botânica/SMA, São Paulo, SP, Brazil

Alcantarea imperialis is a bromeliad widely used in landscaping. It is endemic of the Serra dos Orgãos/RJ/BR. Despite the relevance of this species, there are no studies with this plant and on the activity of the enzyme glutamine synthetase (GS) and glutamate dehydrogenase (GDH). These enzymes are indicators of the metabolism of nitrogen (N) in plants in the presence of nitrate (NO_3^-), which is a major N source available to plants in the soil. This study aimed to evaluate the diurnal and nocturnal activity of GS and GDH in plants of *A. imperialis* cultured in vitro with different concentrations of NO_3^- . In vitro germinated seedlings were transferred to a culture medium with different concentrations of NO_3^- ranged from 5, 15, 30 and 60 mM N. The samples were taken every four hours for 24 hours after the transfer, which began at 10 am, and photoperiod of 12 hours (beginning at 5 am and end at 17h). The results showed that the increase in the concentration of nitrate has a negative influence in the enzymatic activity of GS, and the presence of light act positively on the diurnal activity of the enzyme GS. The GDH enzyme's major activity is in concentration of 15 mM, and the light has no influence on the enzymatic activity.

P526

The Stability of Prolamellar Bodies of Etiolated *Triticum aestivum* Seedlings Studied under Various *In Vitro* Conditions

Beata Mysliwa-Kurdziel¹, Michal Gabruk¹, Pawel Jedynak¹, Joanna M. Grzyb², Sylwia Michlewska³, Slawa Glinska³, Przemyslaw Malec¹

¹Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland,

²Laboratory of Biological Physics, Institute of Physics PAS, Warszawa, Poland, ³Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland

Prolamellar body (PLB) is a paracrystalline structure characteristic for etioplasts of dark-grown angiosperm seedlings, mainly consisting of galactolipids: MGDG and DGDG. Light-dependent protochlorophyllide oxidoreductase (EC1.3.1.33) is the major protein in PLB, mainly in the form of Pchl_{id}:POR:NADPH complexes. Illumination triggers protochlorophyllide photoreduction to chlorophyllide, and further chlorophyll biosynthesis. It also induce transformation of PLB into thylakoid membranes.

We have investigated the stability of PLBs isolated from etiolated wheat (*T. aestivum*) seedlings under different *in vitro* condition by using electron microscopy. Fluorescence spectra were examined at 77 K as the control of the photoactivity of Pchl_{id}:POR:NADPH complexes as well as their aggregation state. Incubation of PLBs in a sugar- and glycerol-free buffer with monovalent (Na⁺, K⁺) or divalent (Mg²⁺, Ca²⁺) cations and detergents (octyl glucoside) resulted in a blue-shift of the main fluorescence band originating from photoactive Pchl_{id}:POR:NADPH complexes, and in a loss of ability to light-triggered chlorophyllide formation. Under continuous PLBs illumination, chlorophyllide was formed, however, a pronounced bleaching of the sample was observed. By using SOSG (singlet oxygen sensor green®), we could detected the appearance of single oxygen, however, an increase of SOSG signal did not directly followed the disappearance of chlorophyllide and protochlorophyllide fluorescence. Alterations of PLB structure were observed under the applied conditions. Biophysical properties of PLBs were further characterised using atomic force microscopy (AFM) as well as by EPR spectroscopy, with using of PLBs labelled with SASL probes.

Acknowledgements: This work was supported by grant 2011/01/B/NZ1/00119 from the National Center of Science of Poland (NCN).

P527

Hormonal and Genetic Regulation of Flavonoid Biosynthesis in Bilberry (*Vaccinium myrtillus* L.) Fruits

Katja Karppinen¹, Elina Hirvelä¹, Tiina Nevala¹, Marko Suokas¹, Nina Sipari², Laura Jaakola³, Hely Häggman¹

¹Department of Biology, University of Oulu, Oulu, Finland, ²Metabolomics Unit, Department of Biosciences, University of Helsinki, Helsinki, Finland, ³Climate lab/Holt, Department of Arctic and Marine Biology, University of Tromsø, Tromsø, Norway

Development and ripening of fruits, stimulated by developmental and hormonal signals, include major metabolic and structural changes such as accumulation of secondary metabolites including pigments and flavours. Bilberry (*Vaccinium myrtillus* L.), also known as European blueberry, is one of the most abundant and economically important wild berry species in boreal forests of the Northern Europe. Bilberries are one of the richest natural sources of health-beneficial flavonoids. Especially anthocyanin pigments are accumulating during fruit ripening. Recent studies have implicated that plant hormone abscisic acid (ABA) has a key regulatory role in the triggering ripening-related processes, including anthocyanin biosynthesis, in non-climacteric fruits but the regulatory mechanisms are not yet understood.

Our aim is to investigate the regulation mechanisms and role of ABA in ripening-related flavonoid biosynthesis in non-climacteric bilberry fruits. The obtained results (Karppinen et al. 2013) have shown that ABA is biosynthesized highly at the onset of bilberry fruit ripening, at the stage when anthocyanin biosynthesis also highly increases. Our results imply that ABA plays an important role in the regulation of ripening in bilberry fruits through transcriptional regulation of key ABA biosynthetic genes. We have currently in progress transcriptome sequencing of different stages of bilberry fruit development to comprehensively study regulation of fruit ripening and flavonoid biosynthesis. Special interest with the transcriptome databases will be the identification of new ripening related regulators.

Karppinen, K. et al. 2013. Phytochemistry 95: 127-134.

P528

Taxonomical Identification of *Fusarium* spp. Caused Cotton Wilt from Different Regions of Uzbekistan Using of Morphological and Molecular Approaches

Lyudmila Glukhova¹, Abdusaid Sheraliev¹, Sharof Egamberdiev², Ilkhom Salakhutdinov², Anvar Sherimbetov¹, Makhmuda Rachbarova¹

¹*Institute of Genetics and Plant Experimental Biology, Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan,* ²*The Center of Genomics and Bioinformatics, Academy of Sciences of Uzbekistan, Ministry of Agriculture & Water Resources of Uzbekistan, and 'Uzpakhtasanoat' Association, Tashkent, Uzbekistan*

Fusarium wilt is one of the most economically damaging cotton diseases worldwide. For reduction of cotton yield losses, new approaches are vital to diagnosis of these pathogens. With this purpose 120 samples of *F. spp.* sampled from different regions of Uzbekistan were studied. Primary taxonomical identification was carried out using modern identification guides, such as Leslie, J.F and Summerell, B.A. (2006) etc. Macromorphological characters of samples were determined on standard PDA medium. Micromorphological differences and topography colonies were carried out on Synthetic Nutrient Agar, SNA. However, it is well-known, that cultures of fungi usually are not stable also the major taxonomical signs cannot be always clearly defined. In this connection, we used the most exact molecular-genetic methods of identification. Analysis of partial sequences of single-copy and high conservative genes such as BT, EF, NIR and rDNA was provided on phytopathogen samples isolated from plants, seeds and soil different part of Uzbekistan (Tashkent, Bukhara, Surkhan-Darya, Namangan, Syr-Darya, Kashkadarya and Andizhan). In results different representatives of *Fusarium* spp. were identified: *F. oxysporum* f.sp. *vasinfectum* (FOV) ; *F. proliferatum* (Tel. *Gibberella intermedia*), *F. equiseti*, *F. solani* (Syn. *Nectria haematococca*), *F. verticillioides* (Syn. *F. moniliforme*), *F. sporotrichioides*, *F. fujikuroi*, *F. semitectum*, *F. lateritium*. Additionally, for some FOV isolates was determined race membership: Race 3 is determined in Tashkent, Syr-Darya, Andizhan and race 4 is determined in Kashkadarya and Bukhara regions. This information will be useful to pathogen management in cotton producing countries to planting of the conforming resistant varieties or taking of adequate measure against *Fusarium* wilt.

P529

First Observations on the Antiviral Properties of *Trichoderma harzianum* Strain T-22 in Tomato Plants

Antonella Vitti¹, Esther La Monaca², Antonio Scopa¹, Ann Cuypers², Maria Nuzzaci¹, Adriano Sofo¹
¹University of Basilicata, Potenza, Basilicata, Italy, ²University of Hasselt, Hasselt, Limburg, Belgium

The study of the biochemical and molecular mechanisms involved in the host-pathogen-antagonist interaction is essential to understand the dynamics of the infectious processes and can be useful for the development of new strategies to control phytopathogens, particularly viruses, against which chemical treatments have no effect. In this work, we demonstrate for the first time the antiviral activity of the rizospheric fungus *Trichoderma harzianum* strain T-22 (T22) against Cucumber mosaic virus (CMV) strain Fny. The molecular and biochemical aspects of the interaction between strain T22 and the tomato plant against CMV are discussed. A particular emphasis has been given on the substances and genes implicated in the plant defense pathways, such as reactive oxygen species (ROS), genes encoding antioxidant enzymes (Cu/Zn-SOD, Mn-SOD, CAT1, CAT2 and APX), and phytohormones responsible for mediating defense responses (salicylic and jasmonic acids). Interestingly, histochemical and morphological analysis revealed an increase in $O_2^{\cdot -}$ and H_2O_2 levels in all the leaf of the plants infected by CMV, indicating the involvement of ROS in plant defense responses. Gene expression analysis (q-PCR) pointed out a clear increase of the oxidative stress in all the plants treated with T22 after the inoculation with CMV. Finally, gene expression analysis of the Coi-1 gene seems to show the activation of a defense response similar to the systemic acquired resistance. The analysis of the results obtained suggests the possible use of T22 as a treatment rather than as a preventive measure.

P530

An International Plant Sentinel Network

Ellie Barham¹, Suzanne Sharrock¹, Charles Lane², Richard Baker²

¹Botanic Gardens Conservation International, London, UK, ²The Food and Environment Research Agency, Sand Hutton, York, UK

The increasing globalisation of trade in plants and plant material, together with the impacts of climate change, has led to a recent increase in the introduction and spread of new and damaging plant pests and diseases. Botanic gardens are in a unique position to help detect potential invasive threats to a country's plant health; within their collections they play host to numerous expatriate plants that can act as sentinels for potentially invasive pests. The International Plant Sentinel Network (IPSN) is being established as part of a European-funded (EUPHRESKO) project led by the UK's Food and Environment Research Agency (FERA). The project will be implemented in collaboration with Botanic Gardens Conservation International (BGCI) and with partners in Europe (Julius Kühn-Institut, Germany, National Plant Protection Organisation, Netherlands and DiBAF, Italy). The network will facilitate collaboration amongst institutions in Europe and beyond, with a focus on linking botanic gardens and arboreta, National Plant Protection Organisations and plant protection scientists. This presentation will describe the aims of the project, including improving the ability of garden staff to identify alien plant pathogens and diseases, by seeking best practise, developing standardised approaches and providing training materials and methodologies for monitoring and surveying. It will also aim to provide professional diagnostic support that can help promote early detection and rapid response to new pest incursions. The network will build on data already available through a number of databases, including BGCI's Plant and GardenSearch databases and CABI's Crop Protection, Forestry and Invasive Species Compendia.

P531

Characterization of Cucumber Responses to Salt Stress and Infection Applied Sequentially

Joanna Chojak, Elzbieta Kuzniak

Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection,
University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Plants are constantly exposed to different environmental stress factors in combination. Integrated whole-plant acclimation response to stresses occurs via signalling molecules, metabolites and phytohormones. Understanding these mechanisms is required to breed plants and to produce crop varieties with enhanced tolerance to changing environmental conditions.

To dissect the mechanisms of plant response to a combination of abiotic and biotic stresses we exposed cucumber plants to short-term salt stress and test their response to subsequent *Pseudomonas syringae* pv *lachrymans* infection. Measuring infection development, proline content, lipid peroxidation and carbonyl group content we monitored plant performance under stress. At the metabolic level we focused on the role of biochemical processes linked to primary carbon metabolism in coordination of the whole plant response to salinity and pathogen infection sensed by roots and leaves, respectively. Finally, at the phytohormone level changes in contents of salicylic acid (SA) and its glucosylated conjugates (SAG) were measured.

Abiotic stress promoted bacterial infection development. The results confirmed that phosphoenolpyruvate carboxylase and NADP-malic enzyme and hence β -carboxylation-related processes could be involved in reprogramming plant metabolism from growth and development to defense. In cucumber leaves SA occurred mostly as SAG. Bacterial infection induced a significant increase in SA and SAG concentrations, however the intensity and dynamics of SA and SAG accumulation differed in leaves of NaCl-treated and non-treated plants.

This study may provide new insights into how response to multiple stress factors is regulated and how the environment can modulate plant-pathogen interactions.

This work was partly supported by grant No 2012/07/N/NZ9/00041

P532

Characterization of the “*Galapagos Enhanced Trichomes (Get)*” Locus: The First Step in the Resistance against Insects

Eloisa Vendemiatti¹, Frederico Almeida de Jesus¹, Maísa Siqueira Pinto¹, Vagner Antonio Benedito², Leonardo Boiteux³, Lázaro Eustáquio Pereira Peres¹

¹Escola Superior de Agricultura “Luiz de Queiroz”- ESALQ/USP, Piracicaba - SP, Brazil, ²Genetics and Developmental Biology Program, Division of Plant and Soil Sciences - West Virginia University, Morgantown - WV, USA, ³Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa de Hortaliças, Laboratórios de Melhoramento Genético Análise Genômica, Ponte Alta, Brasília – DF, Brazil

Trichomes are specialized epidermic structures mostly responsible for plant herbivory protection. The *Solanum* genus has been used as a model to study trichome differentiation. The genus contains a large range of different types of trichomes, including the glandular ones. Glandular trichomes are been called “biological chemical factories”, since they produce and accumulate specialized metabolites. Cultivated tomato (*S. lycopersicum*) does not have type IV glandular trichomes, which are present in some wild species (e.g. *S. galapagense*) and are the main sources of the natural insecticide acyl sugar. The present work is an attempt to characterize a novel natural genetic variation from *S. galapagense* introgressed into *S. lycopersicum* cv. Micro-Tom (MT) that produces type IV trichomes. The locus was denominated *Galapagos enhanced trichomes (Get)*. The most evident feature in the near isogenic line (NIL) harboring the *S. galapagense* allele is the presence of glandular trichomes type I and IV in both sides of the leaves. Since no enhanced AS production and neither insect resistance (*Bemisia tabaci*) were found in the NIL MT-Get we propose that the resistance should be the result of three requirements: i) the capacity to develop special glandular trichomes; ii) the proper localization of the trichomes in the leaves and iii) the capacity of the trichomes to produce specialized metabolites. The cloning of *Get* will give some insights about the development of glandular trichomes, besides to be a useful tool for breeding new resistant varieties with reduced use of pesticides.

P533

***Origanum dictamnus* Oil Vapour Suppress the Development of *Botrytis cinerea* in Tomato Fruit**

Andriana Stavropoulou¹, Nikos Tzortzakis², Dimitris Lydakakis¹, Naresh Magan³, Konstantinos Loulakakis¹

¹Technological Educational Institute of Crete, Heraklion, Crete, Greece, ²Cyprus University of Technology, Limassol, Cyprus, ³Cranfield University, Bedfordshire, UK

Grey mould (*Botrytis cinerea*) development *in vitro* or in cherry tomato (*Solanum lycopersicum* L.) fruit was evaluated after treatment with dittany (*Origanum dictamnus* L.) oil (0, 50, 100 or 250 ppm) and storage at 12°C and 95% relative humidity during or following exposure to the volatiles. *In vitro*, fungal colony growth, spore production and spore germination inhibited with the application of dittany oil with greater effects recorded in higher dittany concentrations. *In vivo*, fruits treated with vapours reduced lesion growth after 7 days of exposure with greater effects marked at the higher concentration (250 ppm). Fungal spore germination was not affected by essential oil application, while spore production, decreased with the presence of essential oil volatiles comparing to the control treatment. The benefits associated with dittany volatiles-enrichment was maintained in fruit pre-exposed to vapours, resulting in suppression in lesion growth while no differences observed in spore germination and spore production. Fruits exposed to essential oil did not differ in quality related attributes such as fruit firmness, total soluble solids, titratable acidity, weightloss, colour (L, C, h) and respiration rates. The results of this study indicate that dittany volatiles may be considered as an alternative to the traditional postharvest sanitizing techniques. Each commodity needs to be individually assessed, and the volatile concentration and sanitising technique optimised, before the volatile treatment is used commercially.

P534

Changing the Contents of Phytohormones in the Leaves and Roots of Wheat with Different Resistance Infected by *Bipolaris sorokiniana*

Lubov Yarullina^{1,2}, Rita Kasimova¹, Albina Akhatova¹, Igor Maksimov¹

¹*Institute of Biochemistry and Genetics of Ufa Scientific Center of Russian Academy of Sciences, Ufa, Russia*, ²*Bashkir State University, Ufa, Russia*

Influence of root rot pathogen *Bipolaris sorokiniana* on the phytohormones content (zeatin, indole -3-acetic acid and abscisic acid) in roots and leaves of wheat with different resistance to the pathogen was studied. Infection of seedlings was performed by watering with a conidia suspension of the fungus in a concentration of 10³ spores/ml. Contents of phytohormones were determined 3, 6 and 9 days after inoculation by immunoassay. Also the rate of disease development was assessed. Inoculation of *B. sorokiniana* significantly changed the level of IAA and ABA in the aerial parts and roots of wheat susceptible cultivar. Thus, the level of IAA in the roots of susceptible plants on 9th day after the infection was lower and ABA - 2 times higher than that of healthy plants. The degree of infestation of susceptible plants within the specified period and was two times higher than resistant. Infected wheat plants of resistant cultivar differed of minor fluctuation of IAA and ABA in the roots and leaves. In resistant wheat plants in response to infection there was an increase of zeatin in several times. In infected plants of susceptible cultivar zeatin concentration decreased during pathogenesis. Analysis of the ratio of IAA, ABA and zeatin in infected (roots) and uninfected (leaves) parts suggests that high level of zeatin in plants contribute the formation of wheat resistance to necrotrophic pathogen *B. sorokiniana*.

P535

Effect of Novel Herbivore on Floral Signalling in *Silene latifolia*: The Interplay between Reproduction and Defence

Giovanni Scopece^{1,2}, Carmen Arena^{1,2}, Maria Litto^{1,2}, Florian Schiestl³, Salvatore Cozzolino^{1,2}, Silvia Fineschi^{1,2}

¹University of Naples, Federico II, Naples, Italy, ²Institute for plant protection CNR-IPP, Sesto Fiorentino, FI, Italy, ³University of Zurich, Zürich, Switzerland

Although the trade-off between defence and reproduction has been investigated in some detail, little is known about how herbivores affect floral signalling. Herbivores may modulate the level of plant reproductive success and may hamper the interaction between plants and their pollinators, thus ultimately they may affect individual fitness. In principle, herbivore attacks may weaken the allocation of plant resources for flower display, reduce plant attraction for pollinators and could change their foraging behaviour. Here we investigated the effect of the foliar herbivory by the invasive *Spodoptera littoralis* on floral signalling and attractiveness to pollinator of the model plant *Silene latifolia*. We found no effect of herbivory on floral traits involved in visual attraction of pollinators (flowering time, corolla diameter, calyx length, petals length), however, *Spodoptera*-infested plants produced a different floral bouquet from control plants. In particular, ocymene was found higher emitted in infested plants and the infested plants were also found more attractive to pollinators compared to un-infested plants likely because of the increased emission of these attractive molecules. Experiment addition of ocymene to *Silene* flowers confirmed the positive role in pollinators attraction of this herbivore-induced compound. This findings showed that exposure to novel herbivores can affect coevolved interactions between plants and their pollinators and that some of these effects are mediated through the emission of herbivore-induced plant volatiles. However, the overall effect on plant pollination success is not necessarily detrimental, as herbivore exposure differently affects the plant investments in defence and the pollinator response.

P536

Interactive Effects of Leafroll Associated Virus (GLRaV-3) and Drought on the Physiology of Two Grapevine (*Vitis vinifera* L.) Varieties

Hanan El aou ouad¹, Rafael Montero², Antònia Romero-Munar¹, Hipólito Medrano¹, Josefina Bota¹
¹Grup de Recerca en Biologia de les Plantes en Condicions Mediterrànies, Departament de Biologia, Universitat de les Illes Balears, Carretera de Valldemossa, km 7.5, 07122, Palma de Mallorca, Balears, Spain, ²Institut de Recerca i Formació Agrària i Pesquera (IRFAP), Conselleria d'Agricultura, Medi Ambient i Territori. Govern de les Illes Balears, C/Eusebio Estada nº 145. 07009, Palma de Mallorca, Spain

Grapevine leaf-roll-associated virus-3 (GLRaV-3) is one of the most widespread viruses worldwide, and one of the most important diseases affecting grapevines (*Vitis vinifera* L.). On the other hand, in the Mediterranean region grapevines usually deal with water deficit during growth period because most of its growth season copes with summer. In the present work, we focused on determining the interaction effects of biotic (GLRaV-3) and abiotic stress (drought) on grapevine growth and physiology (cv. Malvasia de Banyalbufar and Giro-Ros (autochthonous varieties of Majorca (Spain))). Growth parameters, gas exchange, and plant water status (Ψ_{pd}) were compared in virus-free and GLRaV-3 infected potted plants under two irrigation regimes. AN, gs and mesophyll conductance (gm) were affected by virus infection under irrigation and moderated drought. The virus effect on growth parameters was slightly different between two varieties being Malvasia more affected than Giro Ros. No differences were observed in Ψ_{pd} between healthy and infected plants. Limitation analysis (Grasi and Magnani, 2005) revealed that under irrigation, virus infection induced 31% and 12 % of total limitation to AN in Malvasia and Giro Ros respectively. The three partial limitations were similar in Malvasia cv. accounting around 10 % each. By contrast, in Giro-Ros, biochemical limitation was the most affected by GLRaV-3 (8%). Under drought stress, virus infection do not increase the limitation imposed by water deficit. In conclusion, GLRaV-3 infection impairs photosynthesis affecting both, CO₂ diffusion and metabolic processes. Under drought, the reduction of photosynthesis induced by water stress is not increased by virus infection.

P537

First Report on Infection of *Arabidopsis thaliana* Col-0 by Tobacco Necrosis Virus

Renáta Bacsó, Lóránt Király, Zoltán Király

Plant Protection Institute, Centre for Agricultural Research, HAS, Budapest, Hungary

Arabidopsis thaliana is a popular model plant conferring susceptibility to several different pathogens (fungi, bacteria and viruses). However, the effect of Tobacco necrosis virus (TNV) on *Arabidopsis* is not well known. Vannini et al. (2006) reported that TNV causes local necrotic and chlorotic lesions in the Wassilewskija ecotype, but they did not followed replication and systemic spread of the virus. Therefore, the goal of our research was to monitor reactions of the most widely used *A. thaliana* ecotype (Col-0) to TNV infection by assessing in planta virus replication and systemic spread. TNV replication intensity in inoculated *Arabidopsis* leaves was determined by real-time RT-qPCR. A time course analysis revealed that TNV levels progressively increase up to 4 days after inoculation (DPI). Back inoculation of tobacco (cv. Xanthi) leaves was performed mechanically with the sap of TNV-infected *Arabidopsis* leaves. Back-inoculated tobacco leaves displayed symptoms typical of TNV-infection (i.e. local necrotic lesions) within a few days. The present study is the first to show that TNV can infect *Arabidopsis thaliana* ecotype Col-0. This plant-pathogen interaction may provide a new tool for further research on plant defense mechanisms against virus infections.

References

Vannini C., Iriti M., Bracale M., Locatelli F., Faoro F., Croce P., Pirona R., Di Maro A., Coraggio I., Genga A. (2006): The ectopic expression of the rice Osmyb4 gene in *Arabidopsis* increases tolerance to abiotic, environmental and biotic stresses. *Physiological and Molecular Plant Pathology* 69 (2006) 26-42.

P538

Exploring Resistance Response in Melon-Fusarium oxysporum f.sp. melonis Race 1,2 Interaction using High Throughput RNA Sequencing

Maria Silvia Sebastiani^{1,3}, Paolo Bagnaresi², Sara Sestili¹, Chiara Biselli², Luigi Orrù², Valentino Ferrari¹, Giulia De Lorenzo³, Nadia Ficcadenti¹

¹Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Unità di Ricerca per l'Orticoltura (CRA-ORA), Via Salaria 1, 63077, Monsampolo del Tronto (AP), Italy, ²Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Genomics Research Centre (CRA-GPG), Via S. Protaso 302, 29017, Fiorenzuola d'Arda (PC), Italy, ³Dipartimento di Biologia e Biotecnologie "C. Darwin", Università degli Studi di Roma 'La Sapienza', Piazzale Aldo Moro 5, 00185 Roma, Italy

Fusarium oxysporum f.sp. melonis race 1,2 (FOM 1,2) is the most virulent and yield-limiting pathogen of melon (*Cucumis melo* L.) cultivation worldwide. Colonization of plants by FOM leads to necrosis of the infected tissues, collapse of vascular vessels and decay of the plant. Resistance to FOM 1,2 appears to be controlled by multiple recessive genes and strongly affected by environment. A RNA-Sequencing approach was used to investigate the transcriptome dynamic during incompatible and compatible interactions for the identification of candidate resistance genes in the melon-FOM 1,2 pathosystem. The doubled-haploid resistant line NAD and the susceptible cultivar Charentais-T, both inoculated with FOM 1,2, were analyzed at 24 and 48 hours post-inoculation (hpi). The landscape of differentially expressed genes (DEGs) diverged significantly in the two genotypes. NAD, unlike Charentais-T, modulates a higher proportion of up-regulated genes at 24 hpi, suggesting a more prompt response. Several unannotated transcripts were found to be modulated, providing a basis for further exploration of plant defense-related genes. Gene ontology (GO) enrichment of DEGs highlighted that defence response, incompatible interaction and response to stress GO-groups appear as major effectors of resistance to FOM 1,2. Of particular interest were disease resistance genes including FMO1, several RPM1-like and pathogenesis-related thaumatin encoding genes. Other genes involved in cell wall reorganization and hormone signaling pathways were also differentially expressed. To investigate the pathogen behavior in the two genotypes the expression of in planta-specific FOM transcripts was detected. This is the first application of RNA-Sequencing to dissect melon resistance towards this fearful pathogen.

P539

Not Only Plants: The Phytotoxin Fusicoccin Is a General Regulator of the Interaction of 14-3-3 Proteins with Their Protein Targets

Lorenzo Camoni¹, Laura Cervoni², Francesca Cutruzzolà², Cristina Di Lucente¹, Giacomo Janson², Alessandro Paiardini², Stefano Pascarella², Serena Rinaldo², Sabina Visconti¹, Patrizia Aducci¹
¹*Department of Biology, University of Rome "Tor Vergata", Rome, Italy,* ²*Department of Biochemical Sciences, Sapienza University of Rome, Rome, Italy*

Fusicoccin (FC), a phytotoxic terpenoid produced by the fungus *Phomopsis amygdali*, affects several physiological processes in plants, as a consequence of H⁺-ATPase activation. FC stabilizes 14-3-3 interaction with the H⁺-ATPase, thus maintaining the enzyme in its activated state. The 14-3-3 binding site (YpTV-COOH, designed as mode III) is located at the C-terminal end of the H⁺-ATPase and considerably differs to those present in the majority of 14-3-3 clients (mode I and II). This feature explains why FC selectively stabilizes the 14-3-3/H⁺-ATPase interaction.

However, a number of 14-3-3 targets with mode III motifs has been recently identified both in plants and animals. Structure similarity between the H⁺-ATPase and other targets makes conceivable that other 14-3-3 interactions could be stabilized by FC. Accordingly, we recently demonstrated FC ability to stabilize 14-3-3 association to human GPIIb α , a platelet glycoprotein involved in the adhesion of circulating platelets to arteries and capillaries sub-endothelium. As a consequence, FC promotes platelet adhesion and aggregation. This finding proposes FC as a drug-like molecule potentially exploitable to control a number of physiological processes where 14-3-3 proteins take part. Here we show that FC stabilizes 14-3-3 interaction to several plant and animal targets with a mode III motif. Isothermal Titration Calorimetry analysis demonstrated FC ability to stimulate 14-3-3 association to different peptides. Moreover, molecular docking studies provided the structural rationale for the differential FC effect. Our study proposes FC as a promising tool to control cellular processes regulated by 14-3-3 proteins, opening new perspectives on its potential pharmacological applications.

P540

Involvement of Sphingosine Kinase in Attenuation of Pathogen-Induced Cell Death in *Nicotiana tabacum*

Soo Jin Wi, So Yeon Seo, Ky Young Park
Sunchon National University, Suncheon, Republic of Korea

Sphingolipids such as phytosphingosine (PHS) have been suggested to act as second messengers for an array of cellular signaling activities in plant cell, including stress responses and programmed cell death. We previously reported that PHS levels increase at 1h and 48 h in tobacco plants after *Phytophthora parasitica* var. *nicotianae* inoculation using UPLC-Q/TOF MS. PHS treatment significantly increased ROS accumulation from 15min to 3h in not only guard cells but also epidermal cells from tobacco leaves. Further, PHS-induced cell death occurred correspondingly with these rapid increases of ROS and ethylene production at relatively early period of 1h. In addition, PHS-induced rapid cell death was evidenced with increase in transcription of NtMC4. However, PHS treatment did not accompany with further senescence-related ROS and ethylene production, which were responsible to no further severity of cell damage. From 24h after PHS treatment, ROS-detoxifying enzymes and PR-protein were significantly induced. Also, the expression of sphingosine kinase (SphK) was massively increased after 24h. SphK catalyze PHS to PHS-1-P, which is known to positive regulator of cell survival during pathogen infection. SphK transcription was significantly increased at later stage of avirulent elicitation-induced resistant plants, compared with susceptible plants after virulent pathogen infection. When we investigate what is a major component of SphK expression using impaired transgenic plants of ROS or ethylene biosynthesis, ROS levels is a main regulator for SphK transcription. These findings indicate that up-regulation of SphK expression is responsible to attenuation of further cell death progress, and also dues to cell survival in intact plants.

P541

Possible Contribution of Camalexin Biosynthesis to the Quantitative Resistance to Clubroot in a Natural Accession of Arabidopsis

Séverine Lemarié^{1,5}, Christine Lariagon^{1,5}, Jocelyne Lemoine^{1,5}, Nathalie Marnet^{2,5}, Anne Levrel^{1,5}, Mélanie Jubault^{3,5}, Maria Manzanares-Dauleux^{3,5}, Antoine Gravot^{4,5}
¹INRA, UMR1349 IGEPP, F-35653 Le Rheu, France, ²INRA, UR1268 BIA, F-35653 Le Rheu, France, ³Agrocampus Ouest, UMR1349 IGEPP, F-35000 Rennes, France, ⁴Université Rennes 1, UMR1349 IGEPP, F-35000 Rennes, France, ⁵Université Européenne de Bretagne, F-35000 Rennes, France

The use of quantitative partial resistances in plant breeding is thought to be one of the best ways to durably control plant pathogens in agroecosystems. Due to the genetic complexity of those resistances, underlying mechanisms are largely unknown. In this work, we addressed the possible relationship between phytoalexin accumulation and the expression of partial resistance to clubroot in Arabidopsis. Metabolic profiling of roots infected by *Plasmodiophora brassicae* revealed four-times higher levels of camalexin accumulation in the partially resistant accession Bur-0 compared to the susceptible Col-0 accession. To determine the genetic control of these contrasted metabolic responses, two couples of Heterozygous Inbred Lines (HIF) which carry either Col-0 or Bur-0 alleles at the two main clubroot resistance QTL PbAt1 and PbAt5.2, were used. Biochemical analysis of infected roots in these near-isogenic lines revealed that high levels of camalexin accumulation in Bur-0 are mostly controlled by the QTL PbAt5.2. To go further in the understanding of PbAT.5.2 effect, the expression of CYP71A13 and CYP71B15/PAD3 camalexin biosynthesis genes was monitored. Induction of both genes is only observed in HIF carrying the Bur-0 allele, with a maximum at 14 days post inoculation corresponding to the pathogen secondary phase of infection. Using both histological and qPCR quantification approaches, we also show that the camalexin increase parallels the PbAt5.2-mediated reduction of pathogen development. Altogether, our results support the hypothesis that the Bur-0 allele at QTL PbAt5.2 contribute to the clubroot partial resistance through the control of high camalexin accumulation.

P542

A Real Time Quantitative PCR Assay for Quantifying Disease Tolerance in Winter Wheat

Petra Kock-Appelgren^{1,2}, Steven Kildea¹, John Foulkes², John Spink¹

¹Teagasc Oak Park, Carlow, Ireland, ²University of Nottingham, Sutton Bonington Campus, Leicestershire, UK

Disease tolerance is the ability of a crop to maintain yield despite the presence of disease. This self-defence mechanism has potential to be used to protect against yield loss in years of high disease pressure and as an aid in reducing fungicide dependence. Studying disease tolerance is however time consuming and labour intensive, and it is not currently feasible for breeders to incorporate screening for disease tolerance into breeding programs. In this project we are studying tolerance in winter wheat to *Zymoseptoria tritici*, the cause of septoria tritici blotch and the most severe fungal disease of winter wheat in North Western Europe. Control of this disease is currently reliant on fungicides, and can cause losses up to 50% if not properly prevented.

In this study we are investigating if tolerance to *Z. tritici* infection and disease development can be reliably quantified using Real Time PCR. In order to validate the assay, two susceptible winter wheat varieties (Rialto and Xi19) were inoculated with three different concentrations of *Z. tritici* inoculum in a glasshouse experiment. The disease progress was followed by visual disease assessment every seven days. Significant differences in visible disease symptoms were observed between inoculum treatments at 14, 21 and 28 days after inoculation ($p < 0.001$). The pathogen load of the assessed leaves will be determined using Real Time PCR with combinations of different target genes in *Z. tritici* and wheat nuclear and organelle genomes.

P543

Anatomical Characterization of the 'Smut Whip' of Sugarcane Incited by the Fungus *Sporisorium scitamineum*

João Paulo Rodrigues Marques, Claudia de Barros Monteiro-Vitorello, Beatriz Appezzato-da-Glória, Alessandra Palhares, Maria Lucia Carneiro Vieira
Luiz de Queiroz College of Agriculture University of São Paulo, Piracicaba, Brazil

Sugarcane smut is a disease caused by the fungus *Sporisorium scitamineum* that is responsible for losses in sugarcane production worldwide. The most recognized symptom is the development of a whip-like sorus responsible for producing, sheltering and spreading fungal teliospores. In the present work, the lateral buds of *Saccharum* spp. var. RB 925345 were inoculated with a paste of teliospores and transplanted to plastic pots containing substrate and grown for 4 months at 26-30 °C under greenhouse conditions. After the emission, the whip was processed according to standard plant anatomical techniques, and analyzed under light and electron microscopy. Anatomically, the whip is characterized as an elongated internode with three main regions involved: basal, intermediate and apex. The basal region is situated internal in the leaf sheath, and two zones are visualized: a white zone where an intercalary meristem presents a high mitotic activity is seen and a black zone where a sporogenesis proceeding in a mucilaginous matrix within the ground tissue takes place. Parasitic dikaryotic hyphae of *S. scitamineum* are observed inside or between the cells of vascular and ground host tissues. The hyphae were seen throughout the whip. The intermediate and apex are the exposed parts of the whip. In these regions, numerous mature teliospores were observed. The fungus promotes the release of parenchyma cells and the apex becomes necrotic. Characterizing the structural aspects of the whip is very important to understand how *S. scitamineum* interacts with sugarcane tissues and how the plant tissues respond to the pathogen infection.

P544

The "Recovery" Phenomenon in Grapevine (*Vitis vinifera*) cv. Chardonnay Infected by "Bois Noir" Phytoplasma: Investigation of Proteome Modifications by 2DE and Western Blot Analysis

Francesca Degola¹, Paolo Margaria², Sabrina Palmano², Luigi Sanità di Toppi¹

¹University of Parma, Parma, Italy, ²Consiglio Nazionale delle Ricerche, Torino, Italy

The grapevine (*Vitis vinifera*) disease caused by Bois Noir (BN) phytoplasma, a plant phloem-limited pathogens belonging to the class Mollicutes, is an important concern to European vinery economy.

Phytoplasmas have a broad range of plant hosts among monocots and dicots and diseases of many important crops have already been associated with these pathogens. Leaf curling and discoloration, uneven or total lack of lignification of canes, flower abortion and berry withering are the typical symptoms of phytoplasma infection in grapevine. An interesting aspect of the pathogen–plant interaction is "recovery" phenomenon, a spontaneous remission of symptoms in previously symptomatic plants. In this study, we are using 2-DE and Western Blot analysis to explore the proteome changes in leaves and midribs of *V. vinifera* cv. 'Chardonnay' naturally infected by BN, with particular attention to recovered plants.

P545

Development of RTD-Resistant, Elite Tropical Japonica Cultivar by Marker-Assisted Selection for RTSV Resistance and Photoperiod Insensitivity Selection

Woongoo Ha^{1,2}, Junghyun Shim¹, Gideon Torollo¹, Rosalyn B. Angeles-Shim¹, Il-Ryong Choi¹, Rogelio C. Cabunagan¹, Un-Sang Yeo^{1,2}

¹*International Rice Research Institute, DAPO Box 7777, Metro Manila, DAPO Box 7777, Metro Manila, The Philippines,* ²*National Institute of Crop Science, RDA, Suwon, Republic of Korea*

Rice tungro disease (RTD) is a destructive disease of rice in Southeast Asia. RTD is caused by rice tungro spherical virus (RTSV) and rice tungro bacilliform virus (RTBV), which are transmitted by the green leafhopper (*Nephotettix virescens*). In recent years, Southeast Asia has seen a rapid increase in japonica rice consumption. The tropical japonica rice cv. Japonica1 that was developed at IRRI has a high yield potential but is susceptible to RTD. We identified a few japonica rice varieties from South Korea as donor lines with RTSV resistance gene as well as good grain quality. This is the first report on the practical application of MAS for RTD resistance and conventional selection for photoperiod insensitivity for tropical japonica rice breeding.

P546

Preservation and Form Viral Infection of Virus-Free Seed Bulb of Garlic (*Allium sativum* L.) in Korea.

Young-Seok Kwon, In-Hu Choi, Eul-Tai Lee, Cheol-Woo Kim, Eom-Ji Hwang, Sang-Gyeong Bae
Bioenergy Crop Research Center, National Institute of Crop Science, RDA., Muan, Jeonnam, Republic of Korea

Garlic (*Allium sativum* L.) in Korea is one of the important vegetable. Garlic are propagated vegetative, because of this, viruses accumulate and evolve in garlic. Viral diseases of garlic in *Allium* plants have become widespread, causing serious losses in Korea. Although the virus-free seed bulb supply system is needed that can be used for a long time. This research was conducted to investigate the incidence of viral infection and evaluate the effect on the yield in early stages of virus-free seed bulb. Virus infection mainly kind of garlic, GLV, LYSV and OYDV was single or complex infection. When virus-free seed bulb planted in isolation net chamber OYDV occurred most frequently were 96.3%, GLV and LYSV the infection tends to be insignificant or seldom. Virus infections alone tend to look up a lot of 87.8 to 100%, which was estimated to OYDV virus. Complex infection was minimal but, GLV and OYDV 11.4% of the most common infection, plants infected more than three kinds in field mosaic symptoms observed in garlic, 1-2 kinds of infections, the symptoms could not be confirmed. Yield comparison of disease-free bulbs decreased from increase years in field cultivation but increase 18 to 39% than control. Wider planting distance increased weight did not show a tendency to a certain amount.

P547

The Zinc-Binding HIPP3 Protein Regulates LSD1 Dependent Plant Immunity Response and Flowering Time in *Arabidopsis thaliana*

Wiebke Zschiesche

Martin-Luther-University, Halle, Germany

Arabidopsis thaliana HIPP3 (At5g60800) is a member of the HIPP protein family featuring a C-terminal isoprenylation motif, two heavy metal binding HMA domains and four nuclear localization signals (NLS). Microscopy of onion epidermis cells after transient expression of a chimeric GFP::HIPP3 protein confirmed its nuclear localization and ICP-MS analyses of HIPP3 overexpressed in *E. coli* revealed binding of zinc. Transcript levels of HIPP3 are down-regulated during drought stress and in response to ABA treatment, and are clearly up-regulated after infection with *Pseudomonas syringae* pv. tomato. It shows expression patterns as other pathogen related genes with slow induction in PTI, and a fast induction in ETI sparked by the avirulence protein AvRpm1. Two overexpression lines of HIPP3 were affected in flowering showing a clear delay when compared to the wild type. To identify putative target genes of HIPP3, array analyses comparing overexpression line with wild type were performed. Expression of more than 400 genes was clearly affected by overexpression of HIPP3 with similar amounts of genes being up- or down- regulated. Especially genes involved in pathogen response, abiotic stress responses and in seed and flower development were differentially expressed in the overexpression lines, indicating a complex regulatory function of HIPP3. Co-expression analysis of genes affected by HIPP3 overexpression indicates a regulatory function of HIPP3 in the LSD1 and pad4 regulated salicylate pathway of pathogen response and also in seed and flower development. A model is presented showing HIPP3 acting via its bound zinc in the salicylate dependent pathway of pathogen response.

P548

Responses of Avocado (*Persea americana*) to Flooding and *Phytophthora cinnamomi*: A Transcriptomic and Proteomic Approach

Bianca Reeksting^{1,2}, Noelani van den Berg^{1,2}

¹University of Pretoria, Pretoria, Gauteng, South Africa, ²Forestry and Agricultural Biotechnology Institute, Pretoria, Gauteng, South Africa

Avocado (*Persea americana*) is a subtropical fruit tree grown on a commercial scale worldwide. It is a diploid angiosperm with 24 chromosomes and a genome estimated at $\pm 920\text{Mb}$. A major constraint to production is caused by the ubiquitous oomycete *Phytophthora cinnamomi*, a root rot pathogen causing damage to the feeder roots of trees and resulting in branch-dieback, yield losses and eventual tree death. Flooding has been found to exacerbate the root rot (PRR) caused by *P. cinnamomi* and accelerated tree death is seen. Avocado is also susceptible to flooding in the absence of *P. cinnamomi* and even transient flooding severely damages trees. Rootstocks tolerant to PRR are used in the control of root rot; however these rootstocks are often susceptible to flooding, limiting use in flood-prone areas. In this study we used a combination of approaches to elucidate the responses of avocado to both flooding and *P. cinnamomi*. Due to limited genetic resources for avocado, de novo sequencing and assembly of a root transcriptome was performed. Roughly 124Mb of data were generated and assembled into 7685 contigs. Genes involved in defence pathways as well as genes associated with low oxygen stress were identified. Contigs and selected singletons, along with sequence data generated for *P. cinnamomi*, were used in the design of an 8x15k Agilent microarray for expression analysis. Quantitative proteome analysis was also conducted. This study provides resources that may aid in the identification of rootstocks tolerant to a combination of biotic and abiotic stress.

P549

An Integrated Approach to Study the Avocado-*Phytophthora cinnamomi* Interaction

Noelani van den Berg¹, Bianca Reeksting¹, Juanita Engelbrecht², Barry Christie², Waheed Mahomed¹
¹*Forestry and Agricultural Biotechnology Institute (FABI), Department of Genetics, University of Pretoria, Pretoria, South Africa,* ²*Forestry and Agricultural Biotechnology Institute (FABI), Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa*

Phytophthora root rot (PRR) of avocado, after decades of research, remains the most destructive biotic threat to avocado production around the world. Necrotic lesions on the feeder roots result in decline of tree health and eventually tree death. Control strategies rely on the use of phosphites in combination with PRR-tolerant rootstocks. To study this complex interaction we have combined Next generation 454 sequencing with quantitative gene profiling, microscopy, bioassays and plant trials. Bi-flagellate zoospores swam towards avocado feeder roots, encysted and germinated within 2-3 hours post inoculation. Confocal microscopy revealed that tolerance was associated with the production of calloses, while susceptible roots were rapidly colonized despite the production of lignin and tyloses. Furthermore, beta-1,3- glucanase rapidly increased in tolerant rootstocks after *Phytophthora cinnamomi* (Pc) inoculation, while decreasing in susceptible rootstocks. Sequencing identified more than 1000 transcripts that were regulated during the defence response against Pc. Tolerant avocado rootstocks responded early to pathogen infection by inducing several defence-related genes, including PR-genes, lipoxygenase and phenylalanine ammonia-lyase. Defence gene expression was also observed in the susceptible rootstock but usually at a lower level and/or later time point after Pc inoculation. Molecular and phenotypic data have shown that multiple defence mechanisms are at play to protect avocado against this notorious soil-borne oomycete.

P550

Efficacy of *Pseudomonas fluorescens* (PS02 and PS14) Isolates for Biocontrol of Seedling Blight of Wheat Caused by *Fusarium culmorum*

Ali Elagael

University of Tripoli, Tripoli, Libya

Fusarium culmorum one of *Fusarium* species cause seedling blight and foot rot of wheat. There are no cultivars of wheat which are immune to these diseases and chemical control is inconsistent. The aim of this investigation was to isolate natural bacterial antagonists from the wheat rhizosphere and screen them as seed treatments for the control of seedling blight of wheat. 73 of bacterial isolates that isolated from rhizosphere soil using selective media and showed in vitro clearly inhibition zones using a dual plate bioassay were subsequently evaluated as seed treatments (using an agar tube assay) for their ability to control seedling blight of wheat. *Pseudomonas fluorescens* (PS02 and PS14) isolates were selected and identified by conventional methods and confirmed using MALDI-TOF MS. Trials have also been undertaken in a controlled environment trial. *Pseudomonas fluorescens* (PS02 and PS14) were significantly ($P < 0.05$) increased percentage seed germination, enhanced the growth of wheat seedlings (fresh weight and shoot length, and effectively reduced seedling blight of wheat. A small scale field trial confirmed the effectiveness of this isolate. In conclusion, natural rhizosphere bacteria *Pseudomonas fluorescens* have been isolated which show potential for the biocontrol of seedling of wheat.

P551

Phenotypic Plasticity and Epigenetics

Elizabeth Kordyum

Institute of Botany, NAS of Ukraine, Kyiv, Ukraine

The idea, that system stability is determined with the lability of its components, is one of the paradigms of advanced science. In biology, it is a phenomenon of phenotypic plasticity, i. e. the genotype ability to change its expression and realize in different phenotypes in a response to various environment signals owing to this organisms can adapt to temporal and spatial variations of the environment. Phenotypic appearance of changes in gene expression is detected at the level of transcription efficiency, RNA processing, and translation and includes ecologically important patterns – physiological and biochemical, anatomical, morphological, features of developmental biology, reproduction systems, and progeny development. It is of special interest an idea that phenotypic changes are already identified at the transcription level. The epigenetic system is known to be a part of signal perception by a cell and its transfer to changes in gene expression, as well as it has a potential to keep the permanent memory through many cell generations. Therefore, the epigenetic system has to be a key to understand the mechanisms of plasticity of plant responses to environment signals. It is taken into account the wide distribution of vegetative reproduction in plant world, growth modularity and unboundedness, the presence of both numerous cycles in ontogenesis of perennial plants and apomixis – adventive embryony and apospory, as well as individual changeability at the population level. On the basis of current ideas, the questions on possible participation of epigenetic systems for gene expression control in plant phenotypic plasticity are discussed.

P552

Localization of Polysaccharides in Isolated and Intact Leaf Cuticles of Model Species by Enzyme-Gold Labelling

Paula Guzmán, Victoria Fernández, Luis Gil
Technical University of Madrid, Madrid, Spain

The cuticle is a composite, extracellular membrane covering the outer surface of most aerial organs of plants and plays a major physiological and structural role in the protection against multiple potential biotic and abiotic stress factors. Despite polysaccharides constitute an integral part of the cuticle they have hardly been taken into consideration when analyzing cuticular ultra-structure and chemical composition. The presence and general characteristics of cuticle polysaccharides have been demonstrated by staining and spectroscopic methods, but their location in the cuticle remains unclear. According to the prevailing model, polysaccharides are believed to be restricted to the cuticular layer (which is in direct contact with the cell wall) and absent in the cuticle proper (the outermost region of the cuticle). By means of enzyme gold-labelling and transmission electron microscopy, cellulose and pectins were detected for the first time in enzymatically isolated adaxial leaf cuticles of grey poplar, blue-gum eucalypt and European pear, generally appearing along the whole cuticle transversal sections. Nevertheless, the labelling density generally decreased towards the epicuticular wax layer and some differences were observed among the species evaluated. The presence and distribution of cellulose and pectins along intact and even isolated leaf cuticles should be considered for example, when analyzing plant surface interactions or when interpreting organ ontogeny.

P553

Wettability and Other Surface Properties of *Quercus ilex* Leaves as Affected by Leaf Side and Developmental Stage

Victoria Fernández¹, Paula Guzmán¹, Domingo Sancho-Knapik², José Javier Peguero-Pina², Mohamed Khayet³, Luis Gil¹, Eustaquio Gil-Pelegrín²

¹Technical University of Madrid, Madrid, Spain, ²Agrifood Research and Technology Center of Aragon, Zaragoza, Spain, ³Complutense University of Madrid, Madrid, Spain

Plant surfaces have unique properties to fulfill both protective roles against biotic and abiotic stress factors and controlling for example the exchange of gas and water with the environment. Trichomes are among the structural features that may have a major effect on surface topography and wettability at the micro-scale level and are often considered fundamental for plants growing in arid environments. Following a physico-chemical approach, we aimed at evaluating the surface properties of *Quercus ilex* L. subsp. *ilex* leaves to improve our understanding of the functional significance of leaf wettability in relation to the presence of trichomes. The adaxial and abaxial surfaces of young and mature leaves of *Q. ilex* were examined by electron microscopy. The surface free energy, polarity, work of adhesion and solubility parameter of each surface were calculated from contact angle measurements of water, glycerol, and diiodomethane. The chemical composition of isolated trichomes was determined by successive extractions of their components. The differential roughness and chemical composition of the two leaf sides, which varies also with leaf age influences the liquid behavior on these surfaces. The adaxial surfaces have a hydrophilic and wettable character and present drop adhesion in contrast to the highly hydrophobic, non-wettable and drop repellent abaxial leaf sides. The surface free energy, surface polarity and solubility parameter decreased with leaf aging, with generally higher values for the abaxial side. These surface properties have physiological implications and influences water- or pathogen-plant surface interactions.

P554

Impact of Weather Conditions and Light Availability on Dry Calcareous Grassland Vegetation in Experimental Conditions

Tiiu Kupper, Nele Ingerpuu, Priit Kupper

University of Tartu, Institute of Ecology and Earth Science, Tartu, Estonia

Dry calcareous grasslands are a decreasing vegetation type in Europa. In Estonia the main reasons for this are the lack of traditional management and overgrowing with trees.

The aim of the study was to investigate the impact of air humidity manipulation and other environmental variables on cover and species richness dynamics of mosses, lichens and vascular plants. For set up of the experiment soil patches of calcareous grassland vegetation were taken from West-Estonian dry grassland and translocated to the plots (\varnothing 13 m) of Free Air Humidity Manipulation (FAHM) experimental site in East-Estonia in May 2008. Altogether 30 containers with soil patches containing 60 plots of 10 cm \times 10 cm were placed into experimentally afforested plots with three treatments (control, humidification and drying) at FAHM site. Additionally five containers with ten plots were placed on open grassland site nearby. The analysis of vegetation was carried out at the end of July 2008 (initial state) and afterwards in autumn of 2009, 2010 and 2011.

The results show, that there are differences in the growth of mosses, vascular plants and lichens between the study years and between the treatments with different light availability. In the four study years moss cover increased significantly, but the cover of the two dominant species behaved differently. More light-sensitive vascular plants disappeared or decreased drastically in cover.

P555

Senescence-Associated Proteolysis Induced by Abiotic and Biotic Stresses in Barley Leaves

Blanca Velasco-Arroyo¹, Mercedes Diaz-Mendoza¹, Pablo Gonzalez-Melendi¹, M. Estrella Santamaria¹, Ana Arnaiz¹, Jose Dominguez-Figueroa¹, Goetz Hensel², Jochen Kumlehn², Manuel Martinez¹, Isabel Diaz¹

¹Centro de Biología y Genómica de Plantas (CBGP-UPM-INIA). Universidad Politecnica e Madrid. Campus de Montegancedo, Autovía M-40 (km 38), 28223 Pozuelo de Alarcon, Madrid, Spain, ²Plant Reproductive Biology, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK). Corrensstr. 3, 06466 Gatersleben, Germany

Leaf senescence is a strictly controlled process modulated by endogenous and exogenous factors such as hormones, nutrient starvation, dark, temperature, drought, pathogen infection and pests. It is characterized by a massive degradation of macromolecules to relocate nutrients from leaves to growing or storage tissues. Among the 800 proteases encoded by plant genomes, approximately 140 correspond to cysteine-proteases (CysProt). C1A Cys-Prot are the most abundant enzymes responsible for the proteolytic activity during leaf senescence of cereals. The main goal of this work is to identify and characterize the whole C1A CysProt family members of barley involved in leaf senescence, to study their modulation by their proteinaceous inhibitors (cystatins) and to determine their roles mediated by abiotic (darkness and N starvation) and biotic (pathogens and phytophagous pest attack) stresses. Barley has been selected for this study because it is an important crop for feed and food production, represents a useful experimental model for a number of small-grain cereals and can be efficiently transformed for basic and applied uses. To deal with these aims, a molecular and biochemical characterization of C1A CysProt members has been performed, including mRNA and protein expression patterns in response to different stresses, subcellular location, purification of recombinant proteases/ cystatins, or *in vitro* proteolytic assays, using novel and appropriate experimental approaches. To elucidate the *in vivo* participation of these proteins during leaf induced senescence some CysProt or cystatin encoding genes have been silenced and over-expressed in homozygous transgenic barley using *Agrobacterium*-mediated gene transfer and haploid technology.

P556

Stress Escape: A Transcriptomic Analysis of Ethylene and Shade Induced Hypocotyl Elongation in *Arabidopsis*

Debatosh Das, Ronald Pierik, L.A.C.J Voesenek, Rashmi Sasidharan
Utrecht University, Utrecht, The Netherlands

Due to their sessile nature plants have developed strategies to acclimatize to their challenging, dynamic environment. Morphological responses such as accelerated stem elongation allow a plant to escape from stressful conditions. Two environmental conditions eliciting strong shoot elongation are shade and submergence. During shade avoidance, changes in the light environment caused by neighboring plants can elicit rapid shoot elongation to access better lit areas of a canopy. When submerged, rapid accumulation of the volatile hormone ethylene causes vigorous shoot elongation in some plant species to outgrow floodwaters and resume aerial contact. The aim of the current study was to gain a more comprehensive understanding of the distinct and common signaling and regulatory events mediating these strikingly similar phenotypic responses.

To this end, we characterized the transcriptomic responses over time during *Arabidopsis* hypocotyl elongation as a stress escape phenotype in response to ethylene (as a proxy for a flooding signal) and green shade (mimicking vegetation shade). To add spatial, organ-specific resolution and identify distinct responses we harvested the hypocotyl and cotyledon separately. Initial genome wide transcriptome analyses combined with mutant & pharmacological studies revealed distinct signal- and organ-specific temporal regulation of specific gene cohorts and evidence of roles for auxin, brassinosteroid and gibberellin as important players.

We further delineate a possible signal transduction network involving stimulus-specific, and common signal transduction components.

P557

Investigating the Effects of Solar UV-A and UV-B on Leaf Development and Photosynthetic Properties in Sunflower (*Helianthus annuus*)

William Salter, Mark Adams, Tarryn Turnbull
The University of Sydney, Sydney, NSW, Australia

We studied the effects of solar ultraviolet radiation on development and senescence of the primary leaf in the dwarf sunflower (*Helianthus annuus*). Plants were grown outdoors under one of three spectral filters: photosynthetic photon flux density only (PPFD, $\lambda > 400$ nm filter), PPFD & UVA (+UVA, $\lambda > 320$ nm filter) and PPFD, UVA & UVB (+UVB, all wavelengths in natural insolation). We measured leaf gas exchange, morphology and leaf chemistry weekly and harvested plants for plant biomass determination once senescence was observed (after 42 days). During early leaf development plants grown under the PPFD treatment exhibited the greatest rates of triose phosphate utilization ($p < 0.05$), suggesting an enhanced capacity for fast growth. Specific leaf area was least in leaves grown under +UVB ($p < 0.05$), consistent with a light-saturated environment. Photosynthetic rates were greatest 14 days after germination for all treatments, however senescence proceeded quickest in the +UVA treatment as detected by a decreased net photosynthetic rate from 21 days after germination onwards ($p < 0.05$). Spectral environment impacted upon plant structure; plants in the +UVA treatment were significantly taller ($p < 0.05$) and longer between-leaf internodes ($p < 0.05$), suggesting that receipt of PPFD & UVA in the absence of UVB promotes shoot elongation, without any subsequent effect on plant biomass. From this study it can be concluded that solar UV affects the normal development of *H. annuus* leaves, with exclusion of UVA and UVB promoting early leaf growth and exclusion of UVB alone inducing premature leaf senescence.

P558

Quantitative Estimation of Phenotypic Plasticity of *Adonis distorta* Growing on Mount Majella in the Central Apennines

Loretta Gratani¹, Anna Rita Frattaroli², Valter Di Cecco², Giacomo Puglielli¹, Laura Varone¹,
Rosangela Catoni¹

¹Department of Environmental Biology. Sapienza University of Rome, Rome, Italy, ²Department of Life Health and Environmental Sciences. University of L'Aquila, L'Aquila, Italy

Model forecasting changes in species distribution as a function of climate scenarios for the 21st century predict a massive reduction in plant diversity where high mountain systems are likely to be particularly vulnerable. A large adaptability to growth conditions (i.e. large phenotypic plasticity) could result in a lower probability of extinction. *Adonis distorta* Ten. is included in the Regional Red List of the Italian Flora as LR, categorized as DD in the IUCN Red List, listed in the II and IV Annex of the Habitats Directive, and in the Appendix I of the Bern Convention. The species distribution area is restricted to the limestone massifs of the Central Apennines where it grows on high-altitude screes (2,000-2,500 m a.s.l.) characterized by small clasts. The main objective of this research was to analyze phenotypic plasticity of *A. distorta* growing in different environmental conditions (A population at 2675 m a.s.l. on a north-east-facing slope; B population at 2633 m a.s.l. on a south-southwest-facing slope) on Mount Majella (Central Apennines). Plant trait variations between A and B populations was measured by the phenotypic plasticity index sensu Valladares (PI = 0.21, mean of physiological and morphological plant traits). The measured PI reflects a relatively low capability of *A. distorta* to express different phenotypes in different environmental conditions. If *A. distorta* populations do not possess sufficient phenotypic plasticity it could be unable to respond to many drivers of global climate change such as elevated temperatures and reduced water availability, leaving no alternative to extinction

P559

Nitrate Transport as Key Factor Conditioning Responsiveness of *Arabidopsis* Plants to Elevated CO₂ Conditions

Ivan Jauregui^{1,2}, Pedro M^a Aparicio-Tejo^{1,2}, Iker Aranjuelo^{1,2}

¹Instituto de Agrobiotecnología (IdAB), CSIC, Mutilva, Navarra, Spain, ²Universidad Pública de Navarra, Pamplona, Navarra, Spain

Although current atmospheric [CO₂] is limiting for C₃ plants, exposure to elevated [CO₂] have been frequently describe to induce photosynthetic down-regulation. Photosynthetic acclimation is now well documented, however, still some uncertainty remains on the mechanisms leading to it and the implication of roots in such response.

Arabidopsis thaliana plants (grown in a hydroponic system) were exposed (during 8 weeks (prior to flowering) to 400 versus 800 ppm [CO₂]. N was supplied as 0.75 mM KNO₃. Growth chamber conditions were 16/8h photoperiod, 22/18°C thermoperiod, 80% RH, 200 μmol photons m⁻² s⁻¹ PPFD.

Results showed that, even if elevated [CO₂] increased biomass and photosynthetic rates, general decrease in N availability (Rubisco, total soluble protein and N content decreased) was detected in those plants. Parallel to such decline, a noticeable increase in leaf non structural carbohydrate content was observed. Together, this data stated that those plants were subjected to photosynthetic acclimation. Gene expression analyzed by microarrays highlighted the fact that roots were more affected than leaves by [CO₂]: 48 genes were differentially expressed in leaf and 173 in root. Nearly 80% genes were induced in elevated CO₂. Up-regulation of genes involved in root respiration, transport, protein degradation, redox signaling and stress processes were detected. Strong induction of nitrate transporters nrt 1.5 and nrt 2.2 did not prevent nitrate accumulation in roots. Moreover, depleted transpiration pull, remarked that nitrate transport restrictions could have exacerbated leaf N availability under elevated [CO₂]. Leaf N constraints led to the consequent down-regulation of photosynthesis.

P561

Using Non-Invasive Techniques to Determine Grapevine Performance in Response to Different Nitrogen Supply (*Vitis vinifera* L.)

Susanne Tittmann¹, Otmar Löhnertz², Manfred Stoll¹

¹Hochschule Geisenheim University, Institute of general and organic viticulture, Geisenheim, Hesse, Germany, ²Hochschule Geisenheim University, Institute of soil science and plant nutrition, Geisenheim, Hesse, Germany

Climate change experienced by increased temperatures, occurrence of extreme weather events and increasing CO₂ concentration leads in consequence to altered physiology and productivity of crop. The major phenological stages such as budburst or flowering of grapevines are influenced by the increased temperatures and lead to an earlier onset of ripening. Further, the uneven distribution of precipitation plays a key role within the development of vines. Drought during flowering and the initial berry development can result in disadvantageous of growth. Such issues represent future challenges to viticulture. Non-invasive techniques (polyphenolmeter: Multiplex®, FORCE-A; chlorophyllfluorescence) were used to investigate the response of grapevines to different water and nitrogen supply. The results of the polyphenolmeter showed a positive correlation with the nitrogen supply but only a weak feedback to water deficit situation. Chlorophyllfluorescence parameter responds with an increase in non-photochemical quenching and reduced quantum yield. The soluble solids in the berries were not significantly different between the treatments. The effect on berry quality and ripening throughout the season was analysed non-invasively via ImagingPAM and Multiplex. We found a correlation between the ripening status of single berries and the data gathered with non-invasive techniques. The higher nitrogen supply resulted in an increased amount of yeast available nitrogen (NOPA) and amino acids in the must. During the season 2013 a polyphenolmeter attached to a vehicle was used to record leaf parameters and generate maps of vineyard. The uses of such non-invasive techniques have the potential to be implemented in future vineyards management estimating vine's performance.

P562

Characterization of Different Grapevine (*Vitis vinifera* L.) Cultivars Based on Drought Induced Acclimation Mechanisms at Leaf Level

Péter Teszlák, Krisztián Gaál, János Werner, Anna Csikász-Krizsics
Research Institute for Viticulture and Oenology, University of Pecs, Pecs, Hungary

Different impacts of water deficiency on water relation and CO₂ gas exchange in grapevine leaves were studied on two cultivars belong to various ecogeographical groups. Fourteen-year-old vines of *Vitis vinifera* L. cultivars - 'Furmint' (autochthon) and 'Sauvignon blanc' - were investigated in a non-irrigated vineyards on the Mecsek Hills, in Hungary (46°07' N; 18°17' E, 230 m a.s.l.). The cultivars were studied under drought conditions and well-watered status. Drought stress resulted in increasing cell wall rigidity in both cultivars and affected the linear correlation between elasticity modulus (' ϵ ') and relative water content at the turgor loss point (RWC_{TLC}). Water deficiency resulted in increasing ' ϵ ' values (' ϵ ' > 9 MPa) at 'Furmint', indicating decreased cell wall elasticity. Osmotic potential at full turgor (Π_{100}) in stressed 'Sauvignon Blanc' vines was lower compared to the controls. The analysis demonstrated that varieties showed a negative linear correlation between apoplastic water content (A_{WSD%}) and leaf water potential at the turgor loss point (Ψ_{TLP}). Values of C_i increased parallel with increasing net CO₂ assimilation in a range between 18- 27 Pa of C_i both in well watered and stressed vines. In each cultivar, intrinsic water use efficiency (WUE_i) increased at reduced stomatal conductance. WUE_i was significantly (P < 0.05) higher in drought stressed vines compared to the controls in both cultivars irrespective of the different ecogeographical origins.

"This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program'."

P563

The Role of Stored Water in Stem Water Transport Investigated through Linear Variable Displacement Transducers (LVDT): Do Trees Have a Pulse?

Pilar Pita¹, Jaime Puertolas², Luis Gil¹

¹*Universidad Politécnica de Madrid, Madrid, Spain,* ²*The Lancaster Environment Centre. Lancaster University., Lancaster, UK*

Reversible stem swelling and shrinking monitored through linear variable displacement transducer (LVDT) can be attributed to water gain or loss from elastic plant tissues. Flow-induced oscillations of elastic tubes have been documented in many engineering and biomechanical systems. We aimed at investigating whether such oscillatory flow could be detected in stem tissues in order to gain some insight into the contribution of stored water to the ascent of sap.

Four to six LVDT sensors were placed at different heights in the stem of four *Eucalyptus globulus* seedlings. Both increasing soil water or decreasing transpiration led to a steep increase in stem diameter (up to 102 μm in 15 minutes). Maximum daily stem shrinkage (mds) was highest in the most apical stem sections. Maximum stem hydraulic conductivity was lowest in those plants in which the highest mds values were measured, as could be expected from the Ohms law analogy whenever mds values were correlated to xylem water potential.

Data measured every 30 seconds and 2-min means revealed oscillatory patterns of stem swelling and shrinking, with series of up to 24 consecutive waves of quite similar wavelength that could be detected at different heights in the stem and both when the sensor was placed over bark or directly over the xylem.

Oscillatory patterns previously reported in transpiration, stomatal conductance and sap flow had much longer wavelengths (70 min) than those found in the present study (<10 min). The relevance of short-wavelength oscillations in xylem sap transport is discussed.

P564

Comparative Analysis of Physiological Responses of *T. monococcum* and *T. aestivum* to UV-B Irradiation

Irina Moskova, Nina Georgieva, Iskren Sergiev
Institute of plant physiology and genetics, Sofia, Bulgaria

A comparative study on the damages caused by UV-B in einkorn (*T. monococcum*, harvested in Eastern Rhodopes, Bulgaria) and conventional wheat (*T. aestivum*, var. Sadovo-1) was carried out. Recently, the interest to ancient wheats (emmer, einkorn) is rising because they outmatch the traditional varieties of wheat by their higher nutritional value. A major drawback for growing einkorn appears to be the lower yield. Ancient wheats populate mainly mountainous regions, which presumes a higher adaptability to unfavorable environmental conditions. An increased tolerance of einkorn to abiotic and biotic stresses is documented, but the data are rather controversial, most probably due to differences in the subspecies.

The study of the biochemical responses of einkorn to various stress factors will enlarge the knowledge of the possible mechanisms for overcoming the negative consequences, and could give a ground for breeding of new cultivars with increased productivity and resistance. Young wheat seedlings were subjected to UV-B irradiation for 5 h. The samples for analyses were collected after 3 days when there were visible symptoms of damage.

The physiological status was assessed by the changes of plant metabolites related to oxidative stress - free proline, leaf pigments, phenols, free thiols, and activities of antioxidant enzymes.

The data observed showed that the young einkorn plants are more susceptible to applied UV-B irradiation than the conventional variety of wheat.

This work was supported by the grant №BG051PO001-3.3.06-0025, financed by the European Social Fund and Operational Programme Human Resources Development (2007-2013) and co-financed by Bulgarian Ministry of Education and Science.

P565

Role of Isoprene in Tobacco Response and Acclimation to Short-Term UV-B Radiation. A Thermoluminescence Study.

Violeta Peeva¹, Liliana Maslenkova¹, Violeta Velikova¹, Tsonko Tsonev¹, Claudia Vickers², Francesco Loreto³

¹Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria, ²Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St. Lucia, QLD, 4072, Australia, ³The National Research Council of Italy (CNR), Department of Biology, Agriculture and Food Sciences, Piazzale Aldo Moro 7, Rome 00185, Italy

Thermoluminescence emissions from genetically manipulated tobacco plants (*Nicotiana tabacum* L.) able to emit isoprene at levels as naturally emitting species, and their non-emitting controls were compared. In isoprene emitting leaves the B band temperature peak position was always upshifted, thus indicating the more stable stored charge pairs. Moreover, a decrease in the dark-stable proton gradient occurs in these leaves with less downshift of maximal temperature of B band after excitation by flashes.

The UV-B part in the solar spectrum provokes a large number of responses in higher plants and is characterized with high potential to damage the photosynthetic function. We examined the thermoluminescence response of leaves exposed to elevated UV-B radiation (10 W m^{-2}) and the recovery period after irradiation to study whether the presence of isoprene increase the plant tolerance to this kind of stress. Isoprene was found to act with a protective role in various environmental stress conditions (Loreto, Schnitzler (2010) Trends Plant Sci. 15: 154-166; Velikova et al. (2011) Plant Physiol 157: 905-916) by stabilizing thylakoid membranes and/or lowering ROS penetration inside thylakoids. The non-emitting leaves showed the inactivation of a fraction of PSII centers and higher capacity for cyclic electron transport pathway(s) in comparison to versatile and adaptable thermoluminescence response by generation of transthylakoid proton gradient in the emitting leaves. *This work was supported by the grant №BG051PO001-3.3.06-0025, financed by the European Social Fund and Operational Programme Human Resources Development (2007–2013) and co-financed by Bulgarian Ministry of Education and Science.

P566

The Effect of Light Quality on Photosynthetic Responses of *Platanus orientalis*

Vesela Yordanova¹, Violeta Peeva¹, Tsonko Tsonev¹, Violeta Velikova¹, Ivaylo Milenkov², Liliana Maslenkova¹

¹*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, acad. G. Bonchev str., bd 21, Sofia 1113, Bulgaria,* ²*OctaLight PLC, bul. Bulgaria 102, Sofia 1680, Bulgaria*

Platanus orientalis plants were cultivated for 4 months in a growth chamber at different combinations of monochromatic LED lighting and under cool-white fluorescent lamps (CWF) at equal irradiance of $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the tops of the plants, 12-h photoperiod, 25/25 °C day/night temperatures and 60-70 % relative humidity. Three different types of LEDs, emitting in the red (635 nm, R), green (531 nm, G), and blue (463 nm, B) region of the spectrum are used to compose 66:33% RB and 33:33:33% RGB light. The control of the different spectrum combinations is utilized by DMX controller.

The spectral composition of RB light showed a clear positive effect on plant growth characteristics and net CO₂ assimilation in comparison with plants, cultivated under RGB and CWF light. The increase in A_{max} in leaves under RB light was associated with an increase in leaf mass per unit leaf area, chlorophyll content per area and stomatal conductance. PSII activity was also higher in the leaves grown at RB plants, compared to other light conditions.

The obtained results are discussed in terms of the regulatory effects of light quality on the development and the structural-functional relationships of photosynthetic apparatus in isoprene-emitting plants.

Acknowledgments: This work was supported by the grant №BG051PO001-3.3.06-0025, financed by the European Social Fund and Operational Programme Human Resources Development (2007–2013) and co-financed by Bulgarian Ministry of Education and Science and the technical support of the Octa Light PLC in the field of artificial light for HortCultures.

P567

Does Mesophyll Conductance of Wheat Change with Leaf Age and Water Availability

Eisrat Jahan, Margaret Barbour
The University of Sydney, NSW, Australia

Understanding the effect of leaf age on photosynthetic capacity is necessary to estimate the long-term carbon budget of the leaf and the whole plant. Mesophyll conductance (g_m) influences photosynthetic rate and leaf intrinsic water-use efficiency (A/g_{sw}), and has been found to respond with environmental conditions and leaf anatomical traits. To understand the combined effect of leaf and plant age, and water limitation on g_m in wheat (*Triticum aestivum* L.), we used a leaf gas exchange system coupled to a tunable-diode laser absorption spectrometer to measure carbon isotope discrimination and allow estimation of g_m . Significant reductions in g_m were found as leaves aged (88% reduction) and plants aged (58% reduction in g_m of the youngest fully-expanded leaf over five weeks of growth). g_m was lower in droughted plants compared to irrigated, although differences became insignificant as the drought progressed. g_m and A were more strongly correlated than g_m and g_{sc} for both irrigated and drought condition. In this study, plants with high photosynthetic rate and g_m also tended to have high WUE. These results underscore a high level of variability in wheat g_m in response to environmental and physiological conditions.

P568

Biogenic Isoprene - Tool for Stress Protection in Arundinoideae Plants Species

Dilyana Doneva¹, Mastaneh Ahrar², Cecilia Brunetti^{3,4}, Dimitrinka Koleva⁵, Miroslava Stefanova⁵, Violeta Velikova¹

¹Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria,

²Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione

Edmund Mach, S. Michele all'Adige (TN), Italy, ³Institute of Plant Protection, National Research

Council (CNR), Florence, Italy, ⁴Department of Agri-Food and Environmental Sciences, University of

Florence, Florence, Italy, ⁵Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

A large array of volatile organic compounds (VOCs) are emitted into the atmosphere by the leaves of many plant species. Among the biogenic VOCs studied, isoprene is quantitatively the most important. It was demonstrated that isoprene has an important role in plant defence against oxidative stress. In order to expand our understanding of isoprene function in plants, we investigated whether isoprene lack relates to increased amount of other secondary defence compounds under control conditions.

We used plants from Arundinoideae subfamily (*Arundo donax*, *Arundo collina* and *Hakonechloa macra*) which differ in their ability to emit isoprene as a natural metabolite. Plants were evaluated in terms of photosynthesis and chlorophyll fluorescence, amount of xanthophyll pigments and phenolic compounds. Leaf anatomy and chloroplast ultrastructure analysis were also employed to study if the plants have different structural strategies to cope with a stress.

A. collina has the highest capacity to emit isoprene, followed by *A. donax*. *H. macra* does not emit isoprene. No other volatile isoprenoids were detected among all plant species studied. *A. collina* and *A. donax* are characterized with higher photosynthetic rate and higher photochemical efficiency of PSII compared to *H. macra*. A significant lower level of non-photochemical quenching, higher amount of xanthophyll pigments and phenolic compounds, and better preserved thylakoid membranes were detected in *Arundo* species compared to *H. macra*. These data demonstrate that isoprene presence triggers adjustment of plant metabolism and chloroplast ultrastructure to improve plant performance under stress.

Supported by the grant №BG051PO001-3.3.06-0025.

P569

Physiological Differences during Photosynthetic Recovery in Different Functional Groups under Winter Stress Conditions

Fátima Míguez, Beatriz Fernández-Marín, José María Becerril, José Ignacio García-Plazaola
University of Basque Country (UPV/EHU), Leioa, Spain

Sustained downregulation of photosynthesis (winter photoinhibition –WPI-) is a frequent response of woody plants, when low temperatures are accompanied by high solar radiation. Under these conditions, there is an upregulation of photoprotective processes accompanied by a downregulation of photosynthesis. Two recovery types have been described (Verhoeven 2013): rapid form which reverses within minutes to hours and slower, reversible in several days. These processes have been studied in evergreen trees and in crops, but little is known about other organisms that typically live under these stressful conditions in their habitats. So, we wonder whether WPI is present in shrubs, herbs, lichens and mosses and how fast their recovery is. The sampling of representative species from all functional groups was carried out in the north of Spain (~1750 a.s.l). Fluorescence measurements were taken to calculate the rapidly and slowly reversible component of photoinhibition as well as recovery kinetics. Their pigment composition and thylakoid proteins were also analyzed. Results reveal that woody species do not become fully recover in 6 days, indicating they have mostly the slowly component. Mosses and herbaceous do not present a sustained downregulation of photosynthesis during winter. By contrast, lichens present only the rapidly reversible component. Nevertheless, pigment determination show that, in 12 hours under warm conditions, independently of functional group and photochemical efficiency, zeaxanthin disappears, suggesting that the maintenance of WPI is not directly related to the accumulation of zeaxanthin. The patterns of changes in thylakoid proteins were also analyzed in all these species during the recovery.

P570

Improvement of Rape (*Brassica napus* L.) Seeds Germination by Osmopriming: A Combined Transcriptomic and Proteomic Approach

Szymon Kubala¹, Muriel Quinet², Lukasz Wojtyla¹, André Clippe³, Arkadiusz Kosmala⁴, Stanley Lutts², Malgorzata Garnczarska¹

¹Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland,

²Groupe de Recherche en Physiologie Végétale (GRPV), Earth and Life Institute – Agronomy (ELI-A), Université catholique de Louvain, Louvain-la-Neuve, Belgium, ³Institut des Sciences de la Vie, Université catholique de Louvain, Louvain-la-Neuve, Belgium, ⁴Laboratory of Cytogenetics, Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland

To better understand the beneficial effect of osmopriming on rapeseed germination the global expression profiling methods (i.e. transcriptomics and proteomics) were used. The combined approach allowed, for the first time, the comparison of proteomic and transcriptomic data for osmoprimed seeds at the crucial phases of priming procedure (soaking, drying), whole priming process and subsequent germination. Our data showed that a total number of 952 genes and 75 proteins were affected during the main phases of priming and post-priming germination. In general, more genes were up-regulated during osmopriming treatment and post-priming germination. Regarding the different phases of priming, most of genes down-regulated during PEG soaking were affected in opposite way during drying. There were more proteins which abundance decreased in response to PEG soaking and seed drying while most of the proteins analysed during complete osmopriming and post-priming germination showed an increase of their abundance during these processes. Progress towards germination in primed seeds was associated with an increase in protein synthesis potential, post-translational processing capacity and targeted proteolysis. Advanced germination of primed seeds was also linked to higher expression of genes and proteins involved in water transport, cell wall modification, cytoskeletal organization, cell division and oxidative stress management.

This work was supported by grant no. 2011/03/B/NZ9/00068 from the National Science Centre. SK obtained financial support for the best PhD students in PO KL 8.2.2. programme editions: 2011/2012 and 2012/2013 and from National Science Centre DEC-2013/08/T/NZ9/01019. SK is a scholarship holder of The Adam Mickiewicz University Foundation in 2013/2014.

P571

Proline Metabolism in Osmoprimed Rape (*Brassica napus* L.) Seeds Germinating under Salinity Stress

Szymon Kubala¹, Lukasz Wojtyla¹, Muriel Quinet², Katarzyna Lechowska¹, Stanley Lutts², Malgorzata Garnczarska¹

¹Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland,

²Groupe de Recherche en Physiologie Végétale (GRPV), Earth and Life Institute – Agronomy (ELI-A), Université catholique de Louvain, Louvain-la-Neuve, Belgium

Seed priming is a presowing treatment that improves the ability of seeds to germinate. The aim of this study was to determine the effect of osmopriming on the acquisition of salinity tolerance by germinating rape (*Brassica napus* L.) seeds. Germination tests performed in the presence of 100 mM NaCl showed that priming increases the germination rate and uniformity under salinity conditions. Proline level and the expression of genes for key enzymes of proline turnover were studied. In rape seeds germinating under control conditions, proline level was about 2.8-fold higher in primed seeds as compared to unprimed seeds. Under salinity conditions, the primed seeds contained about four times more proline than the unprimed ones. Moreover, P5CSA (encoding one of two isoforms of Δ 1-pyrroline-5-carboxylate synthetase) was up-regulated in primed seeds compared to unprimed seeds and PDH (encoding proline dehydrogenase) was down-regulated under both control and salt stress conditions. These results suggest that the one of the advantages of osmopriming which contributes to enhanced salt stress tolerance of germinating rape seeds is modulation of proline metabolism. Enhancement of proline anabolism with depletion of its catabolism in osmoprimed seeds speak in favor of this hypothesis.

This work was supported by grant no. 2011/03/B/NZ9/00068 from the National Science Centre. SK obtained financial support for the best PhD students in PO KL 8.2.2. programme editions: 2011/2012 and 2012/2013 and from National Science Centre DEC-2013/08/T/NZ9/01019. SK is a scholarship holder of The Adam Mickiewicz University Foundation in 2013/2014.

P572

Relationships among Soil Properties, Functional Leaf Traits, Fruits and Oil Quality in Different Cultivars of *Olea europaea* L.

Costantina Barbarisi¹, Carmen Arena¹, Mariagrazia Volpe², Anna De Marco¹
¹University of Naples Federico II, Naples, Italy, ²CNR-ISA, Avellino, Italy

Extra virgin olive oil (EVOO) is a typical product of the Mediterranean region. The composition of EVOO may be influenced by different factors: cultivar (cv), climatic and edaphic conditions, time of harvesting, stage of maturity and integrity of the fruit, and the oil production technology. Each cultivar is generally associated to specific physical, chemical and biological soil properties as well as to functional leaf characteristics. The aim of this work was to assess the relationships between soil properties, functional leaf traits, fruits and oil quality in different Italian *Olea europaea* L. cultivar (Pisciottana, Ravece, Ortice, Frantoio and Moraiolo). Soils under Ravece showed the highest water content and leaf specific mass (SLA) but fruits and oils presented an extremely low total polyphenols content (TPC). In Ortice cultivar where the lowest soil organic matter content and microbial respiration were found, oil exhibited a reduced amount of TPC and antioxidant activity. Pisciottana cv. characterized by leaves with highest values of C/N and lowest relative water content (RWC) and showed lowest production of total polyphenols in both fruits and oils. Soil under Frantoio and Moraiolo showed the lowest content of OM, CEC, microbial respiration and the lowest SLA. Although these properties suggest a lower productivity, the analyses on fruits and oils revealed the highest content of total polyphenols. Here the 89% of the antioxidant capacity of fruits derived by phenolic compounds.

P573

Drought Response in Seedlings of Norway Spruce Half-Sib Families

Daniel J. Chmura, Marzenna Guzicka
Institute of Dendrology, Kornik, Poland

Norway spruce (*Picea abies* L. [Karst]) is an ecologically and commercially important forest tree species in Central European countries. It is, however, rather susceptible to drought. In the last years spruce health has deteriorated throughout Europe, and droughts may have been a priming factor in this decline. Therefore, increased resistance to drought may be favored in the future, because scenarios of future climate project an increase in the intensity and duration of droughts.

The objective of this study was to investigate the extent of within-species variability of traits associated with drought resistance in Norway spruce. For this purpose, we observed responses to experimental drought of growth and physiology in seedlings of seven half-sib families and two populations (provenance level) of Norway spruce during their third growing season from seed. The three treatments in the experiment involved drought, intermittent drought, and control.

For most traits investigated in the study, variation among families was not significant, but treatments differed significantly. Drought significantly reduced leaf level photosynthesis and stomatal conductance starting already at xylem water potential of about -1.0 MPa. After breaking drought, photosynthesis increased immediately, but did not recover to the control level for at least three weeks. Drought also affected the dynamics of nonstructural carbohydrates in current-year needles, either preventing build-up of starch (drought) or shifting the time (intermittent drought) the peak starch was observed compared to control. We will also present the results regarding growth and hydraulic properties of xylem in examined seedlings.

P574

Induction of Root Fe (III) Reductase Activity in Invasive Woody Plant *Ailantus altissima* (Mill.) Swingle

Danijela Đunisijević-Bojović, Matilda Đukić
Belgrade University Faculty of Forestry, Belgrade, Serbia

Acquisition of iron and phosphorus in the rhizosphere and regulation of their uptake and translocation is of great ecological importance for biomass production of higher plants on calcareous soils. Uptake of Fe in dicots depends on activation of mechanisms, which providing acidification of the rhizosphere and reduction of Fe (III)-chelates to Fe²⁺ ions. These mechanisms are induced under limited Fe availability and there are strongly correlated to Fe deficiency tolerance in annual crops and fruit trees but there are poorly investigated in tree stock for landscape use as well as in invasive plant species.

In our experiments in hydroponic culture of invasive *Ailantus altissima* seedlings exposure to lack of Fe, caused a significant increasing the Fe (III) reductase activity ($P < 0.05$). High induction of Fe (III) reductase activity after 7 days of exposure was found. In plants with chlorosis, reductase activity increased 16.6 times, while in Fe deficient plants without developed chlorosis, this indicator was 19 times higher, compared to the control (Fe sufficient plants). Prolonged exposure to iron deficiency (for 3 weeks) led to a significant decrease of induced reductase activity ($1.45 \mu\text{mol g}^{-1}\text{h}^{-1}$) relative to the measured values after 7 days of exposure ($10.42 \mu\text{mol g}^{-1}\text{h}^{-1}$).

High induction of root Fe (III) reductase activity in invasive woody plant *Ailanthus altissima* indicates good capacity for iron mobilization. It can be considered as good model plant for investigation of nutritional adaptation mechanisms of invasive woody plants on calcareous soils.

P575

Factors of the Variation in Grassland Communities Overgrowing Old Heaps of Zn-Pb Mining Waste

Marcin Woch¹, Paweł Kapusta², Anna Stefanowicz²

¹*Institute of Biology, Pedagogical University of Kraków, Kraków, Poland,* ²*W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland*

The aim of this study was to investigate the variation in plant communities growing on metal-enriched sites created by historical Zn-Pb mining. The study sites (N=65) were small heaps of waste rock covered by grassland vegetation and scattered mostly over agricultural land of western Małopolska (S Poland). The sites were described in terms of plant community characteristics (coverage, species richness and species composition), soil physicochemical properties (pH, texture, heavy metal and nutrient content), and the distance from the forest. Plant communities were classified using phytosociological and ordination (DCA) approaches. Analyses showed that all plant communities belonged to the *Carlino acaulis-Brometum erecti* association, which occurred on heaps in three variants: 1) the typical subassociation (CB), 2) the *festucetum ovinae* subassociation (CBF), and 3) the *rubietum caesi* subassociation (CBR). According to multiple regression results, the heavy metal content in soil and the distance to the forest were the primary factors of the variation in the species composition – the CBF subassociation (distinguished by the abundance of metallicolous plants) replaced the typical one when the metal contamination was high, while the CBR subassociation (distinguished by the abundance of woody plants) dominated near the forest. The species richness (on average, 20, 20 and 17 species per 4 m², for CB, CBF and CBR, respectively) was affected by habitat properties to a much lesser extent than the species composition; the heavy metal content in soil was the most important factor of its variation (negative relationship).

Keywords: heavy metal, historical Zn-Pb mining, species composition, species richness

P576

Development of Cardoon Germplasm Adapted to Climatic Changes from Romania

Petcu Elena¹, Radu Steluta¹, Babeanu Narcisa², Popa Ovidiu²

¹National Agricultural Research and Development Institute, Fundulea, Romania, ²University of Agriculture Science and Veterinary Medicine, Bucharest, Romania

Cardoon (*Cynara cardunculus* L.) is a Mediterranean perennial plant, cultivated increasingly for herbs, vegetal foods and in last years especially as energy crop. In Romania the main barrier to cardoon production is the crop sensitivity to frost. A strategy for valorizing Romanian germplasm of globe artichoke and a foreign germplasm of cardoon has been carried out during last years in frame of Plant Medicinal Breeding program from NARDI Fundulea, Romania. The first objectives of this work consisted in (i) agro-morphological characterization of germplasm using UPOV descriptors, (ii) assessment of the genetic variability existing within and among genotypes and (iii) identification of genetic resources with frost resistance for the development of new genotypes with frost resistance and with high biomass production.

We started the mass selections and selection work was focused on frost resistance, uniformity, biomass yield and bio-compound production.

The results showed some genotypes are very promising for our goals. Moreover, the good range of variability observed among genotypes is important in breeding programs for developing cultivars suitable for Romanian conditions.

P577

Light and the E3 Ubiquitin Ligase COP1/SPA Control the Protein Stability of the MYB Transcription Factors PAP1 and PAP2 Involved in Anthocyanin Accumulation in Arabidopsis

Alexander Maier¹, Andrea Schrader¹, Leonie Kokkelink¹, Christian Falke¹, Bastian Welter¹, Elisa Iniesto², Vicente Rubio², Joachim Uhrig¹, Martin Hülskamp¹, Ute Hoecker¹

¹University of Cologne, Cologne, Germany, ²Centro Nacional de Biotecnología-CSIC, Madrid, Spain

Anthocyanins are natural pigments that accumulate only in light-grown and not in dark-grown Arabidopsis plants. Repression of anthocyanin accumulation in darkness requires the COP1/SPA ubiquitin ligase since *cop1* and *spa* mutants produce anthocyanins also in darkness. Here, we show that COP1 and SPA proteins interact with the MYB transcription factors PAP1 and PAP2, two members of a small protein family that is required for anthocyanin accumulation and for the expression of structural genes in the anthocyanin biosynthesis pathway. The increased anthocyanin levels in *cop1* mutants requires the PAP1 gene family, indicating that COP1 functions upstream of the PAP1 gene family. PAP1 and PAP2 proteins are degraded in darkness and this degradation is dependent on the proteasome and COP1. Hence, the light requirement for anthocyanin biosynthesis results, at least in part, from the light-mediated stabilization of PAP1 and PAP2. Consistent with this conclusion, moderate overexpression of PAP1 leads to an increase in anthocyanin levels only in the light and not in darkness. At last, we show that SPA genes are also required for reducing PAP1 and PAP2 transcript levels in dark-grown seedlings. Taken together, these results indicate that the COP1/SPA complex affects PAP1 and PAP2 transcriptionally as well as posttranslationally. Thus, our findings have identified mechanisms via which the COP1/SPA complex controls anthocyanin levels in Arabidopsis which may be useful for applications in biotechnology directed towards increasing anthocyanin content in plants.

P578

High Relative Air Humidity Increases Leaf Damages Caused by UV Radiation in *Pisum sativum*

Louise Arve, Arupillai Suthaparan, Jorunn Elisabeth Olsen, Sissel Torre
Norwegian University of Life Sciences, Aas, Norway

UV radiation has the highest energy per photon of any part of the solar spectrum and exposure to high levels of UV can cause DNA-, protein- and membrane lipid damage and result in decreased photosynthesis and visible injuries. In plant production UV radiation can be used for growth and disease control. It is therefore important to understand the effects UV radiation has on plants. *Pisum sativum* plants were grown in high (90%) and moderate (60%) relative air humidity (RH) with and without UV radiation from unscreened fluorescent tubes (Q-panel UV 313) 0.1 W/m² 40 minutes every night. Injuries such as chlorosis, necrosis and leaf curling were found in plants treated with UV in high RH. However, plants growing in moderate RH with UV showed no signs of chlorosis or necrosis, but leaf curling was observed. Plants treated with UV radiation showed reduced transpiration both during day and night, compared with the non UV treated plants irrespective of RH. However, desiccation tests using detached leaves showed significantly higher water loss from leaves treated with UV in high RH but not in moderate RH. This indicates that treating plants with UV radiation can cause unwanted injuries and change the water balance of the plants.

P579

UV-B Alleviates the Uncoupling Effect of Climate Change Conditions on Grapeberry (*Vitis vinifera* L. cv. Tempranillo) Anthocyanin-Sugar Accumulation

Johann Martínez-Lüscher^{1,3}, Manuel Sánchez-Díaz³, Serge Delrot^{1,2}, Jone Aguirreolea³, Inmaculada Pascual³, Eric Gomès^{1,2}

¹INRA, Institut des Sciences de la Vigne et du Vin, Villenave d'Ornon, Aquitaine, France, ²University of Bordeaux, Institut des Sciences de la Vigne et du Vin, Villenave d'Ornon, Aquitaine, France,

³Universidad de Navarra, Pamplona, Navarra, Spain

Modeling of future environmental conditions predicts an increase in temperature for most viticultural regions, mainly mediated by the anthropic release of CO₂. Therefore, some wine grape quality traits may get compromised. Furthermore, UV-B may have positive effects on grape composition. The aim of the study was to explore the interactions between temperature, CO₂ and UV-B radiation on anthocyanin and flavonol biosynthesis, in fruit-bearing cuttings under greenhouse controlled conditions. Three doses of UV-B: 0, 5.98, 9.66 kJ m⁻² d⁻¹, and two temperature-CO₂ regimes: 24/14°C (day/night)-395 ppm CO₂ and 28/18°C-700 ppm CO₂ were imposed at fruit set in a factorial design. Flavonol and anthocyanin content were determined (HPLC) in grape skin throughout development. Transcript levels of genes involved in flavonoid biosynthesis were measured one week after veraison. UV-B increased grape skin flavonols. However, lower concentrations were found under elevated temperature-CO₂. Anthocyanins increased faster during the first two weeks after veraison under high temperature-CO₂, due to higher soluble solids content (TSS). At maturity (same TSS), anthocyanins concentration were lower under high temperature-CO₂, thus suggesting that sugars and anthocyanins accumulation were uncoupled. UV-B always increased anthocyanins concentration, especially under elevated temperature-CO₂. Transcript levels revealed the up-regulation of structural (FLS1, CHS, OMT2 and GTS) and regulatory (MybF1 and MybA1) genes of flavonol and anthocyanin biosynthesis under UV-B. MybA1 was further up-regulated by high temperature-CO₂. In conclusion, UV-B alleviated anthocyanin sugar decoupling induced by elevated temperature-CO₂.

Acknowledgements

Spanish Ministry of Science and Innovation [BFU2011-26989], COST Action FA0906 UV4growth, Asociación de Amigos de la Universidad de Navarra.

P580

Stem Respiration Rates Decline with Drought in Two Tree Species of Contrasting Drought Resistance

Jesus Rodriguez-Calcerrada¹, Li Meng¹, Pilar Pita¹, Rosana Lopez¹, Guillermo Gonzalez¹, Jorge Dominguez¹, Owen K. Atkin², Luis Gil¹

¹Universidad Politecnica de Madrid, Madrid, Spain, ²Research School of Biology, The Australian National University, Acton, Australia

Respiration plays a role in plant fitness and its regulation could be an important factor to overcome stress periods. Here we subjected two-year-old seedlings of *Ulmus minor* and *Quercus ilex* to gradual drought. The objective was to evaluate how stem respiration rates were affected by drought and investigate potential factors that might underpin changes in respiration. We measured stem growth with linear variable displacement transducers (LVDTs), leaf photosynthesis at saturating irradiance and 25°C, and stem CO₂ efflux in darkness and 25°C as a surrogate of stem respiration. Upon cessation of watering, stem relative water content remained higher than 75% for 70 days in *Q. ilex* and 10 days in *U. minor*. Below 75% stem relative water content, growth ceased and the stem progressively shrunk as a result of water loss. Stem respiration per unit stem mass was significantly lower in droughted than control plants after 90 days of treatment in *Q. ilex* and 15 days in *U. minor*. For both species, positive correlations were found between stem respiration and relative water content, and between stem respiration and leaf photosynthesis. In view of these results we conclude that stem respiration is affected by drought. Faster water loss in the more drought-sensitive *U. minor* seedlings appeared to result in stem respiration rates declining earlier compared to *Q. ilex* upon drought imposition, although stem respiration was similarly affected by water stress in both species. Drought impacts on stem respiration rates appear mediated by reduced tissue water content, photosynthate production and stem growth.

P581

Leaf Structural Features and Relative Contribution of Photoprotective and Anti-Oxidative Mechanisms in Green Versus Reddish Leaves of Poinsettia (*Euphorbia pulcherrima*)

Julietta Moustaka¹, Eleftherios Eleftheriou¹, Georgia Ouzounidou², Georgia Tanou³, Ioannis-Dimosthenis Adamakis¹, Emmanuel Panteris¹, Grigorios Diamantidis³, Michael Moustakas¹

¹Department of Botany, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece,

²Institute of Food Technology, Hellenic Agricultural Organization-Demeter, Lycovrissi, Attica, Greece,

³Department of Horticulture, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece

Poinsettias are among of the most economically valuable floriculture plants worldwide, having red (bracts), reddish and green leaves due to differential vacuolar anthocyanin accumulation, which is influenced by environmental factors such as temperature and reduced photoperiod. Red leaves were found to be about two-fold thicker than green leaves due to their extensive intercellular spaces in the mesophyll, which moreover was not distinguished in a palisade and spongy parenchyma, as in green leaves. Reddish leaves were of intermediate thickness, with a poorly developed palisade parenchyma. Anthocyanin accumulation in red leaves was localized in both the upper and lower papilla-like epidermal cells, and in peripheral mesophyll cells. Green leaves did not contain pigmented cells, while in the reddish ones they were restricted to the upper epidermis. A differential photoprotective mechanism was revealed between green and reddish leaves, with the second ones exhibiting a higher H₂O₂-scavenging capacity, in part due to their higher ascorbate peroxidase isoform 1 (APX 1) activity. Reddish leaves possessed not only higher maximum quantum yield (F_v/F_m) and actual quantum yield (Φ_{PSII}) than greens, implying a higher electron transport rate (ETR), but also a higher oxidized redox state of quinone A (Q_A) and a reduced non-photochemical quenching (NPQ) for photoprotective heat dissipation. It is concluded that the induction of the NPQ photoprotective mechanism was not necessary in reddish leaves under low light (200 mE m⁻²s⁻¹), in contrast to green leaves, which having lower antioxidant protection and lower anthocyanin accumulation to attenuate light energy, the NPQ photoprotective mechanism was essential.

P582

The Effect of Water Delivery Variation on Resource Allocation of Four Grassland Plant Species, in Monoculture and Mixture

Eamon Haughey^{1,2}, Jennifer C. McElwain², John A. Finn¹

¹Teagasc, Environment Research Centre, Johnstown Castle, Wexford, Ireland, ²School of Biology & Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland

Changes to precipitation patterns may result in a higher frequency of drought and flooding occurrence. The increased instability associated with these changes may affect the functioning of natural and managed ecosystems. We investigate whether fluctuation in the water supply to plants affects resource allocation of biomass, and whether this response differs between plants grown in monoculture and mixture. In March 2013, *Lolium perenne* (perennial ryegrass), *Cichorium intybus* (chicory), *Trifolium repens* (white clover) and *Trifolium pratense* (red clover) seedlings were transplanted into one meter long pipes, diameter 10.15 cm. In each pipe there were; four plants of the same species in monoculture pipes and one of each species in mixture pipes (n = 4 treatment replications). Watering regimes for treatments were varied in three week cycles positively and negatively from the control; moderate treatment $\pm 40\%$ and extreme treatment $\pm 80\%$. Thus, all treatments received approximately the same total amount of water during the 24 week experimental treatment. Leaf material was harvested at a height of 5 cm every six weeks. At the end of the experiment total root biomass measured destructively. There were significant differences between species for both root and shoot biomass. Increased variation in water supply significantly reduced aboveground biomass production for the more productive communities; white clover, red clover and the four species mix but root biomass was not significantly affected. These results indicate that changes to the temporal distribution of precipitation patterns may differentially affect biomass production, with highly productive systems suffering greater impact.

P583

Compartment-Specific Changes in Glutathione and Its Precursor Levels under Different Environmental Situations

Maria Müller, Bernd Zechmann
Institute of Plant Sciences, Graz, Austria

Glutathione is an antioxidant and involved in the detoxification of reactive oxygen species, which are commonly formed during environmental stress situations, and lead to the destruction of biological membranes, proteins, RNA and DNA leading to mutation, cancer and eventually cell death. Changes in glutathione contents are therefore commonly used as stress markers during investigations in most fields of plant sciences.

For this purpose beside other techniques an immunogold cytohistochemical approach was developed and adapted to different plant material in order to detect and quantify subcellular glutathione and its precursors with computer-supported transmission electron microscopy. Surprisingly, plastids contained the lowest amounts of glutathione. Highest glutathione contents were always found in mitochondria, while glutathione-labeling in the cytosol, nuclei and peroxisomes was intermediate. No glutathione was detected in vacuoles and the apoplast.

The development and application of immunogold cytochemical methods to various plants under different environmental conditions revealed that glutathione precursors (especially cysteine) limit the operation of glutathione metabolism. The modification (increase) of cysteine contents in plants resulted in a strong increase in glutathione contents and subsequently in a higher stress tolerance. Thus these studies and methods can now be used for the development of new defense strategies for agricultural use in the future, and can protect farmers from possible crop losses induced by environmental stress situations in the future.

P584

Effects of Available Root-Zone Volume on Yield, Morphological and Physiological Traits and Essential Oil Content in Aeroponically-Cultivated *Ocimum basilicum* L.

Petros Tarantilis¹, K Argyropoulou², George Salahas², G. Kapotis², Dimitrios Savvas¹, E. Paspatis³
¹*Agricultural University of Athens, Athens, Attica, Greece,* ²*T.E.I. of Western Greece, Amaliada, Peloponnese, Greece,* ³*Benaki Phytopathological Institute, Kifissia, Attica, Greece*

A glasshouse experiment was conducted in order to investigate the effects of the available root zone volume on biomass content, morphological and physiological parameters and essential oil content of aeroponically grown sweet basil plants. Three aeroponic boxes (10 m in length, and 0.67 m in width) differing in the depth (0.15, 0.30 or 0.70 m) were used in a fully-automated aeroponic growing system. The results showed that plants cultivated in aeroponic boxes with a depth of either 0.15 or 0.30 m, developed faster and gave statistically higher values of above-ground plant dry biomass production, leaves per plant, and plant height, in comparison with those cultivated in deeper aeroponic boxes (0.70 m). Furthermore, box depths of either 0.15 or 0.30 m were associated with significantly higher net assimilation rates and chlorophyll content, in comparison with a box depth of 0.70 m. In contrast, root dry biomass production was significantly increased when the plants were cultivated in the deepest boxes (0.7 m). At full bloom, the essential oil content was 0.83%, 0.79% and 0.80% in aeroponic boxes with depths 0.15, 0.30 and 0.70 m, respectively. The samples were characterized by high linalool content, which amounted to 63.85 %, 67.02 % and 66.58 % respectively.

P585

Monitoring Plant Odors in Tomato Culture for *In-Situ* Stress Detection

Julie Ripoll^{1,2}, Nadia Bertin², Rachid Al Halabi³, Bruno Buatois³, Michael Staudt³

¹Laboratoire de Physiologie des Fruits et Légumes (EA4279) - Université d'Avignon et des Pays du Vaucluse, Avignon, France, ²INRA Avignon, UR 1115 Plantes et Systèmes de culture Horticoles, Avignon, France, ³Centre d'Ecologie Fonctionnelle et Evolutive CNRS, UMR 5175, Montpellier, France

Plants release a vast variety of volatile organic compounds (VOCs), which are produced either constitutively or are after induction by external stress factors. Stress-induced VOCs reflect metabolic changes that are primary associated with damages and stress signalling, and secondary with defence reactions. To explore the potential use of *in-vivo* monitoring of VOC signatures in crop management, we have started a series of experiments, in which we exposed two tomato cultivars (*Solanum lycopersicum* Mill.) to a range of environmental stresses alone and in combinations including high irradiation, chilling, heat, and drought. The results of our ongoing experiments showed that most volatiles emitted from tomato leaves are cyclic monoterpenes, notably β -phellandrene and 2-carene, which derive from the large storage pools localized inside the trichomes. These emissions are predominantly controlled by the prevailing temperature and the alteration of diffusion barriers occurring during stress exposures. In addition non-stored VOCs were recorded upon certain stress exposures: the acyclic monoterpenes (*E*)- β -ocimene and β -myrcene, the monoterpene peroxide ascaridole; the apocarotenoids 6-methyl-5-hepten-2-one and (*E*)-geranyl-acetone, the amino acid derivatives methyl-1-butanol and 2-phenylethanol, and several lipid peroxidation products such as hexen-1-ol and (*E*)-2-Heptenal. All these volatiles have strong diagnostic potential as stress markers. For example ascaridole was detected only under strong photo-oxidative stress conditions perhaps formed from the reaction of α -terpinene with singlet oxygen. We conclude that gaining knowledge on stress-induced VOCs can be exploited for plant protection and phenotyping issues in horticulture.

P586

Is β -Cyclocitral a Communication Molecule between Stressed Plants?

Beatriz Fernández-Marín¹, Fátima Míguez¹, Astrid Kannaste², Taras Kazantsev², Miguel Portillo-Estrada², Peter Harely², Li Shuai², Ülo Niinemets², José Ignacio García-Plazaola¹

¹University of the Basque Country, Bilbao, Spain, ² Estonian University of Life Sciences, Tartu, Estonia

The overexcitation of photosynthetic apparatus leads to the formation of chlorophyll triplets, which interact with oxygen generating singlet oxygen ($^1\text{O}_2$). In the reaction centres of both photosystems, β -carotene molecules protect the photosynthetic machinery from the effects of triplet chlorophyll and $^1\text{O}_2$. Consequently, β -carotene is a major target of photooxidation, when its protective capacity is surpassed. The oxidation of β -carotene by $^1\text{O}_2$ results in the formation of several oxidation products, some of which are volatiles. Among them, at least one, β -cyclocitral, is a signalling molecule that triggers the activation/repression of many physiological processes. Its role as retrograde signalling molecule has been recently proposed, but to date it has not been studied whether this compound can escape the leaf through the stomata. Thus, we aimed to study the release of β -cyclocitral from photosynthetic tissues from different crop species and lichens (lacking stomata). Our results suggest that, β -cyclocitral is not constitutively emitted under optimum conditions. It is released from leaves under severe stress, only in the presence of light and, particularly, when $^1\text{O}_2$ is produced in photosynthetic tissues. The emissions of β -cyclocitral vary among species and types of stressors but are around 3 orders of magnitude lower than those observed for other more common volatiles and 6 orders lower than those for CO_2 exchange. In agreement with findings in other organisms (fungi and protozoa), our results suggest that β -cyclocitral emission by photosynthetic tissues could function as a signal of organ decadence.

P587

Fruit Yield and Grape Composition of Six Tempranillo Grapevine Clones Grown under Elevated Temperature

Ilhem Mekni¹, Nazareth Torres¹, Mari Carmen Antolín¹, Inmaculada Pascual¹, Fermín Morales^{2,1}
¹Universidad de Navarra, Pamplona, Navarra, Spain, ²Consejo Superior de Investigaciones Científicas (CSIC), Zaragoza, Aragón, Spain

Within a climate change scenario, increases in air temperature are projected, which may negatively impact grape ripening and must composition. In this regard, varietal and genetic diversity may be exploited to maintain grape and wine quality under future warm conditions. The aim of the present study was to evaluate the effect of elevated temperature on fruit yield and grape quality of six Tempranillo grapevine (*Vitis vinifera* L.) clones. Tempranillo selections 260, 1048, 1089, 8, 280 and 843 were grown as own-rooted fruit-bearing cuttings under greenhouse conditions. Two temperature regimes (24°C/14°C or 28°C/18°C, day/night) were applied from fruit set to maturity. Fruit yield, and technologic and phenolic maturity parameters were determined at ripeness. In general, elevated temperature did not affect grape yield, and it only reduced bunch weight in plants of clone 1089, which was the least productive under elevated temperature conditions. Regarding grape quality, clones 8, 260 and 843 were the most affected by elevated temperature, with decreases in malic acid, phenolic richness and anthocyanin concentration, as well as increases in tartaric acid and must tonality. By contrast, clones 280 and 1089 were the least affected by temperature, and the concentration of malic and tartaric acid, phenolic richness, potential anthocyanins and anthocyanin extractability in plants grown at 28°C/18°C were similar to those of plants grown at 24°C/14°C. Results suggest that Tempranillo clones 280 and 1089 may be considered in a future climate change scenario to preserve the effects of global warming, thus maintaining a high level of grape quality.

P588

Effects of Root Zone Temperature on the Total Phenolic Content and DPPH Scavenging Activity in Aeroponically Cultivated Sweet Basil (*Ocimum basilicum*, L.) and Lettuce (*Lactuca sativa*, L.) Plants

Salachas Georgios¹, Argyropoulou Konstantina¹, Papasavvas Angelos¹, Giannakopoulos Evangelos¹, Deligiannakis Yiannis²

¹T.E.I. of Western Greece, Amaliada, Theodoropoulou st., GR-27200, Greece, ²University of Patras, Agrinio, GR-30100, Greece

Basil and lettuce plants were cultivated in a fully automated glasshouse aeroponic system, under different temperatures into the root zone atmosphere (14, 17 and 26⁰C) at 160 ppm NO₃⁻ N into nutritive solution, in order to determine total phenolic content and its scavenging activity on DPPH free radical. In addition, the impact of two additional NO₃⁻ N concentrations (25 and 50 ppm) at the same time period at 17⁰C was also studied. The leaf total phenolic content in basil plants at the highest tested NO₃⁻ N levels (160 ppm) was not statistically affected under the above different root atmosphere temperature conditions, while the decrease in NO₃⁻ N application rates from 160 to 25 ppm resulted in a significant increase in the total phenolic concentration. DPPH scavenging activity was significantly increased both at low temperature and at the lower tested levels of NO₃⁻ N in nutritive solution. In the case of Lettuce plants, leaf total phenolic content was significantly increased as the root zone temperature was decreased from 26 to 14⁰C and was also increased at lower NO₃⁻ N application rates. Leaf DPPH scavenging activity was significantly increased both at lower root zone atmosphere temperatures or at lower NO₃⁻ N application rates.

This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

P589

Morphological and Physiological Trade-Off's in the Stomatal Response of Plant Species to Elevated Atmospheric Carbon Dioxide Concentrations ([CO₂]) under Varying Light Regimes

Aidan Holohan¹, Jennifer McElwain¹, Michael Jones², Christoph Müller³

¹*School of Biology and Environmental Science, University College Dublin, Dublin, Ireland,*

²*Department of Botany, School of Natural Sciences, Trinity College Dublin, Dublin, Ireland,* ³*Institute of Plant Ecology, Justus-Liebig University, Giessen, Germany*

An innate ability to control both aperture and initiation provides plants with two distinct mechanistic options in the control of gas exchange and the maintenance of optimal photosynthetic rates via their stomata. However, although these adaptive responses are often observed, the nature of the stomatal response to elevated [CO₂] may be considered highly variable amongst species and complicated by the addition of what are often conflicting environmental factors. In this instance the diversity of stomatal response to variation in [CO₂] is considered to be physiologically 'active' (via aperture change), morphologically 'passive' (via stomatal density change) or a combination of both, dependent on alterations to the plant growth environment. Here, we test the hypothesis that plants are forced to adopt either an active or passive stomatal response strategy in reaction to elevated [CO₂] when one (in this case light intensity) or more environmental limitations to photosynthesis are imposed. Active stomatal control was assessed as plants conductance (g_s) response to instantaneous changes in external [CO₂] while passive stomatal control over g_s was assessed by analysis of stomatal (density/index) changes between treatments. Findings in this instance demonstrate that plants grown under ambient light and elevated [CO₂] were both active and passive responders exhibiting strong controls over both stomatal initiation and g_s . However, when grown under sub-optimal light intensities plants demonstrated a clear preference for either active or passive stomatal control as plants progressively lost the ability to respond either instantaneously to changes in external [CO₂], or passively, through changes in stomatal initiation.

P590

Polymorphism of Phytochrome 1 from the Fern *Adiantum capillus-veneris* L. Overexpressed in the Transgenic *Arabidopsis*

Vitaly Sineshchekov¹, Larissa Koppel¹, Haruko Okamoto², Masamitsu Wada³

¹Chair of Biochemistry, Department of Biology, M.V. Lomonosov Moscow State University, Moscow 119234, Russia, ²Centre for Biological Sciences, University of Southampton, Southampton, SO17 1BJ, UK, ³Department of Biology, Tokyo Metropolitan University, Tokyo 192-03, Japan

Complex photophysiological behavior of phytochrome A (phyA) in higher plants is connected with the existence of its native post-translationally modified species - phyA' and phyA'' - whose major phenomenological distinction is ability and inability, respectively, to undergo photoreaction at cryogenic temperatures (Sineshchekov, 2010). In this work, we have shown that the origin of the phyA polymorphism can be traced back to its cryptogram ancestor. With the use of low-temperature fluorescence spectroscopy and photochemistry, two isoforms of fern *Adiantum capillus-veneris* L. phytochrome 1, phy1' and phy1'', similar to those of phyA, were detected in etiolated seedlings of transgenic *Arabidopsis* overexpressing it. Their content was similar to that of the endogenous phyA species and their proportion was found to be dependent on the germination-inducing seed preillumination. The transgenic seeds displayed light-independent germination in contrast to the wild-type control, suggesting that fern phy1 can participate in some photophysiological reactions of higher plants. Thus, we may hypothesize that the ability to produce photochemically and functionally distinct phytochrome isoforms is of a general phenomenon - it can be characteristic not only for angiosperm phyA but also for the early land plant phytochromes.

P591

Genomic Study of the Complex Trait of Salt Stress Response in Rice

Inês S. Pires^{1,2}, Jonathan M. Flowers², Sónia Negrão^{1,3}, Margarida M. Oliveira¹, Michael D. Purugganan²

¹ITQB (Instituto de Tecnologia Química e Biológica – Universidade Nova de Lisboa) and iBET (Instituto de Biologia Experimental e Tecnológica), Av. da República, 2780-157 Oeiras, Portugal,

²Department of Biology and Center for Genomics and Systems Biology, New York University, New York, USA,

³Center for Desert Agriculture, Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia

Increase in salinity levels of irrigated soil is becoming a major source of crop yield losses worldwide, with rice (*Oryza sativa* L.) being one of the most salt sensitive crops. We are analysing salt stress response in rice by looking into the phenotype and genotype of 56 rice accessions that represent the existent genetic diversity in this species.

A previous gene-targeted study (Negrão et al., 2013) phenotyped hydroponically these 56 rice accessions under control (0 dS/m) and salinity (12 dS/m) conditions. This phenotyping experiment included measurements of Na⁺ and K⁺ content in shoots and roots, osmotic potential, chlorophyll a and b content, as well as biomass measurements. We have further analyzed this data globally and found that Na⁺ concentration in shoots and shoot biomass are the traits most correlated with presence/absence of salinity stress, while K⁺ levels do not seem to play an important role in rice salinity response.

We also re-sequenced the whole genomes of these 56 accessions to ~14X coverage, identified SNPs, and performed a pilot genome-wide association study. In addition, we looked at families of genes known to be relevant for salt tolerance and observed that some accessions with extreme phenotypes exhibit mutations affecting multiple salt response genes. Our analyses reinforce that salinity response is a complex trait, and that we need to look simultaneously at mutations in multiple genes, such as transporter genes, to explain differences between observed phenotypes under salinity stress.

Negrão S et al. (2013) Plant Biotechnol Journal 11:87-100

P592

Abundance of Cysteine Endopeptidase Dionain in Digestive Fluid of Venus Flytrap (*Dionaea muscipula*) is Regulated by Jasmonates

Michaela Libiaková¹, Kristýna Floková², Ondrej Novák², Andrej Pavlovic^{1,3}

¹*Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia,* ²*Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR and Palacký University, Olomouc, Czech Republic,* ³*Department of Biophysics, Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic*

The trap of carnivorous plant Venus flytrap (*Dionaea muscipula*) catches prey by very rapid closure, within a fraction of a second of the trigger hairs being touch twice. After the rapid closure secures the prey, repeated mechanical stimulation of trigger hairs by struggling prey and generation of action potentials (APs) result in secretion of digestive fluid. Once the prey's movement ceased, the secretion is maintained by chemical stimuli released from digested prey. We investigated the effect of mechanical and chemical (NH₄Cl, KH₂PO₄) stimulation separately on enzyme activities in digestive fluid. No significant exochitinase and β-D-glucosidase activities were detected. Acid phosphatase activity was higher in NH₄Cl stimulated traps while proteolytic activity was higher in both chemically induced traps in comparison to mechanical stimulation. This is in accordance with higher abundance of recently described enzyme cysteine endopeptidase Dionain in digestive fluid of chemically induced traps. Mechanical stimulation induced high level of 12-oxophytodienoic acid (OPDA) but jasmonic acid (JA) accumulates to significant level after chemical stimulation. The mechanical and chemical stimulation can be bypassed by external application of JA, which induce high abundance of Dionain and proteolytic activity in digestive fluid. These results document the role of jasmonates in regulation of proteolytic activity in response to different stimuli from captured prey.

Libiaková M., Floková K., Novák O., Slovák L., Pavlovič A. (2014) paper submitted.

P593

Timothy (*Phleum pratense* L.) - An Important Forage Grass Also for Future Climates?

Venla Jokela, Tiina Toivonen, Mervi Seppänen
University of Helsinki, Helsinki, Finland

It is predicted that climate change will prolong growing season especially in autumn which will result in shorter period for cold acclimation as well as shorter winters. Traits important for winter survival and yield formation are growth cessation in autumn and winter hardiness, whereas vernalization requirement affects the canopy structure of summer growth. These physiological responses are under genetic and environmental regulation.

The aim of this study was to investigate the effect of autumn and winter climate and day length conditions on the growth of timothy genotypes of southern and northern origin.

Predicted future climate conditions were simulated in growth chambers to study autumn growth cessation. Low temperature (5° or 10° C) and 12 h DL prevented the growth of both studied genotypes, whereas at 14 h DL elevated temperature (15 °C) resulted in increased height growth in southern genotype while in northern genotype more side tillers were produced. Fulfillment of vernalization requirement (10 weeks) was seen as higher number of yield-forming tillers in northern genotype, whereas in southern genotype similar response was not observed. The interaction between long photoperiod and vernalization was detected in northern genotype: at 20 h day length no vernalization was required for flowering saturation. Such interaction was not detected in southern genotype where the number of flowering tillers remained at constant level. These results indicate that timothy has potential as a forage grass species also for the future, since there exists genetic variation in important traits for adaptation.

P594

Increased Drought of the Mediterranean Climate and Biotic Interactions: Functional Consequences on Plant Communities in a Shrubland Ecosystem

Natalia Rodriguez-Ramirez^{1,2}, Nicolas Montes^{1,2}, Christine Ballini^{1,2}

¹Aix-Marseille University, Marseille, PACA, France, ²Mediterranean Institute of marine and terrestrial Biodiversity and Ecology IMBE (CNRS 7263 / IRD 237), Marseille, PACA, France

Climate Change is predicted to be particularly pronounced on the Mediterranean basin. With up to 40% decrease in summer precipitation and temperature 2°C to 5°C higher by 2080-2099 than in the 1980-1999, important changes on biodiversity and plant-plant interactions are expected. To evaluate consequences of future drought on the relationship between biodiversity and ecosystem functioning, a large-scale in-situ experiment was set on a Mediterranean woody shrub-dominated ecosystem in southern France (CLIMED project). It consisted in 93 experimental plots (16m², 2m high each): 46 plots are equipped with a rain exclusion device intercepting almost 40% of rain, 47 plots with a similar device in mode "no exclusion" (control). All possible combination of two, three or four dominant species (*Quercus coccifera*, *Rosmarinus officinalis*, *Cistus albidus*, *Ulex parviflorus*) were studied. At the vegetation level the effect of increased drought on diversity (interspecific relationships, functional diversity) and ecosystem functioning (primary production, carbon and nitrogen cycle) were evaluated.

The first results of this experiment revealed that the Shannon-weaver index was negatively correlated to the cover of *Quercus* for both control and exclusion but it's positively correlated with the cover of the other dominant species covers (while only significant with *Rosmarinus* under rain exclusion). The magnitude of the negative correlation between *Quercus* and *Cistus* covers was stronger under rain exclusion.

The resistance to drought of the aboveground biomass depends on the species. *Rosmarinus* seems to be more resistant than other dominant species. The most sensitive seems to be *Ulex*, a legume, with possible consequences on nitrogen cycle.

P595

Chloroplast Protrusions in European Beech Leaves - 2D and 3D Study of the Effect of Elevated Carbon Dioxide

Barbora Radochova¹, Jiri Janacek¹, Martin Capek¹, Jan Michalek¹, Zuzana Lhotakova², Lucie Kubinova¹, Jana Albrechtova²

¹*Institute of Physiology AS CR, v.v.i., Prague, Czech Republic,* ²*Charles University in Prague, Prague, Czech Republic*

Chloroplast protrusions are known as appendices of chloroplast envelopes that sometimes use to form pocket-like structures encompassing mitochondria or microbodies. They enhance membrane transport by increase of the membrane surface area. Moreover, carbohydrate formation may be supported by the increase in stroma volume. Chloroplast protrusions were often observed in plants coping with cold climate and are therefore seen as adaptive structures in plants with a short vegetation period where photorespiration may be one of tools for their survival. Elevated carbon dioxide concentration goes in the opposite way because one of its well known effects on photosynthesis of C3 plants is a decrease in photorespiration.

In the present study we used sun exposed leaves of European beech (*Fagus sylvatica* L.) sampled at the experimental site Bílý kříž (Beskids Mts., Czech Republic) for quantitative assessment of changes in frequency and forms of chloroplast protrusions under elevated carbon dioxide concentration. Samples were chemically fixed and processed for transmission electron microscopy. Proportion of chloroplasts with protrusions was calculated from individual ultrathin sections, and image data of serial sections were used for 3D visualization. Elevated carbon dioxide concentration caused a decrease in occurrence of chloroplasts with protrusions. The pocket-like structures containing mitochondria or microbodies were rather shallow and thus less frequently observed on 2D sections. Chloroplasts protrusions never contained thylakoids or starch, but sometimes small vesicles of inner envelope origin were visible.

This work was supported by the Czech Science Foundation (P501/10/0340) and by the Academy of Sciences of the Czech Republic (RVO:67985823).

P596

Effect of Fertilization and Variety on Yield and Content of Phenols in Onion Bulbs

Pavol Slamka, Andrea Golisová, Martin Krček

Dept. Agrochemistry and Plant Nutrition, Slovak University of Agriculture in Nitra, Nitra, Slovakia

Effect of nitrogen fertilization in combination with sulphur and iron on yield, content of phenols, flavonoids and quercetin was investigated in field experiment. Four varieties of onion (Kamal, Robin, Pueblo and Mundo) were examined under 4 treatments of fertilization. On the average of 2 years the highest yield of onion was achieved with red onion of Robin ($33.50 \text{ t}\cdot\text{ha}^{-1}$) on the treatment fertilized by nitrogen and sulphur. The lowest yield was achieved by white onion of Pueblo ($25.78 \text{ t}\cdot\text{ha}^{-1}$) on the treatment with solo N application. However, this treatment showed the greatest effect on content of phenols in onion of Kamal ($929.4 \text{ mg GA/kg FW}$) and Robin ($864.7 \text{ mg GA/kg FW}$). Regarding phenols, white variety of Pueblo and yellow variety of Mundo were the most efficient when fertilized by N+S+Fe. The highest content of flavonoids in variety of Kamal ($285.3 \text{ mg CAT}\cdot\text{kg}^{-1} \text{ FW}$) was achieved at N+S fertilization and in variety of Robin ($353.0 \text{ mg CAT}\cdot\text{kg}^{-1} \text{ FW}$) at solo N fertilization. The lowest content of flavonoids was achieved in white variety of Pueblo ($115.9 \text{ mg CAT}\cdot\text{kg}^{-1} \text{ FW}$) fertilized with N+S+Fe. From statistical point of view, year showed high significant effect on yield of onion and content of quercetin. Fertilization proved high significant effect on onion yield and content of phenols. A variety as an experimental factor showed highly significant influence on content of phenols, flavonoids, quercetin and yield. Hypothesis that foliar application of fertilizer containing iron can activate enzymes increasing synthesis of quercetin was not confirmed.

P597

What Are Trees Talking About? – the Use of Trans-root Bioelectrical Potential (TRPB) to Study the Response to Environmental Stimuli in *Picea abies*

Robert Bernacik, Pawel Jedynak, Przemyslaw Malec
Jagiellonian University, Cracow, Poland

The Norway spruce (*Picea abies*) populations grown under harsh environmental conditions of the upper treeline promote the formation of tree clusters –spatially isolated groups of tightly growing trees. Root grafts (root fusions) are frequently observed between cluster members. It is believed that accretion of root graft xylems allow for the exchange of molecules and/or electrical signals between individuals¹. The trans-root bioelectrical potential (TRBP) is a parameter used to assess the activity of the root system in trees *in situ*². We have used a continuous monitoring of TRBP to study the putative benefits of tree cluster formation in population of *Picea abies* grown c.a. 1200m a.s.l., in the Western Carpathian Mts. The long-term TRBP measurements were performed in the summer season of 2013, on both selected individual trees and selected tree cluster members, including individuals connected by root grafts. The weather-related parameters (insolation, temperature, rainfall, humidity) were simultaneously monitored. The data analysis showed the existence of correlations between measured TRBP values and environmental parameters both in individual trees and the members of tree clusters. Data comparison revealed structural similarities between TRBP signals observed in particular tree cluster members connected by root grafts. Our data suggests the meaning of root grafts in the cooperative response of tree cluster members to changes in the environment³.

1 Boratynski, A., (1998), *BWN Poznan*,

2 Okamoto H, (1999) *J. Plant Res.* 112: 123-130.

3This study is supported by the Ministry of Science and Higher Education (MNiSzW) of the Republic of Poland (DS-1277/HF/2013, "Generation of the Future")

P598

Early Detection of Kaolin Benefits Application to Minimize Summer Stress Effects in Grapevines Growing in Douro Region Using JIP-Test

Lia Dinis¹, Helena Ferreira¹, Glória Pinto², Carlos Correia¹, José Moutinho¹
¹CITAB, Vila Real, Portugal, ²CESAM, Vila Real, Portugal

In Portugal, the winemaking sector has a crucial economic, social and cultural relevance, especially in the Douro Demarcated Region (Northeast Portugal) which is subject to typical Mediterranean climate with hot and dry summer. These climatic conditions may impair some physiological processes, leading to poor grape yields and quality. Grapevine photosynthetic activity declines during typical summer days with high irradiance and vapour pressure deficit. Many inorganic products have been exogenously applied in order to test the short-term adaptation impact on grapevine physiology and yield under these environmental conditions. One of the most commonly used compounds is Kaolin, which is referenced as a mineral chemically inert to repel insects and prevent disease spread and heat and water stress without restricting leaf gas exchange. However, no relevant work in grapevine grown under severe summer stress is known. The main objective are to compare the responses of leaves (cv. Touriga Nacional) pulverized with 5% foliar kaolin during two years (2012 and 2013) with control plants (leaves without Kaolin). During midday, Kaolin grapevines showed significantly higher F_v/F_M than those plants under control treatment (without Kaolin Pf). The impact of pulverization on Chl a fluorescence were more pronounced during midday where the high irradiance and temperature were highest. Our results clearly demonstrate that decreased susceptibility to photoinhibition in Kaolin application plants was associated with increased active PSII reaction centers and efficient photochemical quenching.

P599

The Effect of Nurse Plants in Caatinga: The Role of *Ziziphus joazeiro* Mart. (Rhamnaceae) in Facilitating Natural Regeneration in Brazil's Northeastern Semi-Arid Region

Givanildo Bernadino de Araujo¹, Renato Soares Vanderlei^{1,2}, Flávia de Barros Prado Moura¹
¹Federal University of Alagoas, Maceió, Alagoas, Brazil, ²Trinity College Dublin, Dublin, Ireland

In semi-arid regions, positive interactions between plant species contribute to mitigate adverse factors such as high light intensity, temperature and low humidity. Northeastern Brazil's Caatinga is composed by thorn scrub and xerophytic vegetation. According to the literature, facilitative interactions become more important in places where environmental conditions are more stressful. This study tests the hypothesis that continuous planting of nurse plants may be a potential alternative to restore Caatinga landscapes. To test it, species richness and abundance and abiotic factors were evaluated in areas influenced by *Z. joazeiro*. This species was chosen as a likely nurse species due to its ecophysiological characteristics such as number of seedlings under canopy, presence of thorns providing protection against herbivores, lack of palatability to livestock and being evergreen. A perimeter was delineated around each plant (n=20) according to the plant's crown. All woody species higher than 0.5 m within this perimeter were collected and identified. Later, this diameter was duplicated to examine the plant's influence beyond the canopy. Control plots with equivalent dimensions were delimited 5 meters away from each plant. Paired Student t-test was used to compare both areas. Under the *Z. joazeiro* canopy, mean richness and abundance were higher than the control areas, evidencing an increase of 1.57 species per meter and 3.29 individuals per meter radius respectively; mean air temperature and light intensity were also lower under the canopy, 8.3 °C (n=10, t=-7.3753, p<0.001) and 54.2 lux (n=10, t=-5.1762, p<0.001) respectively. No significant difference in relative humidity was found. We suggest that restoration based on nurse plants is particularly appropriate in Brazilian Caatinga.

P600

Light Dynamic Response in a High Mountain Plant

Patricia Brito, Águeda M^a González-Rodríguez, J. Roberto Lorenzo, Alicia Perera-Castro
Universidad de La Laguna, La Laguna, Spain

In Mediterranean climates, high mountain plants cope normally with different stressful environmental factors like temperature, radiation or water availability. Light intensity is one of the most relevant factors that can affect the physiological response under these conditions. The aim of this work was study the light dynamic response of *Pteroccephalus lasiospermus*, a Canary endemic species present in Teide National Park (Tenerife), under natural conditions at 2,100 m a.s.l in order to analyze plasticity in photoprotective strategies. For this purpose, plants were growing in pots (well irrigated) with a light reduction (50% solar radiation). These plants were suddenly exposed to high light (above $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$) to evaluate the fast light dynamic response. Physiological response was evaluated by chlorophyll fluorescence, reflectance indexes and photosynthetic pigment composition. Measurements were done in short time intervals (min), directly in the field, during 3 hours after the light exposition. Leaves were sampled and carried out to the lab where predawn conditions were simulated (darkness and water saturation) and measurements were repeated. Ecophysiological performance showed a very high variation in most parameters. Predawn F_v/F_m values were higher than 0.75 showing not permanent damage in PSII, but still displayed high variations despite of leaves were maintained in homogenous conditions during all night. NDVI values were higher than 0.6 in all measurements, and predawn values (0.705-0.782) showed higher rates than field measurements (0.623–0.715). High variation detected in most ecophysiological parameters analyzed under this particular high mountain habitat will be discussed in detail.

Project funding: CGL2010-21366-C04-04 MCI.

P601

GRACE - An European Research Project on GM Crop Impact Assessment

Christian Kohl, Ralf Wilhelm, Joachim Schiemann
Julius Kuehn Institute (JKI), Quedlinburg, Germany

GRACE (GMO Risk Assessment and Communication of Evidence; www.grace-fp7.eu) is an EU-funded research project aiming to substantially increase the transparency and traceability of information dealing with potential risks and benefits associated with the deliberate release of genetically modified plants (GMPs) and their products. GRACE is conducting evidence maps and systematic reviews of potential health, environmental, and socio-economic impacts of GMPs and derived food and feed and is critically reconsidering the gain and necessity of animal feeding trials and alternative omics, *in vitro* and *in silico* studies.

Recent controversies about peer-reviewed papers dealing with impacts of GMPs underline the necessity for an explicit, transparent and unbiased reviewing and collation of published results. Evidence synthesis approaches are already established in other research fields to support evidence-based decision-making. They represent powerful tools to collect, evaluate and summarize accessible research results in order to address a specific scientific issue in a transparent, reproducible and unbiased manner. Thus, their adaption to and implementation in the impact assessment process for GMPs and products derived thereof aims to increase the transparency and reliability of the provided information and to support the impartial updating of science-based decision-making processes.

To provide access to information on key biosafety issues associated with GMPs for risk assessors, policy makers, stakeholders and the public the central access database CADIMA (Central Access Database for Impact Assessment of Crop Genetic Improvement Technologies) is being established. It will support and mirror the evidence synthesis process and assist the dissemination of results and conclusions.

P602

The Leghemoglobin Content in Root Nodules of Various Species of Clover (*Trifolium L.*) Growing in Different Conditions of Moistening

Viktor Khryanin

Penza state university, Penza, Russia

One of the main characteristics of nitrogen-fixing activity of root nodules of legumes is the content of leghemoglobin. This work has conducted a comparison of the contents of hemoprotein of leghemoglobin in nodules of different species of clover growing in different types of humidity. Five species of clover were selected for the study: mountain clover, Alpine clover, creeping clover, meadow clover, plough clover. The study used nodules of plants in the flowering phase. Leghemoglobin was identified with optical density at the isobestic point $\lambda=525$ nm on the spectrophotometer. The study found that the content of leghemoglobin in the root nodules of the types is higher if they grow in areas with sufficient moisture. So, in the floodplain the content of leghemoglobin in mountain clover is 7,67 mg/g WW, Alpine clover - 9,08 mg/g WW, meadow clover - 10,77 mg/g WW, creeping clover - 7,40 mg/g WW, plough clover - 6,21 mg/g WW. On the watershed the content of leghemoglobin in mountain clover is 3,18 mg/g WW, Alpine clover - 3,36 mg/g WW, meadow clover - 2,12 mg/g WW, creeping clover - 1,16 mg/g WW, plough clover - 2,84 mg/g WW. Thus, the content of leghemoglobin indicates the intensity of nitrogen-fixing processes in nodules. The most active in the floodplain are the nodules of Alpine clover and meadow clover. In the conditions of the watershed are the nodules of Alpine clover and mountain clover. The most resistant to changing humidity conditions are the nodules of plough clover.

P603

Contribution of Arbuscular Mycorrhizal Fungi in Attenuation of Heavy Metal Impact on *Calendula officinalis* Development

Marieta Hristozkova, Maria Geneva, Ira Stancheva

Department of Plant Mineral Nutrition and Water Relation, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria

Arbuscular mycorrhizal fungi are prospective tool for enhancing plant tolerance to environmental stress conditions and restoration of naturally or industrially metal-contaminated soils. The study focuses on the evaluation of pot marigold (*Calendula officinalis* L.) response, grown on industrially polluted with Cd and Pb soil to arbuscular mycorrhizal fungi, concerning the growth, mycorrhizal colonization, uptake and distribution of heavy metals in the plant organs. Two isolates of *Claroideoglomus claroideum* (EEZ 35 and EEZ 54) and *Funneliformis mosseae* (EEZ 55) are used. The strains EEZ 54 and EEZ 55 were isolated from soil, naturally enriched with quantity of metals, and EEZ 35- from a place with artificial contamination. Their contribution in attenuation of heavy metals influence on *Calendula officinalis* performance is discussed. Both strains EEZ-35 and EEZ-54 reduced Pb content in the aerial plant parts with about 60% and EEZ-55 with 45% in comparison with the control. Some decrease of Pb was identified in the roots colonized with both *Claroideoglomus claroideum* isolates. Reduction of Cd was noted in the aboveground parts of marigold inoculated with EEZ-35 and EEZ-55. Neither Pb nor Cd was detected in the marigold flowers (*Calendula flos drug*) widely used in pharmaceutical, food and cosmetics industries. The highest biomass accumulation was observed regarding aerial parts of the plants inoculated with EEZ-54, which coincided with the highest percent of colonization and the highest acid phosphatase activity. The beneficial effects of mycorrhizal fungi on plant productivity and soil health are essential to support development of organic and non-pollution agriculture.

P604

Comparative Studies of Plant Growth-Promotion and IAA Secretion with *Pseudomonas fluorescens* and *Pseudomonas putida*

Meliani Amina, Bensoltane Ahmed, Normand Philippe, Oufdoud Khalid, Benedired Loubna
University, Mascara, Algeria

The present study was undertaken to investigate the plant growth promoting traits of a PGPR *P.fluorescens* and *P.putida* like production of indoleacetic acid (IAA), siderophore and phosphate solubilization. In function of the two different types of root response to Fe deficiency (strategies), these isolates were further tested for their effect on seed germination, seedling growth of *Lens culinaris* (strategy II) and *Hordeum Vulgar* (strategy I) under gnotobiotic conditions and for tryptophan (Trp)-dependent IAA production. Both *P.fluorescens* and *P.putida* were shown to produce 3-indoleacetic acid (IAA) in vitro, at concentrations of 89 μgml^{-1} and 116 μgml^{-1} , respectively. Furthermore, high levels of IAA excreted by *P.putida* were most consistent in enhancing plant growth across the calculation of Vigor index. Nevertheless, and on a comparative basis *P.fluorescens* was most promising in promoting plant growth in the case of the plant of strategy I compared to plant of strategy II.

Keys words: PGPR, Screen, *P.fluorescens*, *P.putida*, IAA, Strategy.

P605

Effect of Arbuscular Mycorrhizae Inoculum (*glomus intraradices*) on Seed Germination of Bambara Seed (*vigna subterranean* (l))

Ajiboye Abiodun¹, Ajiboye Modupe¹

¹Osun State University, Osogbo/Osun State/Southwest Region, Nigeria, ²Osun State University, Osogbo/Osun State/Southwest, Nigeria

Bambara seed is highly nutritive crop that is underutilised and consumed in Africa. The viability test for the seeds was between 80-100%. *Glomus intraradice* was found to effectively enhanced the growth of seedlings production against control through direct inoculation. This was evident in the some growth parameters such as plant heights, stem girth, leaf area, dry and fresh weight of the seedlings of 3-4 months old. The mycorrhizal dependency was estimated to be 11.8+4.0g, while that of the non mycorrhizal dependency was 6.8+1.3g. Other chemical components found in the seeds include, iodine, saponification, acidic value and oil contents. Data were subjected to analysis of variance and the experiment was done in triplicates. The seedling establishment of the underutilised seeds should be encouraged in order to substitute the highly proteinous seed of bambara with the high cost of animal protein.

P606

Exposure of *Azolla Filiculoides*-*Anabaena Azollae* Symbiosis to a Cyanobacterial Extract Containing Cylindrospermopsin: Impact on Growth and Biochemical Parameters

C. Santos¹, J. Azevedo¹, A. Campos¹, V. Vasconcelos^{1,2}, A. L. Pereira¹

¹*Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Rua dos Bragas 289, P 4050-123, Porto, Portugal,* ²*Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, 4169-007, Porto, Portugal*

Azolla filiculoides is a heterosporic, free-floating aquatic pteridophyte with worldwide distribution mainly in tropical and temperate regions. Each dorsal lobe has an ovoid cavity in which inhabit a prokaryotic community of several genera of bacteria and a heterocystous filamentous nitrogen-fixing cyanobacterium *Anabaena azollae*. This symbiotic system could be used as biofertilizer in rice fields, feed for livestock or in phytoremediation of industrial and urban polluted wastewaters. However, *A. filiculoides* can grow in eutrophic waters in which can occurs blooms of cyanobacteria producing cyanotoxins such as the case of cylindrospermopsin (CYN). Nevertheless, nothing is known about the phytotoxicity and dynamics of cylindrospermopsin in this symbiotic system.

The aim of our research was the phytotoxicity effects on growth and biochemical parameters of *A. filiculoides*-*A. azollae* after exposure to CYN.

The results point to adaptations in the photosynthetic pigments in both pteridophyte and cyanobiont due probably not only to the presence of CYN, but also due to the presence of other compounds in the crude extract that may function as nutrients mostly in the lowest concentrations (0.5 µg/ml). At the highest CYN (5 µg/ml), the phytotoxic effects are more marked: the pteridophyte showed an abrupt decrease in growth, PC, GST and GR and an increase in chlorophylls, carotenoids, PEC, APC, CAT and GPx.

Funding: Research supported by 1) ERDF through COMPETE and national funds through FCT under the project PEst-C/MAR/LA0015/2013; 2) Porto University under the project IJUP2011_3; and 3) the FSE under the POPH of QREN (fellowship SFRH/BPD/44459/2008 to Ana L. Pereira).

P607

Plant Growth Promotion Effects of Endophyte Community Associated to *Vitis vinifera* cv. Glera

Enrico Baldan¹, Elisabetta Barizza¹, Sebastiano Nigris¹, Stefano D'Alessandro¹, Andrea Squartini², Barbara Baldan¹, Michela Zottini¹

¹Department of Biology, University of Padova, Padova, Italy, ²Department of Agronomy Food Natural Resources Animals and Environment, University of Padova, Padova, Italy

Endophytic bacteria may stimulate host plant growth through any of several possible mechanisms including biological control, induced systemic resistance to plant pathogens, phytohormone production and improvement of nutrient and water uptake. Some of these organisms can be utilized in agricultural and horticultural practices for the purpose of transplant protection against diseases, improvement of establishment and overall performance. It may also improve plant performance in stress environments and consequently enhance yields. In this work we report on the isolation and characterization of culturable bacterial endophytes colonizing Glera grapevine cv. growing in North of Italy (Veneto, Italy). In six different vineyards within the area, 381 culturable strains were isolated and molecularly characterized by Amplified Ribosomal DNA Restriction Analysis (ARDRA). Different biological activity of these endophytes have been evaluated, such as auxin production, phosphate solubilization activity and antifungal properties. Plant growth promoting activity has been tested in in vitro grown grapevine plants and in Arabidopsis plants harboring specific reporter-genes.

P608

Comparison of Perennial Ryegrass and Perennial Ryegrass White Clover Swards at High Nitrogen Fertiliser Application

Michael Egan^{1,2}, Bridget Lynch², Deirdre Hennessy¹

¹Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland, ²University College Dublin, Belfield, Dublin 4, Ireland

There is increased interest in incorporating white clover (WC) into N fertilised grazed grassland in Ireland. The objective of the study was to examine the benefits WC in a high N fertiliser system. A perennial ryegrass (PRG) only sward and PRG WC sward were sown at Teagasc Moorepark. Swards received 260 kg N/ha. Swards were rotationally grazed from February to October 2012. Pre-grazing herbage mass (>4 cm) and sward WC content (>4 cm) were determined twice weekly, and pre- and post-grazing sward height were measured daily. In May, July and August 45 turves (10 cm x 10 cm) were removed at random from each treatment and PRG tiller density and WC stolon mass were determined. Data were analysed using PROC MIXED in SAS. Both treatments had similar herbage production, pre- and post-grazing sward height (14,738 kg DM/ha, 10.6 and 4 cm, respectively). Average sward WC content was 21.6%, ranging from 8.4% in February to 22.9% in October. PRG sward had greater PRG tiller density than the PRG WC sward (5466 vs. 4165/m²; P<0.01). PRG tiller density varied between seasons (P<0.001), peaking in early summer. Stolon mass was greatest in October (47.6 g/m²) and averaged 37.1 g/m². As stolon mass and sward WC content increased, PRG tiller density in the PRG WC sward decreased compared to the PRG sward (P<0.001). Sward WC content remained high throughout the experiment despite high N fertiliser application.

P609

Establishment of White Clover at Different Sowing Rates with Tetraploid, Diploid and Mixed Perennial Ryegrass Swards under Simulated Grazing

Michael Egan^{1,2}, Philip Creighton³, Bridget Lynch², Deirdre Hennessy¹

¹Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, CO. CO. K, Ireland, ²UCD School of Agriculture and Food Science, UCD, Belfield, Dublin 4, Ireland, ³Teagasc, Animal and Grassland Research and Innovation Centre, Athenry, Co. Galway, Ireland

The objective was to identify the effect of clover sowing rate (SR) on sward clover content, perennial ryegrass (PRG) tiller density and stolon mass (SM) in the establishment period. The experiment was a 2x3x5 randomised complete block design with four replicates per treatment. Two sites - MPK and ATH; three PRG swards (27.5 kg seed/ha) - tetraploid, diploid and a 50:50 mix; and five clover (cultivar Iona) SRs - 0, 2.5, 5, 7.5, 10 kg/ha. PRG tiller density and SM were estimated in August and November and sward clover content was measured in August and October. Data were analysed using PROC MIXED in SAS. As SR increased, SM increased ($P < 0.001$). There was no effect of PRG sward on SM. Stolon mass was greater ($P < 0.01$) at MPK than ATH (19.37 and 14.98 g/m², respectively). As SR increased from 0 to 10 kg/ha tiller density decreased from 5967 to 4508 tillers/m² ($P < 0.001$). Tiller density was highest for the diploid sward, intermediate for the 50:50 mix and lowest for the tetraploid sward (5767, 5082 and 4377 tillers/m², respectively, $P < 0.001$). Tiller density was greater at MPK (5669 tillers/m²) than ATH (4482 tillers/m²; $P < 0.001$). Sward clover content was higher ($P < 0.05$) at ATH for SR 2.5 and 7.5 kg/ha compared to the other SRs, and higher at MPK ($P < 0.05$) for SR 5 and 10 kg/ha compared to the other SRs. Sward clover content was not affected by PRG sward. In conclusion, as clover SR increased, SM and clover content increased and tiller density decreased.

P610

Biological Nitrogen Fixation of White Clover (*Trifolium repens*) When Sown with Eight Perennial Ryegrass (*Lolium perenne*) Cultivars at Two Nitrogen Inputs under Animal Grazing

Justin McDonagh^{1,2}, Mary McEvoy¹, Trevor Gilliland³, Michael O'Donovan¹

¹Animal & Grassland Research and Innovation Centre, Teagasc, Moorepark, Co Cork, Cork, Ireland,

²Queens University Belfast, Belfast, UK, ³Agri-food Biosciences Institute, Plant Testing Station, Crossnacreevy, BT5 7QJ, N Ireland, Belfast, UK

Perennial ryegrass is the dominant forage grass species selected for agriculture in Ireland. The inclusion of white clover (WC) with perennial ryegrass in pastures has proven to increase dry matter (DM) production, compared to perennial ryegrass monoculture swards. However, there is a lack of knowledge in the interactions that occur in swards between plant genotypes and managements they are subjected to. The current study evaluated the effect of cultivar, ploidy and nitrogen (N) on seasonal and total clover proportion and the fixation of biological N under animal grazing. The experiment was a randomised block design with 5 replicates. Plots were 54m². Eight cultivars were sown with WC. Two N levels were imposed on the plots: 100 and 250 kg N ha⁻¹. There was no ploidy or cultivar effect on clover proportion, however nitrogen had a significant effect ($P < 0.001$) on clover proportion. The mean clover proportion across the year was 28% at 100 kg N ha⁻¹ biologically fixing a total of 105.7 kg N ha⁻¹. In contrast, at 250 kg N ha⁻¹ the mean clover proportion was at 8%, biologically fixing 23.7 kg N ha⁻¹. Dry matter contribution by WC receiving 100 kg N ha⁻¹ was 2544 kg DM ha⁻¹ while it was only 729 kg DM ha⁻¹ at 250 kg N ha⁻¹. Applying less artificial N resulted in increased clover proportion and a higher biological fixation while having no negative effect on DM yield. Further years experimentation is necessary to determine if these benefits are retained over time.

P612

N Management in Maize Leaves in Relation to Fertilization and Mycorrhizae

Teresa Lehmann, Ewelina Paluch, Wladyslaw Polcyn, Ewelina Stolarska, Marianna Scierzynska
Department of Plant Physiology, A. Mickiewicz University, Poznan, Poland

The aim of the study was question of whether arbuscular mycorrhizal fungi (AMF) can contribute significantly the N nutrition of the plant host. Maize plants (hybrid Opoka) mycorrhized (AMF1) or not (AMF0) with high quality AMF inoculum were grown in phytotron for 8 weeks. After that the commercial fertilizer was introduced for additional 5 weeks at 4 suboptimal doses for plant growth and 1 optimal. AMF colonization was high and similar in the range of all suboptimal fertilization doses but the highest dose strongly reduced the mycorrhization rate. Fertilization stimulated leaf N status (measured fluorometrically as NBI Nitrogen Balance Index) as well as biochemically estimated content of N and P (% of DW) in both plants, but AMF1 plants reached the highest difference in NBI to AMF0 plants at the lower dose of fertilizer. Moreover, at the lowest suboptimal dose presence of fungi allowed plants to reduce the decrease in NBI related to leaf position. Symbiosis also differently regulated root and leaf GS and NR protein expression. These data reveal that AM colonization may assist to host plant in N management.

We acknowledge support for TERI, New Delhi, providing us with spores of *Rhizophagus irregularis*. This work was supported by grant 2011/01/B/NZ9/00362 from Polish National Science Center.

P613

A Cyborg Plant – the Development of an Experimental System for a Long-Term Monitoring of Trans-Root Bioelectrical Potential (TRBP) in Conifers

Robert Bernacik, Pawel Jedynak, Przemyslaw Malec
Jagiellonian University, Cracow, Poland

Xylem serves as a transporting highway, channeling water and ions from roots to leaves. The changes in ion distribution, being an effect of ion uptake from the soil and a subsequent ion transport in the xylem path, generate the so-called trans-root bioelectric potential (TRBP)^{1,2}. Although TRBP has previously been used to monitor the physiological state of plants in the area of plant physiology, agriculture and forestry^{1,2}, the information on its values and variations in conifers, especially those growing in natural conditions, remains limited. To detect TRBP in Norway spruce (*Picea abies*) trees grown in the upper treeline of Carpathian Mts., we have developed a measuring device based on two unpolarizable Ag/AgCl₂ electrodes with liquid junctions² and a waterproof millivoltmeter. The TRBP measuring instruments were accompanied by a portable meteorological station. The experimental system provided a continuous, real-time recording of both TRBP values and several meteorological parameters (insolation, temperature, rainfall, humidity, etc.) in a long-term mode, during vegetational season. The system allowed us to detect correlations between TRBP values and selected parameters in the environment. Our hopes concerning this study are the effects of environmental conditions on the activity of the roots interaction system of coniferous trees in situ³.

¹ Okamoto H, Hasuike S, Masaki N, Shinobe T (1994) J. Plant Res. 107 : 177-180

² Okamoto H, Masaki H (1999) J. Plant Res. 112: 123-130.

³This study is supported by the Ministry of Science and Higher Education (MNiSzW) of the Republic of Poland (DS-1277/HF/2013, "Generation of the Future")

P614

The Growth Zone of Maize Leaves Is an Ideal Model System for Systems Biology Approaches to Investigate the Effects of Drought Stress on Organ Growth Regulation

Viktoriya Avramova, Hamada AbdElgawad, Han Asard, Gerrit Beemster
University of Antwerp, Antwerp, Belgium

Drought is presumably the most important yield-limiting factor under natural and agricultural conditions and therefore detailed knowledge of its impact on plant growth regulation is crucial.

The maize leaf represents an attractive system for growth studies, because of its spatial gradient, allowing sampling of dividing, expanding and mature cells at the same time, and its big size, providing enough material for most molecular analyses, which is a big advantage over the model plant *Arabidopsis thaliana*.

By means of a kinematic analysis we showed that drought inhibits leaf growth in maize by reducing cell division rates in the meristem and cell expansion rates in the elongation zone. A transcriptome analysis provided a molecular basis for the observed inhibition of cell division rates, but also pointed at distinct effects on the development of the photosynthetic machinery, and antioxidant and redox systems. The effects on these regulatory pathways were subsequently confirmed by detailed biochemical analysis of the activity of key enzymes and quantification of metabolites.

We demonstrated the functional significance of the identified transcriptional and physiological changes, showing that 1. Increasing the antioxidant capacity in the growth zone, by overexpression of iron superoxide dismutase, increases leaf growth under control and drought conditions. 2. Increased expression of photosynthesis genes under stress facilitates faster growth upon re-watering compared to unstressed controls.

P615

The *Arabidopsis thaliana* Maternal Effect Embryo Arrest Protein (MEE) Contains a Domain that Functions as an Adenylate Cyclase

David Kawadza, Oziniel Ruzvidzo
North-West University, Mafikeng, South Africa

Adverse environmental conditions are likely to continue, there is thus an urgent need for rational and system-based approach to develop crop plants with increased tolerance to environmental fluctuations. An integrated, systems-based approach may possibly deliver effective biotechnological solutions. Part of this approach involves the study of a special group of proteins: adenylate cyclases (AC) known to systemically affect homeostasis in plants. ACs are enzymes converting adenosine-5'-triphosphate to the second messenger cyclic 3', 5'-adenosine monophosphate (cAMP). In animals and lower eukaryotes, ACs and their product cAMP have firmly been established as ubiquitous signaling molecules with crucial roles in several cellular signal transduction pathways. However, in higher plants only one annotated and experimentally confirmed AC is a *Zea mays* pollen protein capable of generating cAMP and is responsible for regulating pollen tube growth. Recently several candidate AC-encoding genes in the *Arabidopsis* genome have been proposed based on functionally assigned amino acids in the catalytic centre of annotated and experimentally tested nucleotide cyclases in lower and higher eukaryotes. Here we detail the cloning and functional characterization of a candidate AC domain from *Arabidopsis thaliana* in the form of maternal effect embryo arrest protein (MEE-AC; At2g34780). Through a series of complementation tests and enzyme immunoassays, the recombinant MEE-AC was thoroughly prescreened and functionally evaluated for its possible *in vitro* and *in vivo* adenylate cyclase activities. Findings from these tests and assays indicate that the recombinant AtMEE-AC does possess some functional adenylate cyclase activities and is therefore, a *bona fide* higher plant adenylate cyclase with potential roles in cell-signaling and transduction systems.

P616

Classification of 40 Rice E2 Ubiquitin-Conjugating Enzymes and Their Interaction Profiles with 17 Rice ARM-U-Box E3 Ubiquitin Ligases

Hye Jo Min, Ki Youl Park, Hansol Bae, Woo Taek Kim
Yonsei University, Seoul, Republic of Korea

Rice, a monocot model crop, contains at least 48 putative E2 ubiquitin (Ub)-conjugating enzymes. Based on homology comparisons with 40 Arabidopsis E2 proteins and 35 human E2s, 48 rice E2s were classified into 15 different groups. Yeast two-hybrid analyses using the U-box-domain regions of armadillo (ARM)-U-box E3 Ub-ligases and the Ub-conjugating (UBC) domains of E2s showed that, among 40 rice E2s, 11 E2s accounted for 70% of the interactions with 17 ARM-U-box E3s. Thus, a single E2 could interact with multiple ARM-U-box E3s, suggesting the presence of E2 hubs for E2-E3 interactions in rice. Rice SPL11 ARM-U-box E3 displayed distinct self-ubiquitination patterns, including poly-ubiquitination, mono-ubiquitination, or no ubiquitination, depending on different E2 partners. This suggests that the mode of ubiquitination of SPL11 E3 is critically influenced by individual E2s. This work was supported by grants from the Next Generation BioGreen 21 Program (Project No. PJ008152) and the NRF (Project No. 2010-0000782) to WTK.

P617

Regulation of Plant Stem Cell Quiescence by a Brassinosteroid Signalling Module

Josep Vilarrasa-Blasi¹, Mary-Paz González-García¹, David Frigola², Norma Fàbregas¹, Konstantinos G. Alexiou¹, Nuria López-Bigas³, Susana Rivas^{4,5}, Alain Jauneau⁶, Jan U. Lohmann⁷, Philip N. Benfey⁸, Marta Ibañes², Ana I. Caño-Delgado¹

¹Department of Molecular Genetics, Center for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Spain,

²Departament d'Estructura i Constituents de la Matèria, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain, ³Biomedical Genomic Group, Research unit on biomedical informatics (GRIB), Barcelona Biomedical Research park (PRBB), Dr. Aiguader 88, 08003 Barcelona, Spain,

⁴INRA, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, F-31326 Castanet-Tolosan, France, ⁵CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR2594, F-31326 Castanet-Tolosan, France, ⁶Fédération de Recherche 3450, Plateforme Imagerie, Pôle de Biotechnologie Végétale, F-31320 Castanet-Tolosan, France, ⁷Center for Organismal Studies (COS), University of Heidelberg, Heidelberg, Germany, ⁸Department of Biology, HHMI and Duke Center for Systems Biology, Duke University, Durham, NC 27708, USA

The quiescent center (QC) maintains the activity of the surrounding stem cells within the root stem cell niche, yet specific molecular players sustaining the low rate of QC cell division remain poorly unknown. Here, we identified a R2R3-MYB transcription factor, BRAVO (BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTRE), acting as cell-specific repressor of QC divisions in the primary root of Arabidopsis. Ectopic BRAVO expression restricts overall root growth and ceases root regeneration upon damage of the stem cells, demonstrating the role of BRAVO in counteracting Brassinosteroid (BR)-mediated cell division in the QC cells. BR-regulated transcription factor BES1 (BR1-EMS SUPPRESSOR 1) directly repress and physically interacts with BRAVO in vivo, creating a bistable switch that modulates QC divisions at the root stem cell niche. Together, our results define a mechanism for BR-mediated regulation of stem cell quiescence in plants.

P618

Signal Transduction Pathways from Exogenous to Endogenous Salicylic Acid in Wheat

Orsolya Kinga Gondor, Tibor Janda, Gabriella Szalai
*Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences H-2462
Martonvásár, POB. 19, Hungary*

Salicylic acid (SA) is a well-known signal molecule in plants. It is not clear whether the effects of exogenous SA are direct or they are connected with that of endogenous SA.

Mv Emese winter wheat plants were grown in hydroponic solution. SA treatment was carried out either by soaking seeds before sowing or by addition of SA to the hydroponics of seven-day-old plants for a day. Samples were collected after 1 and 7 days of the hydroponic SA treatment. The content of malondialdehyde (MDA), SA, benzoic acid (BA), cinnamic acid (CA), orto-hidroxi-cinnamic acid (oHCA) and flavonoids, namely myricetin, kaempferol, quercetin and rutin was measured in methanol soluble free, methanol soluble and insoluble bound fractions.

Seed soaking did not have any effect on the content of the endogenous SA and its precursors. The hydroponic solution treatment had a hard effect on the level of SA and its precursors. The methanol soluble bound BA content decreased as much as the SA content increased while the free oHCA content increased. The MDA content also increased so it can be concluded that the synthesis of SA could happen via BA pathway and oHCA can serve as an antioxidant. The myricetin and kaempferol content increased in methanol soluble bound fraction, but there was no change in the other fractions. The amount of quercetin and rutin did not change during these treatments.